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289.	Catecholamines: biochemical characterization I	Poster	Thu	8:30 AM
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166.	Characterization of noncholinergic receptors	Poster	Tue	1:00 PM
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344.	Excitatory amino acids II	Slide	Fri	8:30 AM
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301.	GABA and benzodiazepines: binding sites II	Slide	Thu	1:00 PM
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55.	Cardiovascular regulation: functional aspects II	Slide	Mon	1:00 PM
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337.	Corticotropin releasing factor	**Symp.**	Fri	8:30 AM
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315.	Hormonal control of behavior II	Poster	Thu	1:00 PM
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106.	Cortex and cortico-subcortical relationships II	Poster	Tue	8:30 AM
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Theme I: Neural Basis of Behavior

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359.	Alcohol: behavior	Poster	Fri	8:30 AM
357.	Alcohol: environmental influences and electrophysiology	Poster	Fri	8:30 AM
61.	Angiotensin and drinking	Poster	Mon	1:00 PM
185.	Biological rhythms I	Slide	Wed	8:30 AM
313.	Biological rhythms II	Poster	Thu	1:00 PM
312.	Biological rhythms: suprachiasmatic nucleus	Poster	Thu	1:00 PM
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221.	Circuitry and pattern generation II	Slide	Wed	1:00 PM
299.	Clocks in the test tube: toward a mechanistic analysis of circadian oscillators	**Symp.**	Thu	1:00 PM
47.	Drugs of abuse: nonopiates I	Slide	Mon	1:00 PM
331.	Drugs of abuse: nonopiates II	Poster	Thu	1:00 PM
124.	Effects of chronic drug administration: neurotoxicology	Poster	Tue	8:30 AM
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59.	Feeding and drinking: central mechanisms IV	Poster	Mon	1:00 PM
60.	Feeding and drinking: cues for need state I	Poster	Mon	1:00 PM
62.	Feeding and drinking: cues for need state II	Poster	Mon	1:00 PM
264.	Feeding and drinking: cues for need state III	Slide	Thu	8:30 AM
138.	Feeding and drinking: neuropharmacology	Slide	Tue	1:00 PM
177.	Functional reconstruction of neuronal systems	**Symp.**	Wed	8:30 AM
194.	Human behavioral neurobiology	Poster	Wed	8:30 AM
193.	Human neuropsychology I	Poster	Wed	8:30 AM
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192.	Interhemispheric relations	Poster	Wed	8:30 AM
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268.	Invertebrate learning and behavior II	Slide	Thu	8:30 AM
12.	Learning and memory: anatomy I	Slide	Mon	8:30 AM
189.	Learning and memory: anatomy II	Poster	Wed	8:30 AM
100.	Learning and memory: anatomy and physiology	Slide	Tue	8:30 AM
191.	Learning and memory: hippocampal physiology	Poster	Wed	8:30 AM
142.	Learning and memory: pharmacology I	Slide	Tue	1:00 PM
239.	Learning and memory: pharmacology II	Poster	Wed	1:00 PM
240.	Learning and memory: pharmacology of conditioning	Poster	Wed	1:00 PM
190.	Learning and memory: physiology	Poster	Wed	8:30 AM
162.	Monoamines and behavior I	Poster	Tue	1:00 PM
163.	Monoamines and behavior II	Poster	Tue	1:00 PM
340.	Monoamines and behavior III	Slide	Fri	8:30 AM
286.	Motivation and emotion	Poster	Thu	8:30 AM
285.	Motivation and emotion: self-stimulation	Poster	Thu	8:30 AM
157.	Neuroethology: avian auditory system and vocalization	Poster	Tue	1:00 PM
154.	Neuroethology: nonavian auditory system and vocalization	Poster	Tue	1:00 PM
156.	Neuroethology: nonteleosts	Poster	Tue	1:00 PM
155.	Neuroethology: teleosts	Poster	Tue	1:00 PM
115.	Neuropeptides and behavior	Poster	Tue	8:30 AM
81.	Neuropeptides and behavior: opiates	Poster	Mon	1:00 PM
63.	Neuropeptides and behavior: vasopressin and oxytocin	Poster	Mon	1:00 PM
80.	Opiates I	Poster	Mon	1:00 PM
82.	Opiates II	Poster	Mon	1:00 PM
261.	Opiates III	Slide	Thu	8:30 AM
126.	Psychotherapeutic drugs: antidepressants	Poster	Tue	8:30 AM
128.	Psychotherapeutic drugs: anxiolytics	Poster	Tue	8:30 AM
127.	Psychotherapeutic drugs: neuroleptics	Poster	Tue	8:30 AM
2.	Sexually dimorphic behaviors: differentiation in mammals	**Symp.**	Mon	8:30 AM
348.	Sleep	Poster	Fri	8:30 AM
161.	Stress, hormones, and the autonomic nervous system I	Poster	Tue	1:00 PM
307.	Stress, hormones, and the autonomic nervous system II	Slide	Thu	1:00 PM
326.	Stress, hormones, and the autonomic nervous system III	Poster	Thu	1:00 PM

2 SYMPOSIUM. SEXUALLY DIMORPHIC BEHAVIORS: DIFFERENTIATION IN MAMMALS. T. O. Fox, Children's Hosp., Harvard Med. Sch. (Chairman); J. F. DeBold, Tufts Univ.; M. J. Baum, M.I.T.; K. L. Olsen, SUNY, Stony Brook; F. S. vom Saal, Univ. Missouri, Columbia.

The study of sexually dimorphic behaviors, including those involved in reproduction, aggression, play and other activities, has led to the elucidation of many aspects of neural sexual differentiation. Concepts associated with the development and maintenance of these behaviors provide much of the rationale for studies on sexual differentiation of the brain. This symposium addresses several advances in our understanding of sexual behaviors and their determinants, with special attention to generalizations which may be appropriate only within species and consequently to emerging concepts which diverge from previously accepted dogma.

The results of experiments to assess the effects of gonadal steroids on behavior require control of the environmental, social, and hormonal status of the animals and ability of investigators to observe subtleties of behavior. Effects of different doses of androgens and of non-hormonal influences on aggressive behaviors by both female and male rodents will be described (DeBold). The relative influence of androgens and estrogens varies among species and among different endpoints. For example, males of several mammalian species lack the capacity to display many aspects of feminine sexual behavior and neuroendocrine cyclicity in adulthood. This process of defeminization occurs in male rodents in response to the action of estradiol, formed in the developing brain from circulating androgens. Evidence indicates that coital defeminization as observed in rodents does not occur in males of several higher mammalian species. This includes a carnivore, the ferret, despite the presence in males of high levels of neural estrogen biosynthesis and receptors during the perinatal period of sexual differentiation (Baum).

Possible involvement of androgen receptors can be examined, and attempts can be made to distinguish these from the effects mediated by estrogen formation, by analyzing the responses of genetic mutants with androgen resistance. These display defects in androgen receptors along with deficiencies of male traits. Comparisons between mice and rats with androgen resistance are being made for both male and female aspects of reproductive behaviors (Olsen). Male-typical and female-typical behaviors are expressed to variable extents in the other sex. The extent and nature of this phenomenon is species dependent. Intrauterine position correlates with the extent of heterotypical behavior displayed. Parameters of these effects on both females and on males will be discussed. This system provides a means for examining individuality in behavior and for asking how this influences populations and evolution of behaviors (vom Saal).

3 WORKSHOP. RECIPROCAL INHIBITION AND COACTIVATION OF ANTAGONIST MUSCLES: TWO FUNDAMENTAL MODES OF MOTOR CONTROL. D.R. Humphrey, Emory Sch. Med.; A.M. Smith, Univ. Montreal; Y. Lamarre, Univ. Montreal; T.R. Nichols, Univ. Washington; Y. Shinoda, Tokyo Med. Dent. Univ.; E. Bizzi, MIT.

Following the pioneering studies by Sherrington in the decerebrate cat, researchers in motor control have viewed reciprocal inhibition as the major pattern of activation of the antagonist muscles which act about a particular joint. Recent studies have emphasized, however, that many motor activities involve a coactivation of antagonist muscles, either as a means of stabilizing the course of movement of a joint during motor learning or in the presence of unpredictable external perturbations, or of fixing the position of a joint in one plane as a postural support for orthogonal movements about the same joint, or movements of adjacent joints. This workshop focuses on the conditions under which these fundamental patterns of muscle activation occur, and their central correlates. Lamarre will present evidence from studies with normal and deafferented monkeys and humans, which indicates that reciprocal inhibition, coactivation and triphasic patterns of antagonist activation are centrally generated, with peripheral input serving only to modulate these patterns. Nichols will summarize evidence from studies in the cat which indicates that these two forms of muscle activation can be evoked by stimulation of different brain stem sites. He will discuss also the motor control advantages that are offered by these two different patterns of muscle activation. Smith will summarize data from studies of cerebellar neuron activity in the monkey which indicates that a suppression of Purkinje cell discharge may be necessary for switching between reciprocal inhibitory and coactivation modes of control. Shinoda will summarize evidence from studies of branching patterns of single axons in cerebello-thalamo-motor cortex and corticospinal pathways, which indicates that axonal divergence is sufficient to suggest that functional groupings of muscles will ultimately be affected by activity in even single central neurons. Humphrey will present evidence from studies in the monkey which indicates that the efferent outflow of the motor cortex is organized not for the control of single muscles, but for entire sets of muscles that are responsible for certain fundamental movements, and their postural support; in the latter function, antagonist muscles are often coactivated. Bizzi will conclude the formal presentations by summarizing evidence from EMG studies in man concerning the patterns of muscle activation that are seen across the arm, during voluntary, guided movements, in which case both movement and postural support patterns must be generated. The session will close with a round table discussion, in which new avenues of research are suggested and discussed.

PAIN MODULATION: BULBOSPINAL MECHANISMS

4.1 LIGHT AND ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL LOCALIZATION OF SEROTONIN SYNAPSES ON PRIMATE SPINOTHALAMIC TRACT NEURONS. M.A. Ruda and J. Coffield*. NAB, NIDR, NIH, Bethesda, MD 20205.

Serotonin (5-HT) neurons in the brain stem are a major source of descending inhibition in the spinal cord. Spinothalamic tract (STT) cells comprise a major pathway for the transmission of nociceptive information from the dorsal horn to higher centers of the neuraxis. Using the combined techniques of retrograde transport of horseradish peroxidase (HRP) and immunocytochemistry, synaptic interactions between these systems can be identified. To label STT cells, HRP was injected into the thalamus of 2 rhesus monkeys. After 72-84 hrs, the animals were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde in phosphate buffer. The cervical and lumbar spinal cords were sectioned on a Vibratome at 50 μm and processed with $CoCl_2$-intensified DAB to yield a black reaction product in STT cells. To identify transmitter-specific afferent input, the tissue was subsequently processed for PAP immunocytochemistry to produce a red-brown label. Tissue used for light microscopic (LM) analysis was processed with 0.75% Triton X-100 to facilitate penetration of the immunocytochemical stain throughout the section. Tissue for ultrastructural (EM) analysis was initially screened at the LM level, after clearing in glycerin, with a 100X oil immersion objective to identify STT cells contacted by 5-HT immunoreactive axonal endings. LM and EM analyses focused on the somata and proximal dendrites of STT cells in laminae I and V. Most 5-HT contacts were located on proximal dendrites of these STT cells rather than on the cell somata. In lamina I, both small and large bipolar and multipolar STT cells (10-25 μm soma short diameter) received a variable number of 5-HT contacts. In lamina V, larger multipolar neurons (25-35 μm) often received more than five 5-HT contacts while small bipolar neurons received less. More than half of the laminae I and V STT cells sampled appeared to receive 5-HT contacts on their somata or proximal dendrites. At the EM level, STT cells were marked by electron dense HRP granules while 5-HT immunoreactive endings contained PAP reaction product. In laminae I and V, 5-HT axonal endings contacting STT cells varied from 0.8-2.5 μm in length, and contained oval agranular vesicles and a few dense core vesicles. They formed symmetrical or slightly asymmetrical synaptic contacts. STT cells receiving 5-HT synapses typically received synapses from numerous other unlabeled endings. These observations demonstrate that a subset of STT cells in laminae I and V can be postsynaptically modulated directly by descending 5-HT afferent input. Since STT neurons receiving 5-HT input are contacted by numerous other axonal endings, a major site of modulation of the transmittal of noxious information appears to occur directly on the proximal portion of STT cells.

4.2 IONTOPHORETIC STUDY OF BIOGENIC AMINE AND PEPTIDE EFFECTS ON PRIMATE SPINOTHALAMIC TRACT CELLS. Wm.S.Willcockson*, J.M.Chung, Y.Hori*, K.H.Lee*, and W.D.Willis (SPON: J.P.Gallagher). Marine Biomed. Inst. & Depts. of Physiol. & Biophysics and Anatomy, Univ. TX Med. Branch, Galveston, TX 77550.

A full understanding of the role of the spinothalamic tract (STT) in the transmission of pain requires investigation of the neurochemicals mediating synaptic events in this pathway. Proposed mediators include the biogenic amines serotonin (5HT) and norepinephrine (NE), the amino acids glutamate (Glu), γ-aminobutyrate (GABA) and glycine (Gly) and the endogenous peptides enkephalin (ENK) and substance P (SP). 5HT, NE, GABA and Gly have had predominantly inhibitory effects when iontophoresed onto dorsal horn interneurons. Glu and SP usually excite dorsal horn cells.

The compounds studied include Glu, GABA, Gly, 5HT, NE, dopamine (DA), acetylcholine (ACh), methionine-enkephalin (M-ENK), leucine-enkephalin (L-ENK), substance P (SP) and cholecystokinin (CCK). The drugs were applied iontophoretically from 7-barreled glass microelectrodes. Experiments were performed on anesthetized monkeys (Macaca fascicularis). STT cells in the lumbosacral spinal cord were identified antidromically by stimulation in the contralateral thalamus. Drugs were tested for effects on background activity, Glu-induced firing, and activity evoked by pinching the skin.

While Glu excited STT cells and was thus used for tests of the other compounds, the amino acids GABA and Gly inhibited Glu- and pinch-induced activity in all STT cells tested. STT cells were also inhibited by 5HT, NE and DA. No cases of excitation were observed. ACh inhibited the Glu response of most STT cells, although 3 of 21 cells exhibited a slight enhancement of activity.

M-ENK and L-ENK produced strong inhibition of background, Glu-induced, and pinch-evoked activities. Intravenous naloxone reduced these inhibitory effects. SP had complex effects on STT cells. In addition to excitation or inhibition, biphasic effects such as inhibition followed by excitation and excitation followed by inhibition were observed. Commonly, single cells had multiple responses to SP, including any combination of single or biphasic effects depending upon changes in dose or electrode position relative to the cell. CCK was strongly inhibitory on all STT cells tested and when applied simultaneously with L-ENK, inhibition was additive.

Although most of the effects of amines, amino acids and peptides were anticipated from previous work on unidentified interneurons, we did not predict the complex actions of substance P, which suggested more than one type of substance P receptor. Furthermore, we thought CCK would have an action opposite to that of ENK. (Supported by NIH grants NS 09743 and NS 11255 and a grant from The Moody Foundation.)

*Indicates Nonmember of Society

4.3 POSTSYNAPTIC EFFECTS IN SPINAL LAMINA I AND II NEURONS PRODUCED BY STIMULATING MIDLINE MEDULLA AND PONS. A.R. Light Dept. of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27514

Stimulation of midline, medullary-pontine regions has been shown by others to inhibit nociceptive reactivity in animals and to inhibit the responses of neurons to noxious stimuli. The present study used intracellular recordings to examine postsynaptic potentials and conductance changes in marginal zone (lamina I) and substantia gelatinosa (lamina II) neurons following small, single stimuli delivered to the midline medulla and pons. Tungsten monopolar microelectrodes (10 meg-ohm) were used to deliver test volleys (1-10 μ amp, 0.2 msec) to the medulla, and horseradish peroxidase (HRP)-containing micropipettes were used to record neurons intracellularly and to label some neurons for correlative morphological studies. Small hyperpolarizing pulses were delivered through the micropipette to measure the conductance changes. Nociceptive neurons were either inhibited by stimulation of midline medulla or showed no effect. Of those inhibited, all but one demonstrated an IPSP following single stimuli to the medulla. The average latency of the IPSP was 31 msec (range 9.6 - 114 msec; conduction distance ∿ 300 mm) indicating that elements with conduction velocities of ∿ 10 m/sec elicited the effects if produced monosynaptically. The average duration of the IPSP was 206 msec (range of 40 - 450 msec), indicating either very long-lasting synaptic effects or heterogenous conduction velocities of activated descending fibers. These IPSPs all occurred with a small conductance increase, which indicates postsynaptic inhibition, but the small size indicates either additional inhibitory mechanisms or remote placing of inhibitory synapses from the recording site. Half the multireceptive (receiving both innocuous and noxious inputs) and innocuous mechanoreceptive neurons were also inhibited by stimulation of midline medulla. For both classes the IPSP had an average latency of 30 msec and a long duration (average around 250 msec). The IPSPs again occurred with a conductance increase. The other half of the neurons in these 2 classes were excited by stimulation of midline medulla. This excitation was evident as an EPSP which led to an action potential, which in some neurons was followed by a long-lasting IPSP. The EPSP's latency was around 30 msec (300 mm conduction distance) although the duration was much shorter than for the IPSPs (around 12 msec). These findings suggest heterogenous pathways from midline medulla and pons which can selectively postsynaptically inhibit nociceptive neurons and some multireceptive and innocuous mechanoreceptive neurons in laminae I and II, and selectively excite some multireceptive and innocuous mechanoreceptive neurons in these same laminae. Supported by NINCDS grants NS-16433 and NS-00534.

4.4 SIGNAL DETECTION ANALYSIS OF THE RESPONSE PROPERTIES OF DORSAL HORN NEURONS TO NOXIOUS THERMAL STIMULI. K.C. Kajander*, D.C. Tam, T.J. Ebner and J.R. Bloedel. (Spon: G.W. King). Depts. of Neurosurgery and Physiology, Univ. of Minn., Mpls., MN 55455.

Experiments were designed to evaluate the use of signal detection theory for analyzing the capability of lumbar dorsal horn neurons to distinguish between thermal stimuli of different intensities with and without brainstem stimulation. In decerebrate cats, the unitary responses of 113 neurons to noxious thermal stimuli were evaluated with and without electrical stimulation of either the periaqueductal gray (PAG) or the nucleus raphe magnus (NRM). The thermal stimuli consisted of 10° and 12°C temperature pulses with a 2 second rise time beginning from either a 42° or 45°C baseline. The neurons selected for study increased their spontaneous activity in response to the baseline temperature maintained on the hindpaw and responded to the temperature pulse with an increased discharge rate. Each neuron was studied under four conditions: conditions 1 and 2 were the 10°C temperature step (S1) with and without brainstem stimulation, conditions 3 and 4 were the 12°C temperature step (S2) with and without brainstem stimulation. The time of response onset (latency), the duration of the response, and the mean and standard deviation of the discharge rate were determined for each stimulus presentation. Discriminability (d') based on the mean firing frequencies of the responses evoked by S1 and S2 was calculated for appropriate neurons. Electrical stimulation produced increases (13 of 25 cases) or decreases (11 of 25 cases) in d' for different neurons. In only one case was d' unaffected by the brainstem stimuli. These changes in d' did not always parallel changes in mean firing rate, since the latter decreased in all but one case. Two different estimates of criterion, L(x) were calculated. Using estimates based on mean firing frequency of the response in the four different conditions, L(x) was found to always shift to higher values during electrical stimulation. However, when using estimates of L(x) based on the first standard deviation of the firing frequency in the four different conditions, L(x) was calculated to be larger for the S2 conditions than for the S1 conditions when the d' value was greater than 2.2. This relationship was reversed when d' was less than 2.2. These data indicate that changes in neuronal responses reflected in changes of d' and L(x) can occur in the spinal cord as a result of activating descending projections from the brainstem known to be important in altering pain perception. This research was supported by NIH Grants RO1-NS 18338 and RO1-NS 09447.

4.5 INHIBITORY TASK-RELATED RESPONSES OF MONKEY MEDULLARY DORSAL HORN NEURONS. G.H. Duncan*, M.C. Bushnell, R. Bates* and R. Dubner. Neurobiology & Anesthesiology Br., NIDR, NIH, Bethesda, MD 20205.

In previous studies we described mechano- and thermosensitive medullary dorsal horn neurons which, in the behaving monkey, had additional responses associated with significant events within the task. Excitatory task-related responses were studied in detail and were shown to be independent of stimulus intensity or modality. The present study investigated in detail the occurrence of inhibitory task-related responses.

Data were collected from 4 Rhesus monkeys trained to perform detection tasks involving thermal and visual stimuli. Monkeys initiated a trial by detecting the onset of a light cue and depressing a button. They were trained to continue depressing the button for a variable period of 3-8 sec. The monkeys terminated the trial by releasing the button after detecting one of the following events: (1) termination of a warming stimulus (37°-43°C); (2) onset of a noxious thermal stimulus (45°-49°C); or (3) onset of a second light cue. Single unit activity was recorded in the medullary dorsal horn during performance of the behavioral tasks. Projection neurons were identified by antidromic responses to stimulation of the thalamic ventroposterior medial nucleus. A medullary dorsal horn neuron was defined as having a task-related response if the averaged activity associated with a behavioral event was significantly different from the averaged neuronal activity occurring between trials ($p < .05$).

Thirty-three medullary dorsal horn neurons were encountered which exhibited task-related responses. Twenty of these 33 neurons contained inhibitory task-related responses. Both the excitatory and inhibitory responses shared many general characteristics: (1) occurrence in neurons whose response properties were otherwise classified as trigeminal mechanoreceptive, trigeminal thermoreceptive or nonspecific reticular; (2) occurrence in trigeminothalamic neurons; (3) association with behavioral events related to trial initiation, trial continuation or trial termination; and (4) independence of modality or intensity of the relevant stimulus. The most common patterns of task-related response involved either exclusive excitation (13/33) or combinations of inhibition and excitation (16/33). Only 4 neurons demonstrated exclusively inhibitory task-related responses.

We have postulated previously that task-related responses may provide signals for altering the gain-control of somatosensory information or for facilitating appropriate goal-directed motor behaviors (button press, button release, or drinking). The presence of inhibitory task-related responses may serve as an enhancing mechanism for these signals.

4.6 RAPHE MAGNUS INHIBITION OF UPPER THORACIC SPINOTHALAMIC CELL RESPONSES TO INTRACARDIAC INJECTIONS OF BRADYKININ. W. Steve Ammons, Robert W. Blair, and Robert D. Foreman. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Stimulation of the nucleus raphe magnus (RM) inhibits responses of lumbosacral spinothalamic neurons of the monkey (Willis et al, J. Neurophysiol, 40:968, 1977). Our laboratory has observed a similar inhibitory effect of RM stimulation on upper thoracic spinoreticular neurons in the cat (Chapman et al, Neurosci. Abst, 1982). The goal of the present study was to demonstrate effects of RM stimulation on upper thoracic spinothalamic neurons. Experiments were performed on 10 monkeys (Macaca fascicularis) which were tranquilized with ketamine and anesthetized with α-chloralose. The gray matter of the T_1 to T_4 segments of the spinal cord was searched for cells which were antidromically activated by stimuli applied to the ventral posterior lateral nucleus of the thalamus. Each cell was thus documented to be a spinothalamic tract neuron. The somatic receptive field of each spinothalamic neuron was mapped and its response to electrical stimulation of cardiopulmonary sympathetic afferent fibers was determined. Each of the 19 neurons studied received viscerosomatic convergent inputs. RM stimulation (100 Hz, 200 μsec duration, 50-500μ A) inhibited the background activity as well as responses to somatic stimuli of all neurons tested. However, particular attention was devoted to RM effects on spinothalamic cell responses to a noxious cardiac stimulus, intracardiac injection of the algesic chemical bradykinin (2 μg/kg). Bradykinin injection resulted in an increase in cell activity of 13 of 19 neurons from 12 ± 2 to 25 ± 3 impulses per second ($p<.001$). Stimulation of RM (average current 300μ A) near the peak of the response to bradykinin reduced the cell discharge rate to an average of 3±1 impulses per second ($p<.001$). All 13 bradykinin responsive cells were inhibited by RM stimulation. Six of the 13 responsive neurons exhibited a bursting pattern of activity synchronous with the arterial pressure wave. RM stimulation either eliminated the patterned activity or resulted in more random activity in each case. RM inhibition of spinothalamic responses to a noxious cardiac stimulus suggests that the descending systems linked to analgesia may be effective against cardiac pain as well as somatic pain. Supported by National Heart, Lung, and Blood Institute Grants HL22732, HL07430, and HL00557.

4.7 ACTIVITY OF RAPHE-SPINAL(DLF) NEURONS IN RELATION TO NOCIFENSIVE BEHAVIOR Horacio Vanegas, Nicholas M. Barbaro* and Howard L. Fields. Departments of Neurology, Neurosurgery and Physiology, University of California, San Francisco, CA 94143.

The nucleus raphe magnus and adjoining regions of the rostral ventromedial medulla (RVM) project via the spinal dorsolateral funiculus (DLF) to inhibit nociceptive transmission and nocifensive reflexes. We have shown (Fields et al., Soc. Neurosci. Abstr. 8:806; 1982) that, just before heat-elicited tail-flick (TF), the activity of some RVM neurons suddenly increases (on-cells) or abruptly decreases (off-cells). We have now studied whether such neurons project to the spinal cord via the DLF.

Rats were initially anesthetized with pentobarbital (60 mg/kg IP) and later maintained in a lightly anesthetized state by a constant infusion of methohexital (15-30 mg/kg.hr IV). The cervical spinal cord was exposed and bathed in mineral oil. TF was elicited by controlled heating of the ventral surface of the tail to 54°C for a maximum of 10 sec. A stainless steel Pt-Au tip-plated microelectrode was stereotaxically driven into an RVM region where TF could be inhibited by stimulating with continuous trains of 50 Hz cathodal pulses (400 μ sec, ≤ 10 μ A). The same microelectrode was then connected for single unit recording. The cord surface over the DLF was stimulated through bipolar, 0.5 mm diameter silver ball electrodes.

Of 48 cells that were antidromically driven from DLF, as proven by collision tests, 9 (19%) increased their firing rate just prior to TF (on-cells), 9 (19%) abruptly ceased firing just before TF (off-cells), and 30 (62%) showed no change in activity related to TF (neutral cells). Other on-cells (N=6) and off-cells (N=10) could not be driven antidromically from DLF. Calculated conduction velocities (m/sec) were 17.6 (10.9-27.8) for on-cells, 10.3 (5.2-18.8) for off-cells and 12.9 (6.6-28.8) for neutral cells. Histological verification showed that all units were located within RVM at the level of the rostral portion of the VII nucleus. Cells that were antidromically driven from DLF were consistently more caudal than cells that were not driven.

These results show that RVM cells having activity changes that directly precede nocifensive reflexes project to the spinal cord via the DLF. On-cells have a higher mean conduction velocity than off-cells ($p<0.02$, two-tailed t-test), and both values are within the range for myelinated fibers. On- and off-cells which project to the DLF are more caudally located within RVM than those which do not. These results suggest that either the on- or the off-cells, or both, are the output cells for RVM modulation of nociceptive transmission and nocifensive reflexes at the level of the spinal cord.

Supported by PHS Grant DA 01949. HV is on leave from the Instituto Venezolano de Investigaciones Cientificas.

4.8 EVIDENCE THAT MORPHINE PRODUCES ANALGESIA BY DISINHIBITING BRAINSTEM NEURONS Greg Zorman*, Horacio Vanegas, Ian Hentall and Howard Fields (SPON: D. Greenberg). Departments of Neurology, Physiology and Neurosurgery, University of California, San Francisco 94143.

The rostroventral medulla (RVM) contains a class of descending neurons which show an abrupt pause in firing prior to tail flick (TF) elicited by a noxious thermal stimulus (Fields et al, Neurosci. Abstr. 8:806, 1982). To study the contribution of these "off-cells" to the modulation of pain we have recorded their TF related activity after administration of morphine and naloxone.

Male Sprague-Dawley rats (325-375 gm) were initially anesthetized with 70 mg/kg pentobarbital (i.p.) and maintained by constant infusion of methohexital (15-30 mg/kg/hr i.v.) which assured a stable baseline TF latency. A stainless steel Pt-Au tip-plated microelectrode was stereotaxically placed in the RVM at sites where TF could be inhibited by continuous trains of ≤ 10 μA, 50 Hz, 400 μsec monopolar, cathodal pulses. The same electrode was then used for recording the activity of "off-cells" during tail heating. Peristimulus histograms were plotted as a function of either TF or tail temperature: a) before morphine; b) after administration of morphine (2.5 mg/kg i.v.); and c) after administration of naloxone (0.25 mg/kg i.v.). One off-cell was studied per rat.

All off-cells were spontaneously active. Most cells increased their background activity following morphine administration, while one clearly decreased. After morphine the TF was blocked and the pause in off-cell discharge was abolished in all cells. In rats where longterm stable recordings were possible, naloxone restored the TF and, concurrently, the pause in firing. All cells recorded were shown histologically to be in the RVM.

These results suggest that systemically administered opiates produce analgesia by disinhibiting brainstem neurons that control nociceptive transmission at the level of the spinal cord.

Supported by PHS Grant DA01949. HV is on leave from the Instituto Venezolano de Investigaciones Cientificas.

4.9 **CHOLINERGIC INTERACTIONS BETWEEN THE NUCLEUS CUNEIFORMIS AND NUCLEUS RAPHE MAGNUS IN THE RAT.** Michael M. Behbehani, Deborah Haggard*. Dept. of Physiology, Univ. of Cincinnati Coll. of Medicine, Cincinnati, OH 45267.

It is now well established that activation of the cells in the nucleus raphe magnus (NRM) and its adjacent area, the nucleus magno cellularis (NMC) produces analgesia by inhibiting dorsal horn neurons that respond to noxious stimulation. Anatomical studies have shown that a major source of afferents to the NRM is from the periaqueductal gray and the nucleus cuneiformis (NC). In this study we examined the response of the NRM neurons to NC stimulation and tested the possibility that acetylcholine (ACh) may be involved in the interaction between NC and NRM and that it may produce its effect through inhibition of GABA release.

Male Sprague-Dawley rats were anesthetized with 1.2g/kg of urethane and prepared for single unit recording. Two stimulating electrodes separated by 1mm, were placed into the NC area. (AP -5.8, D 5.5, L 1.5.) A five barrel electrode consisting of ACh (0.5M), scopolamine (0.5M), either glutamic acid (200mM), or picrotoxin (5mM) NaCl (2M) and a recording electrode was placed in NRM at coordinate of 12-12.5mm caudal to bregma, 0.0 to 0.1mm lateral and 8.9mm below the surface with the electrode inserted at 20 degrees with respect to the vertical plane.

The activity of 50 cells in the NRM was examined. Thirty percent of the cells responded to NC stimulation by excitation. The response latency ranged between 7 to 35 msec., with a mean of 12 msec. Sixteen percent of the cells were inhibited by NC stimulation and the others did not respond. When the response of the NRM cells to ACh was examined, 50% of the cells that responded to NC stimulation were excited by iontophoretically applied ACh, and their response to NC stimulation and to ACh could be totally or partially blocked by scopolamine. In 20% of the cells ACh and stimulation of NC produced excitation that could not be blocked by scopolamine. In 6% of the cells the response to ACh was blocked, but the response to NC stimulation was not affected and in 14%, the response to NC was blocked but the response to ACh was only partially blocked by scopolamine. In the majority of the cells, scopolamine caused a reduction in the baseline firing of the cells without any effect on the height of the action potentials. Scopolamine, at a dose that blocked the response to ACh, also reduced the response to glutamic acid. Iontophoretically applied picrotoxin caused an increase in the baseline firing of the cells and blocked the response of the NRM neurons to ACh. The results of this study indicate that the major projection from NC to NRM is excitatory and that ACh plays an important role in the interaction between these sites. Since the effect of ACh could be blocked by scopolamine it is concluded that its action is mediated through muscarinic receptors. The action of picrotoxin indicates that the ACh effect may be due to inhibition of GABA release by a presynaptic mechanism.

4.10 PREFRONTAL INFLUENCES UPON PERIAQUEDUCTAL GRAY AND ADJACENT REGIONS. S.G.P. Hardy and H.J. Haigler. Dept. of Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216.

In anatomical studies, it has been demonstrated that the prefrontal cortex (PFC) sends numerous projections to the periaqueductal gray (PAG) and adjacent regions. Furthermore, it has been speculated that the PFC may modulate analgesic functions ascribed to these regions. Neurons in these regions, which alter their firing rates in response to PFC stimulation, are identified in this study. These neurons are also characterized as to whether they alter their firing rates in response to noxious stimulation and the microiontophoresis of various neurotransmitter substances.

In urethane anesthetized rats, bipolar stimulating electrodes were placed in the medial PFC. The electrical stimulus administered to this site was a 12 sec train of 500 μsec square waves, delivered at the rate of 10/sec, and having a peak-to-peak intensity of 300-800 μA. Extracellular recordings were made from spontaneously firing neurons in the region of the PAG, using a 5-barrel micropipette. The recording and balance barrels were filled with 2M and 4M NaCl, respectively, and the remaining barrels were filled with methionine-enkephalin (ME;0.01M), norepinephrine (NE;0.2M) and acetylcholine (ACh;0.02M). Noxious stimulus (foot pinch) was administered to the left hind paw, using an analgesy meter (Stoelting).

Most of the 73 neurons recorded responded to PFC stimulation with a response latency of 15-20 msec. Forty-nine neurons altered their firing rates (71% decreased; 29% increased) following the PFC stimulus. Furthermore, 77% of these neurons also responded to the presence of a noxious stimulus. Of these, 64% increased their firing rates in response to noxious stimulation. The influence of concomitantly-administered PFC and noxious stimuli was studied in 4 neurons, in which PFC stimulation was inhibitory and noxious stimulation was facilitory. In each case the neuronal response to noxious stimulation was completely blocked by the presence of PFC stimulation.

When the previously mentioned neurotransmitter substances were ejected into the recording sites, it was observed that each would occasionally cause the neuron to alter its firing rate in a manner which mimicked the response to PFC stimulation. This mimicry was elicited in 100%, 52% and 29% of the neurons studied with ME, NE and ACh respectively.

The results of this study demonstrate that one of the functions of the PFC is to inhibit nociresponsive neurons of the PAG region. Furthermore, the results of this study suggest two putative neurotransmitters which may mediate this PFC effect, i.e. ME and NE.

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4.11 THE PERIAQUEDUCTAL GRAY PROJECTIONS TO THE RODENT SPINAL TRIGEMINAL, RAPHE MAGNUS, GIGANTOCELLULAR PARS ALPHA AND PARAGIGANTOCELLULAR NUCLEI ARISE FROM SEPARATE NEURONS. A.J. Beitz, M.A. Mullett* and L.L. Weiner*. Dept. of Vet. Biology, Univ. of Minnesota, St. Paul, MN 55108.

Possible collateral branches of midbrain periaqueductal gray (PAG) axons which distribute to the nucleus raphe magnus, nucleus reticularis gigantocellularis pars alpha, nucleus reticularis paragigantocellularis and the spinal trigeminal nucleus were analyzed with the double fluorescent retrograde tracer technique, developed by Kuypers and co-workers (1980). Twenty-five adult Sprague-Dawley Rats received injections of the tracers fast blue (FB) and nuclear yellow (NY) into various combinations of the above four nuclear groups. Fast blue (3-5%) was typically injected 48-84 h prior to killing the animal while NY (1-2%) was injected 24-36 h prior to perfusion. The animals were subsequently perfused with 125 ml of hypertonic saline (1.5%) followed by 1000 ml c 10% formalin in citrate buffer (pH 7.18). Thirty-five micron frozen sections were subsequently cut, air dried and viewed with an Olympus BH-2 fluorescent microscope. The location of NY-labeled, FB-labeled and double labeled neurons within the PAG were then plotted for each case. Double labeled neurons were identified by the presence of a light blue fluorescence in the cytoplasm of the cell body and dendrites and an intensely yellow or white fluorescent nucleus. With the exception of a small number of double labeled neurons observed in the PAG following injections of fluorescent dyes into the nuclei reticularis paragigantocellularis and gigantocellularis pars alpha, no double labeled cells were found in this midbrain region following injections of tracers into various combinations of the above four nuclear groups. Injection of the retrograde tracers into these nuclei resulted in labeling predominantly within the ventrolateral, lateral and dorsal portions of the PAG. No double labeled neurons were observed in the majority of our cases despite the fact that the location of FB- and NY-labeled cells within the PAG overlapped extensively. The results of this investigation indicate that the nucleus raphe magnus, the nuclei reticularis gigantocellularis pars α and paragigantocellularis and the spinal trigeminal nucleus are innervated by seperate neuronal populations within the PAG.

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4.12 PYRAMIDAL TRACT MEDIATION OF CORTICOSPINAL INHIBITION. E. Carstens, M.J. Guinan*, S. Pretel & S.N. Suberg. Dept. Animal Physiol., Univ. of Calif., Davis, CA 95616.

Electrical stimulation in the medial posterior sigmoid gyrus (hindlimb region of somatosensory cortex) inhibited the responses of contralateral spinal dorsal horn neurons to noxious skin heating in the cat (Pretel et al., Soc. Neurosci. Abs. 8:767, 1982). We have investigated the role of the pyramidal tract in mediating this corticospinal inhibition by determining if (1) medullary pyramidal stimulation inhibits dorsal horn neurons in a similar manner, and (2) corticospinal inhibition is blocked by lesion of the medullary pyramid.

The responses of single lumbar dorsal horn units to noxious heating of glabrous footpad skin, or to other stimuli, were recorded in cats anesthetized with sodium pentobarbital and ventilated with 70% N_2O. Electrical stimuli (single 0.1 msec pulses, or 100 msec pulse trains at 100 Hz, 3/s, up to 300 µA) were delivered through bipolar electrodes positioned in the posterior sigmoid gyrus, medullary pyramid, and in some experiments in the cerebral peduncle or internal capsule (IC).

Single-pulse stimulation in the medullary pyramid (contralateral to the spinal unit and rostral to the pyramidal decussation) orthodromically excited 18 of 19 tested units in laminae IV-VI. Pyramidal stimulation using pulse trains inhibited the responses of each of 24 units evoked by noxious skin heating. Thresholds for inhibition were lower than those for excitation. The responses of dorsal horn units to a series of graded heat stimuli increased linearly from threshold (40-44°C) to 52°C. When the series was repeated during pyramidal train stimulation (15-70 µA), the slope of the linear temperature-response line was reduced with little change in threshold in 8 units, as was the case with cortical stimulation. Stimulation in the IC or cerebral peduncle bilaterally also had a similar effect in 9 units. Inhibition produced by pyramidal or IC stimulation was unaffected following systemic administration of the serotonin antagonist methysergide (1 mg/kg) in 4 units, similar to the lack of effect of methysergide on corticospinal inhibition.

Inhibition of dorsal horn unit heat-evoked responses produced by cortical stimulation was reduced or abolished following electrolytic lesion of the medullary pyramid contralateral to the spinal unit in 3 cases. Corticospinal inhibition was not affected by a similar lesion below the level of the pyramidal decussation in a fourth unit.

The results indicate that corticospinal inhibition may be mediated by axons descending in the medullary pyramid.

CELL AND TISSUE CULTURE: NEURONS, GLIA, AND NEURONAL MODELS

5.1 DISSOCIATED CHICK SUPERIOR CERVICAL GANGLION NEURONS IN CULTURE. A.D. Zurn* and F.L. Mudry* (SPON: B. Fulpius). Dept. of Biochemistry, University of Geneva, Switzerland.

Superior cervical ganglia from 8-day chick embryos are dissociated with trypsin and cultured on polyornithine-coated culture dishes conditioned with medium from chick heart cells. The cells survive for 3-4 weeks when grown in Eagle's minimum essential medium (MEM) supplemented with heat-inactivated horse serum (1.5%) fetal calf serum (1.5%), glucose (0.3%), glutamine (2mM), KCl (35mM), choline chloride (150µM), MEM amino acid mix and vitamin mix, penicillin-streptomycin and 1µg/ml nerve growth factor (NGF). The cultures contain 5-10% non-neuronal cells as determined either by morphological criteria or by fluorescence microscopy using tetanus toxin and anti-tetanus toxin antibodies. 95-100% of the neurons survive up to 10-12 days in culture. Their number then gradually decreases to 50-60% of the initial amount after three weeks. The size and shape of the neurons (phase-bright, neurite-bearing, tetanus-toxin-positive cells) is heterogeneous. They all take up norepinephrine (NE) as detected by autoradiography after incubation with 6×10^{-7} M (^{3}H)-NE. Incubation of three-week-old cultures with the radiolabeled precursors (^{3}H)-choline and (^{3}H)-tyrosine reveals that the sympathetic neurons synthesize up to 30 times more catecholamines (CA) than acetylcholine (Ach). Both Ach and CA synthesis increases with time in culture, CA synthesis increasing at a two-fold higher rate than Ach. In the absence of NGF, only little CA is synthesized but the amount of Ach is approximately the same as in the presence of NGF. Thus there seem to be two subpopulations of sympathetic neurons in the chick superior cervical ganglion, a cholinergic one surviving in the presence or absence of NGF and an adrenergic one surviving only in its presence. (Supported by the Swiss National Science Foundation grant 3.685.0.80).

5.2 THE INFLUENCE OF SERUM ON THE DEVELOPMENT OF SYMPATHETIC NEURONS IN CULTURE E. Wolinsky*, P.H. Patterson, S.C. Landis, and A. Willard, Dept. Neurobiology, Harvard Medical School, Boston MA 02115

Culturing neurons under serum-free conditions offers greater control of the extracellular environment than the usual serum-supplemented conditions. Iacovitti et. al. (Neurosci. 7:2225, 1982) have reported that neuronal survival and tyrosine hydroxylase activity are comparable between serum-containing and hormone supplemented cultures of sympathetic neurons, but that unlike conventional cultures, serum-free cultures do not produce acetylcholine or store significant amounts of norepinephrine, and contain few varicosities and vesicles. We have modified the N2 serum-free recipe of Bottenstein and Sato (PNAS USA 76:514, 1979) by replacing the basal medium mixture of F12 and DME with L15. Under these conditons, varicosities and vesicles are abundant and of normal appearance, and metabolic labelling studies indicate that norepinephrine synthesis and storage in these serum-free cultures is similar to that in serum-containing cultures. Survival and tyrosine hydroxylase levels are also normal, and serum-free cultures resemble conventional cultures in their binding of a panel of monoclonal antibodies, response to heart cell conditioned medium by induction of acetylcholine synthesis (Patterson and Chun, Devel. Biol. 56:263, 1977), and response to chronic depolarization by increased tyrosine hydrosylase activity (Walicke, Campenot, and Patterson, PNAS USA 74:5767, 1977).

The neurons grown in the L15-based N2 medium do not produce detectable acetylcholine. We attribute this to the absence of a cholinergic inducing factor normally presented to the cultured cells at low levels in adult rat serum. Serum dose-response experiments support this view. Interestingly, serum from neonatal rats does not induce acetylcholine synthesis. This could reflect a schedule of cholinergic factor production consistent with the appearance of cholinergic properties in the sympathetic innervation of the rat sweat gland (Landis and Keefe, Devel. Biol., in press, 1983).

Neurons grown in the modified N2 medium display a high frequency of electrical coupling, as was observed by Higgins and Burton (Neurosci. 7:2241, 1982) using the original N2 formulation. We are exploring the effects of serum, heart cell conditioned medium, and the individual N2 hormone supplements on coupling. The utility of serum-free conditions is exploited in this systematic testing of individual hormones and nutrients at known concentrations.

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5.3 EFFECT OF EXTRACELLULAR MATRIX (ECM) ON DOPAMINE RELEASE FROM PHEOCHROMOCYTOMA (PC 12) CELLS. S.L. Kozak* and C.L. Bethea. Division of Reproductive Biology and Behavior, Oregon Regional Primate Center, Beaverton, OR 97006.

Substrates of various origins can affect morphology, growth and functional properties of many cell types. PC 12 cells (a clonal line of rat pheochromocytoma) synthesize, store and secrete dopamine as well as other transmitters. These cells are rounded and loosely attached when cultured on plastic, but become flattened and exhibit significant neurite extension when cultured on ECM secreted by bovine corneal endothelial cells (Fugi et al., J. Neurosci. 2:1157, 1982). To determine if functional properties could be altered along with the morphological changes induced by this substrate, we examined dopamine release from PC 12 cells cultured on ECM vs. plastic. Cells were seeded on ECM and plastic (25 and 50 x 10^4 cells/17mm well). After overnight attachment, the medium was replaced and aliquots were harvested and acidified at 1,2,3,6 and 18 h. Medium dopamine was (1) detectable by radioenzymatic assay after 3 h; (2) reflected cell number; (3) increased with time; and (4) was higher from cells on ECM than plastic at both cell concentrations.

Dopamine release from 25 and 50 x 10^4 cells was then examined at 12,24 and 72 h and was higher from cells on ECM than plastic. The cells were harvested into 0.1N PCA at 72 h for dopamine and DNA content determinations. There was no difference in the dopamine content or DNA content of cells on ECM or plastic therefore dopamine content/μg DNA was not different, whereas medium dopamine/μg DNA was 1.7 fold higher from cells on ECM than plastic (ECM = 98 ng/μg DNA vs. plastic = 55 ng/μg DNA). Dopamine content of cells on ECM and plastic averaged 5.6% of medium dopamine.

In several experiments dopamine release, dopamine content and DNA content were examined in cells on ECM and plastic at 3,6,12, 24,48 and 72 h. Medium dopamine levels from ECM cultures showed an increase over plastic cultures of 49% (3 h, n=5); 62% (6 h, n=4); 45% (12 h, n=4); 88% (24 h, n=4); 53% (48 h, n=3); and 52% (72 h, n=3) with a mean increase of 58% for all time points. Both medium and cellular dopamine and DNA content increased with time indicating growth contributed to the rising levels of medium dopamine. However no difference was observed in dopamine or DNA content between cells on ECM vs. plastic. Higher medium dopamine/μg DNA in ECM cultures confirmed the stimulatory effect of ECM on dopamine release. In summary, the maintenance of PC 12 cells on ECM promotes neurite extension and enhances the release of dopamine. Although the mechanism by which ECM enhances dopamine release is unresolved, these data provide further evidence of substrate influence on cellular function.

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5.4 SPONTANEOUSLY RELEASED PROTEINS FROM CULTURES OF SENSORY GANGLIA INCLUDE PLASMINOGEN ACTIVATOR AND A CALCIUM DEPENDENT PROTEASE. Randall N. Pittman, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Two dimensional gel electrophoresis and fluorography were performed on proteins spontaneously released into culture medium from dorsal root, nodose and trigeminal ganglion neurons. Each population of sensory neurons released a limited number of proteins in the pH (4.6-7.5) and MW ranges (10-280K) studied. DRG neurons released about 6-7 major proteins into the medium, two of which were not released by sympathetic neurons and one that did not appear to be released by neurons from nodose or trigeminal ganglia. Neurons from nodose ganglia released 6-8 proteins of which at least one was not released by sympathetic, DRG, or trigeminal ganglion neurons. Proteins released by sensory neurons were distinctly different from those released by nonneuronal cells.

A number of possible functions may be associated with proteins released by neurons including process outgrowth, recognition and synapse formation. It has been suggested that migrating granule cells release plasminogen activator, a highly specific serine proteinase (PNAS 78:7810; Nature 298:753), and it has been shown that growth cones of neuroblastoma cells release plasminogen activator (Sci. 213:1532). The proteins released by sensory neurons were analyzed for plasminogen activator activity as well as for other specific proteinases. Because serum contains protease inhibitors, techniques were developed for growing DRG and trigeminal neurons chronically in serum-free medium. Under these conditions cultures can be obtained which contain only neurons or neurons plus satellite cells. In cultures of DRG neurons grown for 4 weeks in serum free medium, a typical urokinase-like plasminogen-dependent protease activity was released into the culture medium which had a MW of 51K. A protein with similar properties was also found associated with membrane fractions from these cultures. Larger quantities of plasminogen activator were released from co-cultures of DRG or trigeminal neurons and satellite cells, suggesting that satellite cells either induce neurons to release more activator or are also releasing plasminogen activator. In addition to the plasminogen activator, a calcium-dependent protease activity was also released into the medium by cultures containing neurons and satellite cells but not from neuron-alone cultures. This neutral protease had a MW of 60K, and required mM concentrations of Ca^{2+} for activity. Assay conditions which should have revealed release of lysosomal proteases were negative; therefore, the proteases characterized here are probably not resulting from cell lysis but rather appear to be spontaneously released. Total cell homogenates, however, did contain a protease with characteristics similar to cathepsin D (the major lysosomal protease in brain).

5.5 MORPHOLOGICAL AND BIOCHEMICAL EFFECTS OF NGF OR APPLIED ELECTRIC FIELDS IN THE PRESENCE OR ABSENCE OF NON-NEURONAL CELLS OF CHICK DORSAL ROOT GANGLIA. B. F. Sisken, S. Estes*, E. Barr*, and R. Kryscio*. Wenner-Gren Res. Lab, Dept. of Anatomy, and Dept. of Statistics, Univ. of Kentucky, Lexington, KY 40506.

Eight day chick embryo dorsal root ganglia (DRG) were cultured in complete medium containing 10% dialyzed fetal bovine serum and 600 mg% glucose, or in complete medium with 8 ug/ml cytosine arabinoside (ara C). This drug specifically inhibits DNA synthesis and is commonly used to reduce the mitotically-active non-neuronal cells without affecting the neuronal cell population. It was used, therefore, to allow a reasonable assessment of neuronal regeneration at both the morphological and biochemical levels.

Neurite outgrowth, protein content and ^{3}H-proline incorporation into TCA-precipitable proteins were determined in four treatment groups: control, 2.5s NGF (10 nM), single pulse electromagnetic fields (PEMF) and 10nA direct current (DC). All parameters were assayed for each group at 3 and 6 days in vitro (DIV). Confirmatory evidence was obtained by grain localization on radioautograms after incorporation of the labeled amino acid precursor. In normal media, both electrical treatments stimulated neurite outgrowth at 3 DIV. The values were intermediate to that produced by NGF. All group values decreased by 6 DIV. Conversely, protein content increased with time due to the growth of the non-neuronal cells. In the ara C cultures at 3 DIV, neurite outgrowth increased in control cultures approximating the values of both PEMF and DC cultures; NGF cultues were unaffected at this time and remained significantly higher than any of the other groups.

In ara C cultures at 6 DIV, dramatic differences were noted. Neurite outgrowth in control, PEMF and DC groups increased while the NGF group decreased significantly. The protein content in the NGF cultures did not change with time.

Protein synthesis was comparable in all four groups at 3 DIV and 6 DIV, but decreased at 6 DIV in ara C medium. Radioautograms of such cultures revealed heavy label in neurons and neurite especially in the ara C series. At 6 DIV, these labeled neurites extend out radially in all groups but those treated with NGF; in these ganglia, the labeled neurites form dense circular rings around the centrally-placed neurons. Our conclusions are that ara C addition promotes neurite production by decreasing non-neuronal overgrowth demonstrating the independent ability of neurons to grow neurite processes.

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5.6 DIFFERENTIATED SCHWANN CELLS CULTURED FROM ADULT SCIATIC NERVES CONTAIN ASTROCYTE TYPE INTERMEDIATE FILAMENTS, K.L. Fields*. (SPON. R. Katzman). Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

In work done on tissue sections, we found that antisera raised to the 49,000 MW protein of CNS 10nm astrocyte filaments stain long,thin elements in rat sciatic nerve. This same pattern is seen with all anti-GFAP antisera, and the staining is absorbed by purified GFA protein. The pattern is different from that found with anti-vimentin or anti-neurofilament antisera (Yen and Fields, J. Cell Biol. 88:115, 1981).

In teased nerves, the staining is associated with non-myelinated nerve fibers, or the cells associated with them, not with myelinated nerve fibers or collagen. The staining network looks just like the classical osmium-fixed , teased nerve fibers described by Tuckett in ox splenic nerve (J. Physiol.19:267,1896) (Gould and Fields, Trans. Am.Soc.Neurochem. 13:59,1982).

In dissociated cell cultures from adult rat sciatic nerve, cells staining with anti-49K serum are present. They are round or elongated bipolar cells, and are stained by anti-vimentin or anti-49K sera only after alcohol fixation, demonstrating that the antigen is cytoplasmic, rather than extracellular. Flat fibroblastic cells do not stain with anti-49K sera, nor do Schwann cells from neonatal rats. All the 49K-antigen positive cells have the Schwann cell surface antigen Ran-1, whereas none of them have a fibroblast surface antigen, Thy-1.

Cytoskeletal preparations from adult nerve cultures, but not from neonatal nerve cell cultures, contain a protein band at 50,000 MW, which binds anti-49K antibody in immunoblotting experiments.

These results indicate that all Schwann cells in nerves do not have the same type intermediate filaments, but that some of them, apparently those associated with C-type fibers, have the same protein subunit as astrocytes.

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5.7 MYELIN-DEFICIENT MOUSE MUTATION: COMPARISON WITH ALLELIC SHIVERER MUTATION IN SITU AND IN VITRO. X.-Y. Shen*, A. L. Hall*, M. K. Wolf and S. Billings-Gagliardi. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

Myelin-deficient (shi^{mld}), a mouse mutation producing CNS hypomyelination, is allelic with shiverer (shi) and closely resembles shi except that the deficiency of myelin basic protein (MBP) is not as complete. We are outcrossing shi^{mld} to a B6C3H hybrid stock for accurate comparison with shi and all other hypomyelinated mutants. Affected shi^{mld}/shi^{mld} mice were examined at N4 and subsequent outcrosses (more than 90% comparability). Blocks of cerebellum and optic nerve were taken from P-18 to P-24 mice, and primary explants from P-0 affected cerebellum were maintained in vitro for 18-24 days, for examination by light and transmission electron microscopy. Both in situ and in vitro, shi^{mld} resembles shi in almost all ultrastructural features. Both mutants are deficient in CNS myelin; however, our B6C3H affected animals appear to make more myelin than others studying these mutations have reported. In both mutants, oligodendrocytes produce bundles of redundant microprocesses and myelinate inappropriate targets, many myelin sheaths are incorrectly wrapped, and most sheaths are incompletely compacted. The major feature distinguishing shi^{mld} from shi is the presence of a true major dense line (MDL) in some shi^{mld} myelin sheaths. The region of MDL usually forms one or two complete lamellae, or a patch containing small parts of several adjacent lamellae. Rarely, an entire myelin profile shows the MDL throughout all its lamellae (as many as 7). Injected normal optic nerve oligodendrocytes produce normal myelin around the shi^{mld} axons in the immediate vicinity of the optic nerve fragment, just as they do in jp, jp^{msd}, qk, and shi. We conclude: 1. Both shi and shi^{mld} appear to make more myelin on the B6C3H background than on other backgrounds. The remainder of the genome influences the quantity of myelin formed. 2. The amount of MDL in B6C3H mutants appears to be in keeping with the amount of MBP. Affected shi CNS has no MBP and no MDL, while affected shi^{mld} CNS has some MBP and some MDL. 3. Other cytological abnormalities seen in these two mutants do not seem to be influenced by the small amount of MBP in shi^{mld}. We speculate that the shi locus does not concern the message for MBP, but rather a regulatory factor. Abnormality of this factor causes both the deficiency of MBP and MDL and the other cytological abnormalities, but by different mechanisms.

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X-YS is a visiting scholar from the Shanghai First Medical College, People's Republic of China, supported in the U.S. by an award from the Norton Company of Worcester, MA.

5.8 SHIVERER OLIGODENDROCYTES FORM SHIVERER MYELIN AROUND NORMAL AXONS. M. K. Wolf, G. Schwing-Stanhope*, A. L. Hall*, and S. Billings-Gagliardi. Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605.

Normal oligodendrocytes in severed fragments of immature optic nerve, explanted onto cultures of cerebellum deficient in myelin, colonize the cerebellar tissue and myelinate its axons. They are equally successful whether the culture is from a mutant mouse with CNS hypomyelination or from a normal mouse stripped of its own mature oligodendrocytes and myelin by treatment with cytosine arabinoside (Ara-C). We now find that oligodendrocytes from B6C3H hybrid shiverer (shi) mutant optic nerve, colonizing normal cerebellum demyelinated by Ara-C, form myelin with defects identical to those of shi myelin in situ or in cultures of shi cerebellum. Four cultures were produced from each P-0 B6C3H hybrid normal cerebellum. Two were left untreated to test in vitro myelination of that cerebellum; two were grown in medium without embryo extract and with added Ara-C, 4.5 µg/ml, for 7 days in vitro, then washed free of Ara-C and fed medium with embryo extract. One such Ara-C treated culture tested the completeness of myelin suppression in that cerebellum; the other received an explant of optic nerve from a known homozygous P-8 to P-10 shi/shi mouse. All cultures were processed for transmission electron microscopy at 17 to 20 days in vitro. Ara-C treated cultures lacked mature oligodendrocytes and myelin, but contained abundant astrocytes. Ara-C treated cultures with shi optic nerve contained numerous myelinated axons near the optic nerve fragment. All this myelin had the ultrastructural features of shi myelin: sheaths often incorrectly wrapped and usually not compacted; absence of a true major dense line; bundles of redundant oligodendrocyte microprocesses; inappropriate myelination of these bundles and of oligodendrocyte cell bodies. Ultrastructurally normal myelin was not seen. We conclude: 1. The added optic nerve fragments are the source of myelinating glia in these cultures: if immature normal glia survive the Ara-C treatment, they do not produce myelin. Therefore, these cultures can be used to study the response of mutant oligodendrocytes to normal axons. 2. Oligodendrocytes of shi express every major feature of the shi disease in the presence of normal axons and astrocytes. This is further evidence that all these defects are intrinsic to oligodendrocytes. 3. Since the disease is expressed in the presence of genetically normal astrocytes, it seems unlikely that shi astrocytes have any direct role in the disease.

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5.9 IDENTIFICATION AND CHARACTERIZATION OF CELL TYPES IN CULTURES OF RAT RETINA USING MONOCLONAL ANTIBODIES. K. Akagawa* and C. J. Barnstable. (SPON: D. L. Edwards). Department of Neurobiology, Harvard Medical School, Boston MA and The Rockefeller University, 1230 York Avenue, New York.

To identify and characterise the cell types of rat retina in vitro, we have studied the expression of cell-type specific antigens using monoclonal antibodies both in monolayer and aggregation cultures.

Photoreceptors which are labelled by antibody RET-P1 are small and round cells which have no or one small process. Though they do not develop outer segments in monolayer cultures, they form characteristic rosette structures in aggregation cultures, in which outer segment markers appear to develop.

Amacrine cells can be labelled by HPC-1, raised against rat hippocampus, using either immunofluorescence or PAP staining. Most of the labelled cells are bipolar and have long processes.

Bipolar cells and photoreceptors are labelled by an antibody, RET-B2. In monolayer cultures of 2 day old retina the antigen only appears after 7 days in vitro. The bipolar cells extend long processes and have many different morphologies, some of which are very similar to those of the amacrine cells. Thus without antibody markers it would have been impossible to distinguish amacrine and bipolar cells in these cultures.

Ganglion cells are the only cells that are labelled by anti Thy-1 antibody in vivo. In monolayer cultures from 2 day old rats Thy-1 +ve cells were found in cell clumps but not in the dispersed cell monolayer. This suggests that the triggering of Thy-1 expression is dependent upon some cell interactions. Such interactions are not necessary for the maintainence of expression of the antigen since in cultures of retinas from older animals that are already expressing Thy-1, solitary ganglion cells can be found that are Thy-1 +ve.

We have also studied the uptake of various transmitter candidate amino acids using autoradiography. Cells which take up GABA in monolayer culture have long processes whereas those taking up glycine have small short processes. Many of the GABA cells appear to be amacrine cells. Aspartate is taken up into many types of cells including small round cells that have the morphology of the RET-P1 + ve photoreceptor precursors. In aggregation cultures, cells labelled by GABA and glycine uptake were located on the surface of the aggregates.

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5.10 A MONOCLONAL ANTIBODY TO THY-1 ENHANCES PROCESS REGENERATION BY DIFFERENTIATED RAT RETINAL GANGLION CELLS IN CULTURE. Dana Leifer*(1), Stuart A. Lipton*(1,2) and Colin J. Barnstable(1,3) (SPON: T.N. Wiesel). Depts. of Neurobiology(1) and Neurology(2), Harvard Medical School, Boston, MA 02115; Dept. of Neurobiology(3), The Rockefeller University, New York, NY 10021.

Solitary retinal ganglion cells can be identified in culture with fluorescent markers. Ganglion cells are labelled by the retrograde transport of fluorescent dyes injected into the projection sites of the optic nerve two days prior to retinal dissociation. In culture, the identity of ganglion cells is verified by a second label, 2G12, a monoclonal antibody against Thy-1, that in retina is specific for ganglion cells.

As previously reported (Suppl Invest Ophthal 24, 138, 1983), a significantly greater proportion of ganglion cells regenerate processes when plated on anti-Thy-1-coated glass coverslips than on plain glass, or on glass coated with collagen.

In this study we tested the specificity of this effect and its relationship to adhesion. The action of anti-Thy-1 was not a non-specific property of immunoglobulin bound to the glass coverslip since: (i) IV2G10, an antibody against a cytoplasmic antigen, did not enhance process outgrowth of ganglion cells, and (ii) anti-Thy-1 had no effect on process outgrowth of amacrine cells, identified autoradiographically by uptake of tritiated glycine and GABA.

There appeared to be a requirement for the Thy-1 antibody to be bound to the coverslip since adding antibody at varying concentrations 3-4 hours after plating on plain glass caused no enhancement of process growth.

We tested whether the effect of anti-Thy-1 was related to adhesion of cells to the substrate. Antibody RET-N2 reacts with an antigen on the surface of all retinal cells, including ganglion cells. Although approximately the same number of ganglion cells adhered to both substrates, RET-N2 did not enhance process regeneration like anti-Thy-1. Fibronectin was also ineffective in promoting regeneration. On the other hand, poly-L-lysine coated coverslips stimulated ganglion cell process outgrowth. From these results, it is not yet clear whether the effect of anti-Thy-1 is a consequence of the strength of adhesion or of a more specific surface modulation involving Thy-1, or both.

The ability to identify and grow retinal ganglion cells in culture has also enabled us to study their membrane properties using intracellular and patch-clamp pipettes.

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5.11 ANALYSIS AND PURIFICATION OF NEURONS BY IMMUNOFLUORESCENCE AND FLUORESCENCE-ACTIVATED CELL SORTING. P.A. St.John and J.L. Barker, Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

Experiments with cell cultures of neurons and myotubes have provided important insights into mechanisms of synaptogenesis at this peripheral synapse. Cell cultures may provide similar insights into synaptogenesis in the central nervous system. The spinal cord, where the cellular neurobiology in vivo has been particularly well studied, is a good subject for studies in vitro. However, previous work on spinal cord cultures has shown that they contain a variable mixture of cell types. In an effort to gain control over the types of neurons present in cultures, we have begun to use a fluorescence-activated cell sorter to separate different subpopulations of cells from the embryonic mouse spinal cord and dorsal root ganglia.

All experiments were performed on dissociated spinal cord and dorsal root ganglion cells from 13-day embryonic mice, cells of the age used for cell cultures. In the first series of experiments, individual cells, not reacted with fluorescent probes, were examined in the cell sorter for amount of forward-angle light scatter, a measurement influenced by cell size and makeup. Forward-angle light scatter was sufficient to distinguish live cells from dead cells and debris in the absence of staining, and sorting based on this criterion produced a population of live cells with over 95% purity (based on trypan blue exclusion). The purified, sorted cells could be grown in culture, and neurons survived in these cultures for at least 3 weeks.

In initial studies to determine the feasibility of sorting subpopulations of cells using fluorescent probes, we have labeled fixed, permeable cells with antibodies to intracellular antigens. Cells in suspension were fixed and reacted with antibodies against several neuropeptides and transmitter-related enzymes. Although immunoreactivity for the antibodies tested could not be detected unambiguously in a fluorescence light microscope, the greater sensitivity of the cell sorter showed that the cells did contain specific immunoreactivity for antibodies to the following: glutamic acid decarboxylase (up to 50% of cells), glutaminase (30%), vasoactive intestinal peptide (10-20%), substance P (up to 10%), somatostatin (up to 30%), and neuron-specific enolase (15-25%). In future experiments, fixed and labeled cells will be purified with the cell sorter for use in producing cell type-specific, surface-directed antibodies.

In other experiments, live, unfixed cells were analyzed or sorted following labeling of motor neurons by retrograde transport from muscle injections (Schaffner, St. John, and Barker, this volume), or labeling by a neuronal surface-directed monoclonal antibody (the A2B5 clone) or lectins. Experiments are in progress to grow these specifically labeled and sorted cells in culture.

5.12 PURIFICATION OF EMBRYONIC MOUSE MOTONEURONS BY FLOW CYTOMETRY. A.E. Schaffner, P.A. St.John and J.L. Barker, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

One approach to a better understanding of how the nervous system works might involve the purification of homogeneous populations of nerve cells. This would simplify the morphological and electropharmacological characterization of the cells as well as their interactions with presynaptic and postsynaptic neurons and targets. Two laboratories have reported successful purification of embryonic chick motoneurons by employing the techniques of retrograde transport of a fluorescently labeled lectin with subsequent sorting of specifically labeled cells in a flurorescence-activated cell sorter (FACS) (Okun and McPheeters, Soc. Neurosci. 6: 733; O'Brien et al., Soc. Neurosci. 8: 129). We have isolated motoneurons from mammalian embryos using similar techniques. Limbs of 13 day mouse embryos were injected with 10mg/ml of fluoresceinated horseradish peroxidase or wheat germ agglutinin. After 4-8 hours of retrograde transport at 37°C, spinal cords and dorsal root ganglia were mechanically dissociated and the cells analyzed and/or sorted on a FACS. An initial analysis indicated that 22% of the cells were specifically labeled. When cells were sorted into specifically labeled and unlabeled populations and assayed for choline acetyltransferase (ChAT) activity there was a 3.8-fold enrichment in ChAT activity (cpm/cell) in the specifically labeled population compared with unlabeled cells. To test whether labeled dorsal root ganglion (DRG) neurons might be contributing to the high yield of labeled cells, DRGs were removed from the cords and DRGs and cords analyzed separately. In this experiment, approximately 9% of spinal cord cells were specifically labeled while 36% of cells from the DRG were specifically labeled (presumably from sensory connections). Thereafter all DRGs and meninges were routinely stripped from the cords. We have used two approaches to determine the location of labeled cells in situ. Several embryos were injected and processed for cryostat sectioning. Fluorescent cells in the spinal cord were confined to the anterior horn. In one embryo fluorescence was also seen in a DRG. In another experiment, cords were dissected into "dorsal" and "ventral" halves and analyzed separately using flow cytometry. There was no specific fluorescence ($< 0.1\%$) in cells from the dorsal cord. However, $> 5\%$ of cells from the ventral cord were specifically labeled. We are presently working on conditions that will allow the survival of sorted motoneurons in culture. In other experiments, purified motoneurons will be used as antigens to generate antibodies against surface determinants on motoneurons or cholinergic neurons in general. Such antibodies should provide a powerful tool for identifying these cell types both in vivo and in vitro and elucidating their involvement in neuropathologies.

5.13 THE DEVELOPMENT OF GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVITY IN MOUSE SPINAL CORD CULTURES. M.T. Caserta and J.L. Barker, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

Spinal cord (SC) neurons grown in tissue culture have been shown to be an excellent system for studying the electrophysiological responses of neurons to gamma aminobutyric acid (GABA), a major inhibitory transmitter in the vertebrate central nervous system. It has been shown that most mature cultured SC neurons respond to GABA and that these neurons are invested with terminals that react with antibodies to glutamic acid decarboxylase (GAD), the biosynthetic enzyme for GABA. We have examined developing SC neurons in culture by immunohistochemical localizaton of GAD at the light and electron microscopic (EM) levels with a well-characterized sheep antibody to rat brain GAD (Oertel, et al. Neuroscience 6: 2689).

SC cultures were prepared from dissociated SC and dorsal root ganglion (DRG) cells of 12-13 day mouse embryos and were grown for up to 4 weeks. In mature cultures (2 weeks or older), GAD immunoreactivity was confined to terminals which could be seen enveloping neurons of varying size and morphology. However when cultures were pretreated with colchicine, GAD containing cell bodies were visualized. These GAD-positive neurons were small to intermediate in size ($< 20\mu$ diameter) and were usually found within clusters. Large neurons resembling motoneurons or DRG cells were not labelled. EM immunocytochemistry demonstrated numerous GAD-positive terminals synapsing on dendritic and axonal processes as well as cell bodies. The vesicles in these terminals were either round or pleomorphic. No terminals with solely flattened vesicles were ever observed. GAD-positive cell bodies could be demonstrated as early as 4 days in culture without colchicine pretreatment. Most of the immunoreactivity was localized to the Golgi region of the soma with a few labelled terminals visible. EM analysis showed that many synapses had already been formed in these young cultures and that a few axodendritic profiles were immunoreactive at this stage.

The development of another neuronal enzyme, neuron specific enolase (NSE), was compared to GAD and found to develop much later in cultured SC neurons (2 to 3 weeks). This late development of NSE is consistent with previous in vivo developmental studies. These results suggest that the early maturation of GAD-containing neurons is not a generalized phenomenon of cells in tissue culture but specific to this subpopulation. Work is now in progress to compare the development of SC GABAgeric neurons in vivo to their development in vitro. What role this inhibitory transmitter, GABA is playing in the developing SC is an important question that can best be addressed in tissue culture which allows ready access for biochemical, morphological and electrophysiological analysis.

6.1 EFFECTS OF ACTIVITY DEPENDENT NEURAL FACTORS ON MUSCLE GROWTH AND MAINTENANCE IN THE YOUNG CHICK EMBRYO. J.W.Bloom*, E. Cosmos and J. Butler*. (Spon: R. Butler). Neurosciences Dept. McMaster University Health Sciences Centre, Hamilton, Ontario L8S 3Z5.

Although brachial muscles of the chick embryo do form in the complete absence of innervation, both their growth rate and, eventually, their survival are dramatically reduced (Butler, J. et al., J.Exp.Zool.224:65,1982). These observations stress an important role for activity-dependent and/or neurotrophic factors on early muscle growth and maintenance. To differentiate between these possibilities, activity-dependent neural influences were manipulated independent of neurotrophic influences by pharmacological neuromuscular blockade(reduced activity)and by electrical stimulation(increased activity)of intact chick embryos. Electrical stimulation was repeated in embryos in which the brachial musculature had been rendered aneurogenic by neural tube removal. Anterior(ALD)and posterior(PLD)latissimus dorsi muscles were analyzed histochemically and quantitatively.

In intact embryos treated with d-tubocurarine, chronic reduction of activity by 40-60% was noted from 4-11 days in ovo; however, only a modest decrease in the growth of the ALD and PLD muscles was observed. Further, chronic augmentation of activity from day 6-10 via spinal indirect stimulation did not increase, and in some cases reduced, the growth of normally innervated ALD and PLD muscles. These experiments indicate that in the intact embryo rather large perturbations, both upward and downward, of the amount of activity reaching the musculature have little effect on muscle growth or maintenance during the first half of embryonic development. We are attempting to achieve 100% activity reduction by α-bungarotoxin treatment since very great reductions in activity markedly reduced growth of leg muscles in intact embryos (Laing, N., J.E.E.M. 72:269, 1982).

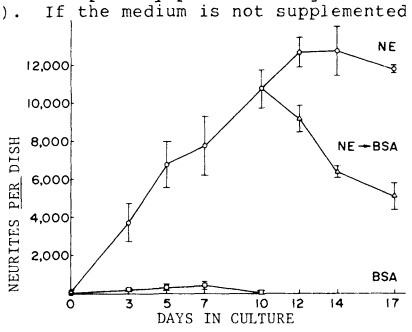
To test the role of activity in the absence of trophic factors, aneurogenic muscles were chronically electrically stimulated for 2-4 days beginning on day 6 or 7. When activity alone was added back to the aneurogenic ALD muscle, its growth was increased by 100% compared to unstimulated aneurogenic controls. In addition, the aneurogenic PLD muscle, which usually degenerates by day 7.5, was rescued by direct stimulation and continued to grow throughout the experimental period.

Thus, activity plays an important role in early muscle development as demonstrated by the marked enhancement of the growth (ALD,PLD) and survival(PLD)of aneurogenic muscles when activity alone is experimentally imposed upon them.

(Supported by grants from MDAC and MDA).

6.2 NEUROTROPHIC SUPPORT OF SPINAL CORD NEURONS IN CULTURE. H. Popiela, R. Beach, and B. Festoff. VA Medical Center, Kansas City, MO 64128.

Guided by the hypothesis that peripherally-produced neurotrophic factor (NTF), retrogradely transported to central neurons, is extractable from nerve fibers while in transit, we extracted adult chicken peripheral nerves (NE) using a similar procedure as for extraction of muscle-directed NE (Popiela, 1978, Exp. Neurol. 62: 405-416; Popiela et al.,1982, J. Neurosci. Res. 8:547-567). NE was added to serum-, hormone-, and factor-supplemented medium and assayed on dissociated spinal cord neurons from 8 day old chick embryos. In the presence of NE, neurons are well maintained and respond by prolific outgrowth of neurites (fig.,NE). If the medium is not supplemented with extract but bovine serum albumin is substituted instead, neurons show poor neuritic outgowth and they die within 10 days (fig.,BSA). If NE is withdrawn at 10 days from NE-treated cultures neurites diminish, concomitant with death of somata. Control tissues, such as skeletal muscle or brain, contains a fraction of the neurotrophic activity found in nerves when added at similar protein concentrations. NE is not replaceable with a muscle-directed factor previously purified from NE (NTF) nor with transferrin including several chicken transferrins. These studies show that adult chicken peripheral nerves contain a factor supporting spinal cord neuron maintenance and neurite outgrowth that is not replaceable by transferrins.



Supported by NIH (RO1NS17197), ALSSOA, Medical Research Service of the VA, and the University of Kansas Regional ALS Research Center.

6.3 TROPHIC INTERACTION IN HIPPOCAMPAL CELL CULTURE MEDIATED BY NTF.
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A dissociated cell culture system of the developing hippocampus in serum-free medium has been established in our laboratory in recent years. By using a coverslip technique we demonstrated that primary glialcells produce a soluble trophic factor which promotes survival and outgrowth of hippocampal neurons (W.Seifert et al. 1981, 1983).

This neurotrophic factor has been further characterized by a rapid and quantitative bioassay based on this coverslip technique (H.W.Müller and W.Seifert, 1982, W.Seifert and H.W.Müller, 1983).

Studies in our laboratory have demonstrated that NTF is a relatively heat-stable and protease-resistant molecule of low molecular weight. It seems to be specific for central neurons and did not exhibit trophic activity for peripheral neurons in cell culture (H.W. Müller, S.Beckh and W.Seifert, 1983).

NTF is produced by primary cultures of astrocytes and secreted into serum-free medium in a time-dependent manner. Experiments will be discussed concerning
(i) the survival activity and the neurite-promoting activity of NTF, and
(ii) the problem of short-term versus long-term survival of neurons in this serum-free culture.

Screening of tissue extracts with our bioassay has revealed rather high NTF-activity in adult rat brain and even higher activity in embryonic brain. This fact might suggest a biological significance for NTF in development and maintenance of CNS neurons. Experiments on the developmental appearance of NTF will be discussed.

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6.4 Substance P and Somatostatin Regulate Sympathetic Noradrenergic Function. J.A. Kessler, J.E. Adler, I.B. Black. Division of Developmental Neurology, Cornell University Medical College, New York, New York 10021

Peptidergic-noradrenergic interactions were examined in the rat sympathetic superior cervical ganglion (SCG) in explant and dissociated cell culture. The putative peptide transmitters, substance P and somatostatin each increased activity of the catecholamine-synthesizing enzyme, tyrosine hydroxylase (TOH), after one week exposure in culture. Maximal increases occurred at 10^{-7}M for each peptide, and either increasing or decreasing the concentration reduced effects. By contrast, the peptide had no effect on TOH activity in ganglion homogenates, excluding a direct effect on the TOH enzyme or the radiochemical catalytic assay. Consequently, substance P and somatostatin increased TOH activity through processes that transpired in culture and not through direct interaction with the apo- or holo-enzyme. Similar increases in TOH were produced by the metabolically stable substance P agonist, [pGlu^5, MePhe^8, Sar^9]-substance P(5-11). Moreover, the substance P antagonist, D-pro^2, D-phe^7, D-trp^9-substance P, prevented the effects of the agonist, suggesting that the elevation of TOH activity was mediated by peptide interaction with specific substance P receptors. Our observations suggest that peptides may modulate sympathetic catecholaminergic function.

6.5 EFFECTS OF CONDITIONED MEDIA ON NEURITE OUTGROWTH AND SURVIVAL OF PURIFIED MOTONEURONS. A.L. Calof and L.F. Reichardt. Div. Neurosci., Dept. Physio., Univ. Calif., San Francisco, CA 94143

We are interested in the role of target tissue in guiding the growth and synaptic differentiation of spinal motoneurons, and have used a modification of the retrograde transport/fluorescence-activated cell sorting technique of McPheeters and Okun (Soc. Neurosci. Abstr. 6: 733) to purify motoneurons. Lucifer Yellow VS is coupled to wheat germ agglutinin, the conjugate is purified by gel filtration, and it is injected into all limbs of stage 28-29 chick embryos. After 24 hrs. in organ culture, examination of fixed sections of these embryos reveals label in the spinal cord confined to the ventral horn. Single-cell suspensions show 5-7% of cells labeled with small, brightly-fluorescent cytoplasmic granules.

For sorting, spinal cords are dissociated to single cells and then sedimented through BSA to remove debris. Cells are sorted using a Becton-Dickenson FACS IV equipped with logarithmic amplification (LA). Emitted fluorescence passes through a 475nm long pass filter prior to analysis and sorting. LA reveals a peak of fluorescent events with intensities well above background (autofluorescence), and comprising 4-7% of scatter events. This peak is sorted, yielding purities of about 80%.

Purified motoneurons display rapid neurite outgrowth when plated onto polylysine (PLYS)-coated dishes preincubated with serum-free medium conditioned by cultures of chick myotubes (MCM_{SF}). They respond similarly to substrata treated with bovine corneal endothelial cell conditoned medium (BCE-CM_{SF}). We have sought to establish the identity of the MCM_{SF} factor with the BCE-CM_{SF} factor, which has been partially purified in our laboratory (Lander et al., J. Cell Biol. 94: 574-585). Rat sympathetic neurons respond to MCM_{SF} as well as BCE-CM_{SF}. Thus, media conditioned from both cell types can promote neurite outgrowth from cells with axons in the periphery. The MCM_{SF} factor is sensitive to trypsin, but not to collagenase, neuraminidase, or chondroitinase ABC; and it has a buoyant density in CsCl similar to that of the BCE-CM_{SF} factor. Like BCE-CM_{SF}, the neurite-outgrowth promoting effects of MCM_{SF} can be mimicked by conditioned media from other cell types (chick fibroblasts, chick embryonic spinal cord).

When plated at adequate density, motoneurons extend neurites and survive on MCM_{SF}-treated substrata for ~1d in serum-free medium. This response is not seen on PLYS alone, or on laminin-treated PLYS. MCM_{SF} added to the culture medium enables motoneurons to survive for >7d in culture, but this survival effect so far does not appear to be specific to medium conditioned by myotubes. Supported by grants from NSF and MDA to LFR.

6.6 SPECIFICITY OF NEURONOTROPHIC INFLUENCE ON TARGET-DEPRIVED NEURONS IN THE DEVELOPING RAT VISUAL SYSTEM. F. Haun and T. J. Cunningham. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Neurons in the developing CNS are particularly vulnerable to separation from their target tissue. This sensitivity may be due to removal of target-derived neuronotrophic influences operative during normal development. We have been investigating this possibility in the developing visual system of the rat. Large unilateral lesions of the visual cortex are made in newborn Long-Evans rats; these lesions destroy all cortical targets of the dorsal lateral geniculate nucleus (dLGN). Immediately following the lesion, we transplant cell suspensions of E14-15 fetal posterior cortical neurons into the lesion cavity to determine if these transplanted cells affect the otherwise rapid degeneration of the ipsilateral dLGN. In a previous report (Anat. Rec. 205:77A) we showed a temporary saving of at least twice the volume of the affected dLGN, compared to control animals with lesions but without transplants. In the present study we compared the effectiveness of transplants of E14-15 posterior cortex with transplants of E14-15 cerebellar plate (plus subadjacent brainstem) to determine whether this temporary delay of dLGN degeneration is specific to the type of CNS tissue transplanted. We also compared the numbers of ^{3}H-thymidine labeled neurons in the dLGN of E14 versus E16 labeled host rats to determine whether the effect is specific to particular neuronal populations of the host nucleus. Five days after the lesion, the dLGN of animals without transplants shows a severe depletion of both E14 and E16 labeled neurons (with 4% and 20% respectively of those on the unoperated side remaining). Rats with cerebellar transplants gave similar results. In animals with posterior cortical transplants we find not only a significant saving of dLGN volume but specificity of the neuronal populations which contribute to this volume. The percentage of remaining E14 labeled neurons is comparable to animals without transplants. The survival of later-generated E16 neurons however is increased by more than two-fold. We conclude that: a) fetal cortex transplants exert at least a temporary survival-promoting influence on target-deprived or axotomized neurons of the developing dLGN, an effect that is relatively specific to the appropriate target; and b) the particular dLGN neurons protected by the transplant are less mature. Notably, these two kinds of specificity have been shown using an in vivo assay, which suggests the possibility of studying trophic relationships among visual system neurons developing in situ.

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6.7 RETINOIC ACID EFFECTS ON THE NERVE GROWTH FACTOR RECEPTOR. B. E. Haskell*, R. W. Stach, K. Werrbach-Perez* and J. R. Perez-Polo. Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch, Galveston, Texas 77550.

Retinoic acid (RA) mimics some of the effects of nerve growth factor (NGF) on explanted chick embryo dorsal root ganglia (DRG) and on human neuroblastoma cell lines. Specifically, NGF stimulates profuse neurite outgrowth from these targets, an event often used to assay for NGF activity. DRG exhibit neurite development in vitro in response to retinoic acid both in serum-supplemented medium and in the chemically-defined, hormone supplemented N-2 medium of Bottenstein and Sato. Maximal response to RA occurs in N-2 medium after 3 days in the presence of 5×10^{-7} M retinoic acid. In LAN-1 neuroblastoma cells, increased neurite outgrowth is associated with a dramatic increase in NGF binding to neurites and cell bodies as determined by immunofluorescence using affinity purified rabbit antibodies directed to mouse β-NGF. At concentrations of RA and treatment times deemed optimal for these responses there is an inhibition in cellular proliferation of LAN-1 cells. Also, RA has been reported to increase the apparent number of receptors for insulin and epidermal growth factor and to modify cell surface glycoproteins in vitro. Since the nerve growth factor protein (NGF) is a well characterized neuronotrophic factor acting on vertebrate embryonic sensory and sympathetic neurons and human neuroblastoma lines, it was of interest to determine if receptors to NGF were affected by RA and to correlate these changes with morphological aspects of differentiation directed by NGF in vitro. NGF acts on responsive cells by binding to two different cell surface membrane receptors. These two receptors have equilibrium dissociation constants of approximately 10^{-11} M for the high affinity receptor (type I) and approximately 10^{-9} M for the low affinity receptor (type II). Kinetic analysis of NGF receptors can be carried out on whole cell preparations or solubilized receptors with no detectable change in ligand binding kinetic properties or structural properties of the receptor molecules. Also, quantitative determination of the presence of solubilized NGF receptor by Rosenthal and kinetic analysis demonstrated a five-fold increase in the number of receptors present after 3 days in RA but no detectable change in receptor number after one day. These results are in agreement with the hypothesis that retinoic acid may increase the number of demonstrable NGF surface receptors on LAN-1 cells.

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6.8 CELLULAR INTERACTIONS IN DEVELOPMENT OF THE PRIMARY OLFACTORY PATHWAY. A. I. Farbman and M. I. Chuah*, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60201

It has been shown in an earlier study from this laboratory that the olfactory bulb, the target organ of olfactory receptors, exerts an effect on development of the receptors. The effect is expressed as an approximate doubling of the amount of a specific olfactory marker protein (OMP) in organ cultures, i.e., when olfactory mucosa taken from fetal rats (day E15) is explanted in combination with presumptive olfactory bulb (POB) and grown for 7 days, about twice as much OMP is found than in cultures of olfactory mucosa alone. OMP was estimated in cultures by two methods: 1) a solid phase radioimmunoassay using ^{3}H-labeled OMP, 2) a quantitative immunohistochemical procedure in which positively stained receptor cells were counted and compared in cultures of mucosa with and without POB. The cell counts indicated that the increased amount of OMP in explants with the POB was due to an approximate doubling of OMP-positive receptor neurons. In order to determine whether the increased OMP in cultures including POB came about as a specific influence of the bulb, mucosa was cultured in combination with other tissues including cerebrum, cerebellum, cervical spinal cord and heart, all taken from E15 embryos. Radioimmunoassays of these cultures showed they contained the same amounts of OMP as in explants of mucosa alone, i.e., no enhancement was produced by these tissues. Therefore, the facilitating or enhancing effect of the bulb seems to be specific. It was of interest to determine whether a diffusible factor from the bulb could be identified. We made "sandwich" cultures in which olfactory mucosa and POB were co-cultured on opposite sides of a thin (25 μm) Millipore filter, 0.45 μm pore size; in addition thin (12.5 μm) Nucleopore filters, pore sizes, 3 and 8 μm, were used in these sandwich cultures. Diffusible factors, if they existed could pass through these pores. The radioimmunoassay results indicated there was no enhancement of OMP in these cultures even when the amount of POB tissue was increased 3X. Light and electron microscopic examination of these sandwich cultures showed that the growing olfactory axons did not pass through the filter pores to contact the bulb. The results indicate that the presumptive olfactory bulb specifically increases OMP level by a mechanism that requires direct contact between receptors and bulb.

We are grateful to Dr. Frank Margolis, Roche Institute of Molecular Biology, Nutley, N.J. for his generous gift of OMP, ^{3}H-OMP and antibody to OMP. This research was supported by USPHS grants #NS-06181 and NS-18490.

6.9 ENHANCEMENT OF DOPAMINE NEURON SURVIVAL AND AXONAL PROLIFERATION BY MEMBRANES FROM STRIATAL TARGET CELLS. A. Heller, P.C. Hoffmann and B. Wainer. Depts. of Pharmacological and Physiological Sciences and Pediatrics, Univ. of Chicago, Chicago, Il. 60637

Embryonic mouse dopamine (DA) neurons of the rostral mesencephalic tegmentum (RMT) when dissociated into single cells can be cultured either alone or in combination with dissociated cells from target areas such as the corpus striatum (CS). In rotatory cultures the cells reassociate to form aggregates. Only in the presence of target cells do the neurons develop and maintain axonal patterns similar to those seen in vivo (Hemmendinger et al., PNAS 78:1264,1981). In RMT-CS coaggregates there is a 4-fold enhancement of DA neuron survival compared to cocultures with cells from non-target areas (Hoffmann et al., Brain Res. In press). Since the aggregates are formed from single cells, the information necessary for these interactions appears to be intrinsic to the neurons, themselves. In order to identify factors responsible for these phenomena, membrane preparations from areas containing target and non-target cells were added to single cell suspensions of the RMT along with single cells from a non-target area, the tectum (T), the latter being added to maintain equal numbers of dissociated cells in the various combinations. Membranes were obtained by high speed centrifugation (106,000 x g) following tissue homogenization in distilled water. The following combinations were examined: 1) RMT-CS, 2) RMT-T-CS, 3) RMT-T with T-membranes and 4) RMT-T with CS-membranes. The resulting aggregates were cultured for 8 days, exposed to 10^{-6}M DA for 10 min and processed for histofluorescence. The aggregates were sectioned at 10 um and examined under the fluorescence microscope. For each combination sections were randomly selected and the number of DA neurons counted, yielding the following values: RMT-CS, 94; RMT-T-CS, 93; RMT-T with T-membranes, 24; RMT-T with CS-membranes, 65. To assess the presence of axons 3 observers independently judged whether or not each of the sections contained DA axons by observing randomized color photomicrographs. The results expressed as numbers positive/total number of sections observed were: RMT-CS, 21/26; RMT-T-CS, 23/25; RMT-T with T-membranes, 2/27; RMT-T with CS-membranes, 18/28. The supernatant fractions from the membrane preparations did not affect either cell survival or axonal development. These results suggest that macromolecules associated with the membranes of embryonic CS cells are responsible for the ability of such target cells to enhance the survival of central DA neurons and the proliferation and maintenance of their axons in the 3 dimensional coaggregate system in culture. (Grant No. MH 28942).

6.10 AGE-DEPENDENT REQUIREMENTS FOR HETEROLOGOUS NEURITE EXTENSION FACTOR AND NERVE GROWTH FACTOR IN CULTURED SYMPATHETIC NEURONS. M. D. Coughlin. Dept. of Neurosciences, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

The superior cervical ganglion (SCG) of the 14 gestational day (E14) mouse embryo extends neurites and differentiates biochemically when cultured in the absence of added nerve growth factor (NGF). In contrast, ganglia from newborn (NB) mice require added NGF for survival in culture. Moreover, neurite outgrowth in culture requires a neurite extension factor derived from conditioned medium (CMF) or an antigenically similar factor produced by neurons themselves (Coughlin and Kessler, J. Neurosc. Res. 8: 289, 1982).

Whereas maximum survival and neurite extension of dissociated sympathetic neurons in culture is obtained in the presence of both added NGF and added CMF, the relative requirements for these factors change during development. Maximum survival of E14 neurons requires added CMF and is not significantly altered by the presence of antiserum to NGF (anti-NGF). Although NGF promotes survival of E14 neurons over control levels, survival is approximately half of that obtained with CMF alone. In contrast, survival and neurite outgrowth of NB SCG neurons absolutely requires NGF. Although CMF alone enhances survival of NB neurons, this effect is reversed by addition of anti-NGF, suggesting that such survival depends on endogenous NGF carried into culture. In the presence of antiserum to CMF (anti-CMF), neurons survive in the presence of NGF but fail to extend neurites.

These results suggest that at early embryonic stages, sympathetic neurons do not require added NGF for survival but do require exogenous sources of a neurite extension or axonal guidance factor. At later stages, SCG neurons develop both a requirement for NGF and the capacity to respond to NGF with production of an autologous neurite extension factor antigenically similar to CMF.

6.11 PRIMARY CULTURE OF DISSOCIATED FETAL MESENCEPHALIC RAPHE: DIFFERENTIAL STIMULATION OF SEROTONERGIC GROWTH BY TARGET TISSUE. Efrain Azmitia, Patricia M. Whitaker, Jean Lauder, and Alain Privat. Dept. of Anatomy, Mount Sinai Sch. Med., N.Y.,N.Y.

Fetal mesencephalic dopaminergic neurons show enhanced maturation when co-cultured with striatal target cells (Prochiantz et al, 1979). Mesencephalic 5-HT neurons have multiple forebrain but not hindbrain targets (Azmitia and Segal, 1978). A tissue culture system was developed using micro-titration plates to study the effects of fetal cells from hippocampus (HIP), spinal cord (SC), olfactory bulb (OB), striatum (ST), and cortex (CTX). Tissue from rat fetuses (E 14-16) were dissected and dissociated in Versene 1:5000 (GIBCO). The cells were centrifuged (500 X g, 10 min), resuspended in complete medium (Eagle's minimum essential medium, Hank's buffer, non-essential aminoacids, 1% glucose, and 10% fetal calf serum-GIBCO), and 200 ul final volume plated in Linbro tissue culture 96 multi-well plate (area=0.28 cm^2) on collagen. The cultures (3-6 wells per condition) were incubated at 37°C in a water-saturated 95% O_2/5% CO_2 atmosphere. Initial plating density (IPD) was determined with a Levy Hemacytometer Chamber. Serotonergic cells were immunocytochemically stained in the wells using an antibody raised against 5-HT conjugated to hemocyanin. ^{3}H-5-HT uptake was measured in each well (NEN, 24 Ci/mmole, 5 X 10^{-8} M, 20 min incubation at 37°C in Hanks buffer with 1% glucose, non-specific uptake determined in presence of 10^{-5} M fluoxetine). Protein determination were made in each well using a modified Lowry. Uptake of ^{3}H-5-HT was linear within an IPD of 0.5-2 10^6 cells/cm^2. The number of 5-HT immunoreactive cells was also linear within this range (0.5% of total cells). Each 5-HT cell concentrated approximately 1 femtomole of serotonin within 20 min. ^{3}H-5-HT uptake (IPD=10^6 raphe cells/cm^2) was stimulated by IPD of 10^6 cells/cm^2 from HIP (11 fold), SC (9.7 fold), OB (10.7 fold), CTX (6.4 fold), and ST (5.3 fold) after 5 days in co-culture. The stimulation appears less if the uptake is expressed per ugm of final protein in each well rather than as a function of IPD: HIP (4.6 fold), SC (4.3 fold), OB (3.2 fold), CTX (3.4 fold), and ST (2.8 fold). The target stimulation on an IPD of 0.5 X 10^6 raphe cells was 36,2.8,1.6,5.9, and 0.7 fold without protein and 5.7,5.3,3.4,1.8, and 5.3 fold with protein correction (respectively). The results suggest that a wide variety of target tissues can stimulate mesencephalic serotonergic neurons. The effects of pharmacological manipulations of serotonergic function on ex vivo maturation is currently under investigation. Research supported by NSF grant 79-06474.

6.12 STIMULATION OF NEURITE OUTGROWTH BY ADULT AMPHIBIAN HEART-CONDITIONED MEDIUM. H.T. Whelan, P.C. Letourneau*, M.H. Tuszynski*, and K.F. Swaiman*. Div. of Pediatric Neurology, and Dept. of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455.

Conditioned culture medium has been used for characterizing many neurotrophic substances. Much of the work thus far has concentrated on medium conditioned by embryonic heart cells in culture.

Central nervous system regeneration, even through adulthood, occurs in urodele amphibians, such as Ambystoma tigrinum (the tiger salamander). Large scale organ-by-organ screening for possible neurotrophic factors was thus undertaken using adult tiger salamanders with spinal cord lesions in hopes of finding neurotrophic mediators effective in adults.

The ability to stimulate neurite outgrowth was assayed in dissociated neuron cultures from 11-day-old chick embryo dorsal root ganglion. The ability to increase choline acetyl transferase (CAT) activity was assayed in dissociated neuron cultures from 16-day-old fetal mouse cortex. Two to four weeks after spinal cord transection, organ homogenates from brain, spinal cord, heart, kidney, liver, skeletal muscle, gonads, spleen, pancreas, blood, and regenerating tail-bud at the site of cord transection were individually prepared and aliquots added to the culture medium.

The results were compared with those achieved by adding β-nerve growth factor (β-NGF), chick embryo heart cell-conditioned medium (HCM), or both to similar neuron cultures. Control cultures contained medium with no additives.

Although several tissue types (i.e. heart, brain, cord, spleen) appeared promising initially, only heart homogenate repeatedly produced extensive neurite outgrowth and produced an increase in CAT activity (10%). These changes paralleled the effects of β-NGF and HCM.

Further characterization of the neurotrophic effects of the salamander heart homogenate was achieved by fractionation of cell components by ultracentrifugation. Effects on neurons in culture were enhanced in the nuclear and microsomal fractions, especially after disrupting the organelles and centrifuging away the debris.

This activity is eliminated by heating or excessive freeze-thawing, suggesting a protein neurotrophic factor. Further characterization is under way.

7.1 A LATE AND LONG-LASTING POST-ACTIVITY POTENTIATION IN THE SKELETAL MUSCLE OF THE RAT. J. García Ramos. Department of Physiology and Biophysics, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, D.F. and CEUNICYT, Universidad de Colima, Colima, Col., México.

After a brief period of relatively high frequency activity not producing fatigue of the gastrocnemius-soleus muscle of the anesthetized rat, a late or delayed potentiation lasting for more than 60 min was observed. The improved responses to test shocks appear well separated from the postetanic potentiation.

This delayed potentiation was induced with indirect stimulation through the sciatic nerve as well as with direct stimulation applied to the previously denervated (5 days) or to the curarized muscle. The late potentiation was transiently blocked by the protein-synthesis blocking agents (cycloheximide and chloramphenicol) and not present at low temperatures (below 35 degrees, centigrade). During this potentiation neither the electromyograms nor the impedance changes of the whole muscle showed any correlated variation. Several brief periods of relatively high frequency stimulation applied at intervals from 1 to 60 min showed additive effects. In opposition, the lack of that stimulation may result in reduction of the responses to test shocks.

These observations suggest that relatively rapid activity of a muscle causes certain biochemical changes of transient duration which may constitute the basis for the muscle hypertrophy and for its better performance during training. The reversion of these biochemical reactions at rest could be responsible of the fall in muscle efficiency and the atrophy of disuse.

7.2 MUSCLE AFTERDISCHARGE MASKS NEUROMUSCULAR BLOCKADE IN MODERATE HYPOCALCEMIA. B.W. Allen*, G.G. Somjen, & D.B. Sanders. Dept. Physiology & Div. Neurol., Duke Univ., Durham, NC 27710.

We recorded [Ca^{2+}] with a calcium-selective electrode (Ionetics, Inc.) in a flow cell in an extracorporeal loop connected to a carotid artery in cats. EMG and contractions evoked by stimulating the peroneal nerve were recorded from EDL muscle. We already reported (Allen et al., Fed. Proc. 41:1734, 1982) neuromuscular blockade and slowed relaxation of the contraction, caused by lowering arterial [Ca^{2+}] by i.v. citrate. We now find that contractile force initially increased as arterial [Ca^{2+}] was lowered, even as the amplitude of the transmitted EMG action potentials was depressed. Enhanced contractions and slowed relaxation were accompanied by EMG afterdischarges in the wake of stimulus-evoked action potentials. Multiple firing of the muscle membrane explains the apparently enhanced strength of contraction. The EMG afterdischarges, and the spontaneous discharges appearing in profound hypocalcemia, had the character of single muscle fiber potentials, not of motor unit discharges. (We have recently seen similar EMG signs in a patient with severe hypocalcemia.) In profound hypocalcemia, and during recovery from it, contractions evoked by trains of stimuli (but not by single shocks) were more depressed than the corresponding EMG action potentials. This suggests failure of excitation-transmission coupling, possibly due to loss of calcium from sarcoplasmic reticulum.

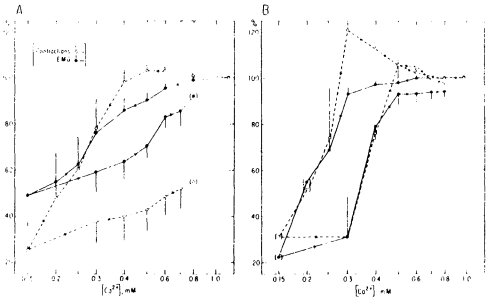

Fig. 1: Amplitudes of contractions and EMG action potentials evoked by motor nerve stimulation as functions of arterial [Ca^{2+}] during hypocalcemia and recovery. Note hysteresis. A: stimulation by trains of shocks; B: single shocks.

(In part supported by NIH Grants NS 18670 and NS 18949).

7.3 ADAPTATION IN FUNCTION OF THE PHARYNGEAL CONSTRICTOR MUSCLES OF THE RHESUS MONKEY. A.J. Miller, L. Rowe*, G. Chierici*, and D. Clendenning*. Craniofacial Center, Department of Growth and Development and Department of Otolaryngology, University of California, San Francisco, CA 94143

The palatopharyngeus muscle and pharyngeal constrictor muscles were studied to determine their function with changes in head posture, upper airway obstruction, swallowing and inducement of laryngospasm. Pairs of fine wires were placed in an array along a rostro-caudal axis of the pharyngeal wall, 2-4 mm lateral of the midline to record the electromyography activity (EMG). A flexible fiberoptic scope was used to monitor pharyngeal movements. EMG activity was synchronized with the view of the pharyngolaryngeal movements on a split-screen and recorded on videotape.

Tonically discharging motor units were recruited in certain regions of the pharynx including the caudal superior pharyngeal constrictor (SPC) and the rostral middle pharyngeal constrictor (MPC). This tonic activity could remain even during phasic contractions of these muscles during swallowing. Not all fibers of the pharyngeal constrictors were recruited during the peristaltic-like contractions of the pharynx during swallowing. Rhythmic respiratory discharge could be induced within the pharyngeal constrictor muscles with nasal obstruction, flexion of the head, or a change from the supine to erect position. Fibers of the SPC discharged during inspiration while the inferior pharyngeal constrictor fibers (IPC) were active during expiration. These findings suggest that certain fiber groups of the pharyngeal constrictors are important to postural maintenance of the pharynx, hyoid bone, and thyroid cartilage.

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7.4 RANDOM REINNERVATION OF LG AND SOL MUSCLES BY THEIR COMMON NERVE? M.J. Gillespie, T. Gordon and P.R. Murphy. Depts. of Physiology & Pharmacology, University of Alberta, Edmonton, Alberta, T6G 2H7.

In order to determine whether nerve fibers show a preference for their former muscles we have examined the contractile and histochemical characteristics of two muscles in the rat hindlimb, fast lateral gastrocnemius (LG) and slow soleus (SOL) after reinnervation by their common nerve. The LGS nerve was cut before its entry into the LG and sutured to the surface of the muscle thus presenting that muscle with fast and slow nerve fibers. The nerve fibers must course through LG as they normally do to reinnervate SOL muscles. After 3-14 months reinnervated LG and SOL weighed 68% and 59% of control muscles respectively, while twitch and tetanic forces were similarly reduced. For reinnervated SOL muscles contraction time (CT) was considerably faster than control values (42.5 vs 72 msec), while half relaxation time (1/2RT) was similar. Though CT in reinnervated LG muscles was almost normal (35 vs 32 msec) 1/2RT was longer than control (37 vs 30 msec).

This data might be explained if the LG became reinnervated by more slow nerve fibers than normal leading to a longer relaxation phase with the fast muscle fibers determining the normal CT. Similarly, reinnervation of soleus by more fast nerves than normal could account for the decreased CT and normal 1/2RT. This prediction was born out when the proportion of fast and slow motor units in the reinnervated muscles were determined physiologically (Table 1). Results show a considerable increase in the proportion of S units with a concomitant decrease in FR units in reinnervated LG. SOL muscles were composed of 70% fast contracting units and 30% S units. The CT and 1/2RT of FF, FI, FR and S motor units in the reinnervated muscles were normal so that the data supported the above interpretation of the whole muscle contractions.

Thus the nerve-muscle specificity that dictates connectivity in the embryo does not appear to operate in adult reinnervation. Rather the very similar proportions of slow and fast motor units in both reinnervated muscles resembled the proportions of fast and slow fibers in the common LGS nerve. This is consistent with the idea that slow and fast muscles show no preference for their former nerves and indeed suggests that reinnervation may occur in a random fashion.

Percentage of motor unit types

Muscle	Control				Reinnervated			
	FF	FI	FR	S	FF	FI	FR	S
LG	33	14	43	10	45	12	13	30
Soleus	-	-	20	80	6	38	25	31

7.5 INCREASED GROWTH FACTOR-LIKE ACTIVITY FROM HYPERTROPHIED SKELETAL MUSCLE. M.G. Bissell and S. Kaufman* Lab. of Neurochemistry, NIMH Bethesda, MD 20205

Passive mechanical stretching of skeletal muscle leads to hypertrophy of the tissue in response. Using a technique developed in this laboratory for stretching monolayers of chick embryo myotubes in vitro, it has been shown previously that skeletal myotubes respond to passive stretch by increased amino acid uptake (as measured by uptake of aminoisobutyric acid, AIB), increased incorporation of amino acids into, and accumulation of total cellular protein. These increases have been shown to be inhibited by ouabain, after a 30 minute lag period, and by tetrodotoxin. Stretch has also been shown to be associated with an early increase in the Vmax of the membrane Na/K-dependent ATPase, as measured by rubidium-86 uptake. These stretch effects take place in serum free medium and have been shown to be mimicked by the addition of serum to unstretched cultures.

More recently, we have carried out additional studies on a whole animal model of skeletal muscle hypertrophy. This in vivo tenotomy model involves the surgical section of the Achilles tendon of the gastrocnemius muscle of one limb while a "sham" operation is carried out on the other limb. Following the operation, the weight-bearing load is redistributed from the gastrocnemius to the two smaller synergist muscles, the soleus and plantaris, which rapidly hypertrophy. Using this system, we have seen significant increases in wet weight of the soleus and plantaris, as early as four to six hours post-tenotomy. We have made aqueous extracts of hypertrophied soleus and plantaris muscles from rats 48 hours after cutting the tendon. These extracts, like serum, cause dose-dependent increases in AIB uptake and amino acid incorporation when added to unstretched chick myotube cultures. Similar extracts from the corresponding nonhypertrophied muscles on the unoperated contralateral hindlimbs of the same animals show a significantly smaller effect when added to the cultures. The substance (or substances) responsible for this effect, like the similarly active serum component, is heat labile and retained by a dialysis bag. The active factor (s) from rat hindlimbs is degraded by treatment with α-chymotrypsin. Its effect on AIB uptake is not additive with 10% stretch. Like the effect of serum, its effect on AIB uptake is sensitive to actinomycin D and ouabain, but unlike stretch it is effective within 5 minutes of application. Further characterization of the substance or substances responsible for these effects are currently underway.

7.6 NEUROMUSCULAR FUNCTION IN A HEALTHY AGED POPULATION. A.A. Vandervoort* and A.J. McComas. Departments of Neurosciences and Medicine, McMaster University Medical Centre, Hamilton, Ontario, Canada, L8N 3Z5.

In view of the importance of maintaining normal mobility in the ageing population we have studied function in two opposing groups of leg muscles, the ankle dorsiflexors (DF) and plantarflexors (PF), in a large group of elderly subjects. Attempts have been made to answer three major questions. First, to what extent does muscle strength decline with age? Second, can descending motor pathways be effectively utilized in activating motoneurones? Third, other than in maximal force generation, are there changes in the contractile properties of aged muscles? The findings in 70 healthy men and women, aged 60-100 years, were compared with those in 28 young adults aged 20-32 years; all subjects were volunteers and the study was approved by the local ethics committee. The results of the study disclosed: (i) relatively small (15-20%) decreases in maximum voluntary DF and PF torques in 60-69 year old men and women compared with controls, (ii) normal activation of DF and PF motoneurones during maximal effort, even in the oldest subjects, (iii) slowing of the isometric twitch between the third and seventh decades, and (iv) reduction in the maximum voluntary: twitch torque ratio for aged PF muscles. Ultrasonic imaging of calf musculature indicated that the reductions in PF voluntary and twitch torques were greater than the loss of muscle cross-sectional area. We conclude that the PF and DF muscles of elderly subjects remain effective for daily activities despite the loss of functional muscle mass.

Supported by the Muscular Dystrophy Association of Canada.

7.7 ELECTRICAL STIMULATION EXERCISE CONDITIONING PROTOCOL FOR PARALYZED MUSCLE. J.A. Gruner, R.M. Glaser*, S.R. Collins*, S.D. Feinberg*. Dept. of Physiology, Wright State Univ. School of Medicine, Dayton, OH 45435.

Electrical stimulation has been shown capable of restoring function to paralyzed muscle in individuals where there is no lower-motoneuron involvement. Few studies, though, have carefully examined the relationships between various exercise parameters, such as work load, repetition rate, and duration on the rate of increase and final levels of strength and endurance obtained in electrically stimulated muscle. Moreover, such studies have rarely been concerned with developing exercise protocols which may safely be prescribed for paraplegic individuals whose physical condition, including cardiopulmonary fitness and skeletal integrity, may vary greatly and are difficult to accurately assess.

An electrical stimulation exercise system was designed which could be safely applied to most paraplegic individuals. The exercise system consists of a platform, backboard, and ramp which the individual is wheeled up onto. A system of weights and pulleys is built into the platform so that when the knee is extended, a cable attached to the subject's ankle will raise a weight behind the subject. Knee extension is produced by stimulating the quadriceps muscle using 0.2 ms pulses at 30 hz delivered via surface electrodes (3M). An Apple II+ computer controls the stimulus amplitude via feedback so that the weight can be lifted a preset distance (for a constant work load). A typical exercise session consists of 2 bouts of 20 leg lifts with each leg extending alternately once every 30 seconds. Bouts are 10 minutes apart. The work load for a given exercise session is adjusted according to the individual's past achievement using the following algorithm. If the first 20 lifts are not successfully completed, the weight for that leg is decreased by 1.25 lbs. If both bouts are completed successfully in two successive sessions, the weight is increased by 1.25 lbs on the next session.

Six subjects have been exercised 3 sessions per wk, starting at 2.5 lbs. Three subjects increased in load at the maximum rate until reaching about 15 lbs after 23 sessions (1 subject) and 23 lbs at the end of 36 sessions (2 subjects). Two other subjects initially dropped to 1.25 lbs where they were held for 10 to 14 sessions, after which they began to steadily increase in load. In these subjects, the rate of increase of the muscle's work capacity should be reflected by the rate of increase in weight lifted. Some spasticity was often present in antagonist muscles so that the amount of work measured externally may have underestimated the actual work performed. Also, it was noted that some individuals who could not voluntarily extend their knees or produce measurable quadriceps EMG activity could "voluntarily" extend the duration of the stimulation-induced contraction.

7.8 MUSCLE GAIN CHANGES INDUCED BY INTERMITTENT SUBMAXIMAL ISOMETRIC EXERCISE. T.M. Banas* and P.E. Crago, Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

Muscle fatigue, when viewed as an internal disturbance to neuromuscular regulation, can potentially be used in the study of stretch reflex function if the exercise does not compromise the interpretation of the electromyogram (EMG) as a measure of motor neuron pool output. We found that intermittent submaximal exercise can substantially reduce muscle force generation without reducing muscle action potential conduction velocity. This is in contrast to sustained maximal voluntary contractions where conduction velocity has been shown to decrease, thus limiting the reliability of the EMG in reflex assessment.

Torque was measured at the interphalangeal joint of the thumb of adult normal subjects during isometric contractions of the Flexor pollicus longus (FPL). The joint was held at 10 degrees of flexion. EMG was measured from surface electrodes over the FPL or from intramuscular wire electrodes. Muscle gain was calculated as the slope of the linear regression of torque on EMG . Steady state values of torque and smoothed, rectified EMG were measured as the average values during the last half of two second contractions at torque values chosen randomly between three and fifteen N-Cm. The subjects rested for five seconds between contractions. The power spectral density of the raw EMG was calculated from ten similar contractions at an intermediate torque level.

The torque-EMG relationship and the power spectral density were measured before, a few minutes after, and at intervals of 30-45 minutes after a period of exercise. The exercise consisted of 30-60 isometric contractions at 15 N-Cm torque for 11 seconds, with 10-20 seconds rest between each contraction. The slope of the torque-EMG relationship was only slightly reduced a few minutes after the exercise, but was reduced by 1/3 to 1/2 after 30-45 minutes. The slow decrease in gain that took place after the exercise may have been due to a gradual decrease of an exercise induced potentiation, but this hypothesis was not tested directly. Typically the slope remained low for the next 30-45 minutes and then began to increase.

The power spectral density was measured at the same EMG level both before and after the exercise. The torque was typically 10 N-Cm before the exercise and was recalculated on the basis of the new torque-EMG relationships after the exercise. The shape of the spectrum was changed very little by the exercise, except for a possible slight shift to higher frequencies. Thus it appears unlikely that there were any changes in muscle fiber conduction velocity accompanying the reduction in muscle gain.

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7.9 ELECTROPHYSIOLOGIC DIFFERENCES BETWEEN MAMMALIAN FAST AND SLOW MYOFIBERS. J.A. Florendo,* J.F. Reger,* and P.K. Law (SPON: R. Caldwell). Depts. of Anatomy, Neurology, and Physiology Biophysics, Univ. of Tennessee Ctr. Hlth. Sci., Memphis, TN. 38163.

Miniature end-plate potentials (mepps) and indirectly elicited action potentials were recorded in vivo at 37°C from surface fibers of the fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus (SOL) muscles of 3 to 4-month-old Bar Harbor 129 mice.

Mepps of the EDL exhibited a significantly higher frequency, smaller amplitude, and shorter duration than the mepps of the SOL. Histograms demonstrated that EDL and SOL fibers had overlapping but skewed distributions in all three parameters.

	EDL	SOL
Frequency (hz)	8.79 ± 4.50	2.40 ± 1.36
Amplitude (mv)	0.75 ± 0.15	1.02 ± 0.21
Duration (msec)	1.29 ± 0.31	2.92 ± 0.89

Mean ± SD from 52 EDL and 56 SOL fibers. All differences significant at $P < 0.005$ by Student's t-test.

Action potentials of EDL fibers exhibited a significantly greater amplitude and shorter duration than SOL fibers. A single stimulus elicited several action potentials from an EDL fiber but only one action potential from a SOL fiber. Histograms demonstrated that EDL and SOL fibers had overlapping but skewed distributions of amplitude and duration.

	EDL	SOL
Amplitude (mv)	96.30 ± 3.55	79.75 ± 8.17
Duration (msec)	0.99 ± 0.06	1.28 ± 0.10

Mean ± SD from 86 EDL and 77 SOL fibers. All differences significant at $P < 0.005$ by Student's t-test.

Fast- and slow-twitch muscle fibers can thus be identified and distinguished on the basis of these electrophysiologic parameters.

(Supported by MDA, NSF PCM79-21008 and USPHS RR-03166 to PKL)

7.10 GPI-1B AND GPI-1C AS GENOTYPE MARKERS IN NORMAL/DYSTROPHIC MYOBLAST TRANSPLANT STUDY OF IMMUNOCOMPATIBLE MICE. P.K. Law. Departments of Neurology and Physiology/Biophysics, Univ. of Tennessee Ctr. Hlth. Sci., Memphis, Tennessee 38163.

We have shown that surgically transplanted mesenchymal cells from the limb-buds of normal mouse embryos improved the structure and function of dystrophic mouse muscles (Muscle & Nerve 5:619-627, 1982). In order to study the mechanisms responsible for such improvement, we seek immunocompatible host and donor mice that exibit distinct genotype markers and the trait of dystrophy. These conditions are satisfied with mice of C57BL/6 $+/+$ $gpi\text{-}1^{c/c}$ strain (courtesy of Dr. A. C. Peterson, Montreal) and C57BL/6 dy^{2J}/dy^{2J} $gpi\text{-}1^{b/b}$ strain (Bar Harbor). The survival and development of donor myoblasts in host muscles will be analyzed with electrophoresis of muscle isozymes of GPI. We report here a reliable and convenient GPI electrophoresis method using 1% agarose gel films (Corning 470100).

The mouse soleus, weighing about 7 mg, was thoroughly homogenized in 0.12 ml of ice-chilled double-distilled water. The sample was centrifuged at 600g for 10 min. and then at 1300g for 10 min. at room temperature. 0.5 µl of the supernatant was applied to each well of the agarose film. Electrophoresis was conducted at pH 8.6 in a LKB 2117 Multiphor, anode to cathode, for 3.5 hours at 5 mA per channel or 2.5 V/cm field strength. The gel bed was cooled by running water at 4°C. The staining solution used was specific for GPI. It was modified from Eicher and Washburn (Proc. Natl. Acad. Sci. USA 75:946-950, 1978), and consisted of 8 ml of 0.2 M Tris-HCl, pH 8.0; 20 µl of G6PD (0.3 IU/ul); 0.4 ml of Mg acetate (53.6 mg/ml); 0.6 ml of fructose-6-phosphate (100 mg/ml); 0.4 ml of phenazine methosulfate (2.5 mg/ml); 0.4 ml of MTT tetrazolium (10 mg/ml); 0.4 ml. of NADP (10 mg/ml). Staining time was 4 min. at 37°C. Densitometric tracings of the gel bands were analyzed and the gels photographed.

Donor muscles exhibit GPI-1C and host muscles exhibit GPI-1B.

The test muscle receiving myoblast transplant may exhibit a variety of electrophoretic patterns which can be interpreted as follows: (1) GPI-1B, GPI-1C. A mosaic muscle with donor and host cells. Mosaic fibers are not present. (2) GPI-1B, GPI-1C, GPI-1BC. A mosaic muscle with host fibers, donor fibers and mosaic fibers. (3) GPI-1C, GPI-1BC. A mosaic muscle with donor fibers and mosaic fibers. Host fibers have degenerated. (4) GPI-1B, GPI-1BC. A mosaic muscle with host fibers and mosaic fibers. (5) GPI-1BC. A mosaic muscle with only mosaic fibers. (6) GPI-1C. Host cells are replaced by donor cells. Fusion between donor and host cells does not occur. (7) GPI-1B. Donor cells do not survive in the host muscle. (Supported by MDA)

7.11 OCULOMOTOR NUCLEUS INNERVATION OF THE LATERAL RECTUS MUSCLE IN THE CAT. J.R. McClung*, S.J. Goldberg, J.S. Nelson* and C.H. Fowlkes*. Dept. of Anat., Med. Coll. of Va.-VCU, Richmond, VA 23298.

Most motoneuron pools are organized such that all of the cells are localized in a specific nucleus and innervate a single muscle. There are examples, however, in both the skeletal and extraocular systems where unusual innervation patterns exist. The motoneuron pool innervating the cat retractor bulbi muscle, for example, is divided and contained in both the principal and accessory abducens nuclei and the oculomotor nucleus. Some of the VIth Nerve retractor bulbi axons also branch and innervate the lateral rectus muscle. Anomalous innervation of the lateral rectus muscle by the oculomotor nerve has been observed in human congenital abnormalities of the abducens nucleus (Duane's Syndrome), and some IIIrd Nerve innervation of the lateral rectus muscle has recently been suggested to occur normally in the cat.

The present studies document that, in the cat, the motoneurons which innervate the lateral rectus muscle are found in the ipsilateral principal abducens nucleus in expected numbers and are present in small numbers in the accessory abducens and oculomotor nuclei.

The lateral rectus muscle nerve was dissected from the muscle and the cut proximal end was sealed in plastic tubing and exposed to a 50% solution of HRP. This carefully isolated preparation allowed the use of TMB histochemistry for localization of the retrogradely labeled motoneurons without evidence of HRP leakage or spreading to other orbital structures. In addition, stimulation of the abducens nucleus elicited expected twitch contractions of 12 to 15 grams and simultaneous stimulation of the oculomotor nucleus added 300 to 500 milligrams in twitch tension. Oculomotor nucleus stimulation alone elicited consistent responses in the lateral rectus muscle both before and after total bilateral extirpation of the abducens nuclei. These procedures eliminated any participation by either principal or accessory abducens motoneurons in the response.

These findings show the lateral rectus, a primary ocular rotatory muscle in the cat, to have a diverse innervation derived from three brainstem nuclei and this emphasizes problems both in defining a motoneuron pool and in understanding the manner in which motoneurons are recruited at widely separated sites.

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8.1 TWO CLASSES OF GABAERGIC NEURONS REPRESENT THE MAJORITY OF NEOSTRIATAL NEURONS IN THE RAT. W.H.Oertel and E.Mugnaini. Neurological Clinic,Technical University,Munich,FRG and Department of Biobehavioral Sciences,U.Conn.,Storrs,CT 06268,USA.

The rat neostriatum contains evenly distributed medium size neurons with scattered large neurons inbetween in a ratio of approximately 50:1.Retrograde transport studies have demonstrated that the majority of the medium size neurons project to the globus pallidus, the entopeduncular nucleus and substantia nigra pars reticulata.Physiological,biochemical and cytochemical reports indicate that the neostriatal neurons include GABAergic,cholinergic and peptidergic cell populations.In the present study on the rat neostriatum we have reinvestigated the immunocytochemical localization of glutamic acid decarboxylase(GAD),the enzyme which synthesizes GABA,with a sheep antiserum to rat brain GAD.

Untreated rats and rats that had received a stereotaxic injection of colchicine(10-20µg) into the neostriatum 12-50 hr prior to sacrifice were used.The animals were perfused with a zinc-formaldehyde fixative.Floating brain sections were processed for GAD-like immunocytochemistry with the PAP method of Sternberger.

The neostriatum of untreated rats contained scattered distinctly GAD-immunoreactive pleomorphic medium size to large neurons.The majority of medium size neurons were not immunoreactive.After colchicine injection,however,the majority of medium size neurons throughout the neostriatum exhibited distinct GAD-immunoreactivity. Thus,the rodent neostriatum contains two classes of GABAergic neurons: a minor cell class which can be demonstrated immunocytochemically with the employed GAD-antiserum in untreated animals,and a major cell class that is stained only following topic injection of colchicine.Cells belonging to the first category vary considerably in shape and size,whereas cells of the second category are more homogenous and appear to correspond to the principal neostriatal nerve cell, commonly referred to as medium size neurons.

Previous investigations have indicated that neostriato-pallidal and neostriato-nigral terminals are numerous and,at least to a large extent,use GABA as their transmitter.Such GABAergic neostriatal projections may originate from the major population of GAD-positive neurons.Apparently,only by blocking axoplasmic transport with colchicine, the concentration of GAD in these nerve cell bodies reaches levels detectable by our technique.The minor population of GAD-positive neurons stainable without colchicine treatment may have particular functional and anatomical features.

The data indicate that two categories of GABAergic neurons represent the majority of rat neostriatal neurons. Localization of neurons synthesizing acetylcholine or neuropeptides and their relation to GABAergic neurons is presently under investigation.

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8.2 IMMUNOHISTOCHEMICAL LOCALIZATION OF LEU-ENKEPHALIN AND GLUTAMIC-ACID-DECARBOXYLASE IN THE NUCLEUS CAUDATUS OF THE RAT. M. Morelli, M. Del Fiacco*, J.-Y. Wu and G. Di Chiara. Institutes of Exp.Pharmacology and Toxicology, and Anatomy, University of Cagliari, Italy. Baylor College of Medicine, Houston U.S.A.

GABA-ergic and Enkephalinergic neurons have been described as projecting from the nucleus caudatus to the substantia nigra of the rat. These neurons are considered to play a very important role in modulating striatal motor responses such as catalepsy, turning behaviour and stereotypy. In order to study the precise localization of these neurons we applied the immunocytochemical technique to consecutive (6 µm) sections of the nucleus caudatus of the rat. Male rats were injected with 30 µg of Colchicine in the caudal part of the caudate head in order to block the axonal flow. The dilution of the specific Leu-Enkephalin (L-Enk) and GAD (glutamic-acid-decarboxylase) antibodies was 1:600 and incubation was carried out at 4°C overnight. The sections were processed using the PAP immunoreaction technique. Several sections incubated with non immunized rabbit serum showed no reaction products. In the normal caudate nucleus, a dense network of GAD and L-Enk immunoreactive fibers could be observed throughout the entire area. In these animals immunoreactivity in neuronal perikarya was not observed. In the caudate nucleus injected with Colchicine, L-Enk and GAD containing perikarya could be demonstrated throughout the entire extent of the nucleus. L-Enk immunoreactive cells were clustered in the ventral and lateral part of the posterior half of the head of the nucleus caudatus, while only few positive cells were found in the medial and dorsal part of it. In the body of the nucleus caudatus L-Enk containing perikarya were concentrated in its ventralmost part. GAD immunoreactive cells in the nucleus caudatus were more evenly distributed. The major concentration of CAD positive somata was found in the external part of the caudatus, both dorsally and ventrally; only relatively few somata were stained in the medial part of the nucleus. Thus the topography of Leu-Enkephalinergic somata in the nucleus caudatus is in part superimposable to that of GAD positive somata. Both GAD and L-Enk positive somata were of medium size. The possibility that GABA and L-Enk coexist in striatal neurons will be discussed.

8.3 DEPLETION OF STRIATAL SOMATOSTATIN BY LOCAL CYSTEAMINE INJECTION. M. F. Beal and J.B. Martin, Department of Neurology, Harvard Medical School, Boston, MA 02114.

The presence of somatostatin in the striatum is of particular interest since alterations in concentrations of this peptide have been found in degenerative neurologic illnesses. It has been recently demonstrated that sytemic cysteamine results in reduction of somatostatin-like immunoreactivity (SLI) in rat brain including the striatum. In the present study we examined the effects of local cysteamine injections in the rat striatum. Injections were made in a volume of 1.0 or 2.0 µl at the coordinates 8.4 mm anterior, 2.7 mm lateral and 4.5 mm ventral to the dural surface. Animals were subsequently decapitated and the brains sectioned at 2 mm intervals with dissection of the striatum, cortex and hippocampus. SLI was measured by radioimmunoassay. In an initial experiment 7.5 µg, 15.0 µg, 30.0 µg, 50.0 µg and 100.0 µg of cysteamine were injected into 6 animals in each group and 30 µg of ethanolamine was injected into controls. Animals were sacrificed at 2 hours. A dose response was demonstrated with a maximal depletion of approximately 50% of the total striatal SLI seen at the 30 µg and higher doses. The cortex and hippocampus were unaffected. A time course experiment was done using a dose of 30 µg of cysteamine in 1 µl and 30 µg of ethanolamine in controls. A significant depletion of striatal SLI to approximately 50% of controls (1.09 ± .06 to .57 ± .03 ng/mg protein at 1 hour) was found at the time intervals 1,3,6,24 and 72 hours. In addition at the 3rd hour and at subsequent time intervals there was an approximately 20% depletion of SLI in the contralateral striatum. Initial results showed a decrease in striatal dopamine turnover. Histologic examination of the two highest doses of cysteamine (50 and 100 µg) using Nissl stained serial sections showed that glial scarring was localized to the needle tract and directly adjacent neurons appeared normal. These studies show that local injections of cysteamine result in depletion of SLI and may be useful in studying the interactions of somatostatin with other known striatal neurotransmitters.

8.4 THE RELATION OF STRIOSOMES IN THE CAUDATE NUCLEUS OF THE CAT TO THE ORGANIZATION OF EARLY-DEVELOPING DOPAMINERGIC FIBERS, GAD-POSITIVE NEUROPIL, AND CAT-POSITIVE NEURONS. A.M. Graybiel, M.-F. Chesselet, J.-Y. Wu, F. Eckenstein, and T.E. Joh, Dept. of Psychol. and Brain Sci., Mass.Inst.Tech., Cambridge, MA 02139

In the caudate nucleus of the cat, histochemically distinct tissue compartments called striosomes have been identified in cross sections as 0.3-0.5mm-wide regions in which the neuropil is low in acetylcholinesterase (AChE) activity and high in met-enkephalin-like immunoreactivity. These zones, in turn, have been correlated in serial-section analyses with patches or gaps in cortical and thalamic afferent-fiber distributions, with zones of weak efferent-cell labeling and with clusters of ^{3}H-thymidine (^{3}H-thy) labeled cells in cats exposed to the ^{3}H-thy as E23-E30 fetuses. We report here immunohistochemical experiments designed to determine the relation of this macroscopic ordering to the distributions of three key neurotransmitter systems in the striatum: the cholinergic, as marked by antibodies to choline acetyltransferase (CAT); the GABAergic, as marked by antibodies to glutamic acid decarboxylase (GAD); and the early forming "islandic" dopaminergic, as marked by antibodies to tyrosine hydroxylase (TOH).

For the TOH experiments, we studied late fetal and neonatal kittens, in which early forming dopaminergic fibers form discrete patches visible in TOH preparations. We pre-exposed the animals at E25-E27 to ^{3}H-thy and used autoradiography to identify the striosomes as clusters of ^{3}H-thy-labeled neurons. Serial-section comparisons showed that these ^{3}H-thy clusters (the striosomes) were in register with the TOH patches (the dopamine "islands").

For the GAD experiments, serial sections through the caudate nucleus of adult cats were processed alternately for AChE and for GAD-like immunoreactivity by Sternberger's PAP method. GAD-positive cell bodies were rarely seen but the striatal neuropil was GAD-positive and there were patches of very intense GAD-like immunoreactivity. These GAD-positive patches were strictly aligned with (and often somewhat smaller than) the AChE-poor striosomes.

For the CAT experiments, we visualized both the AChE-poor striosomes and AChE-positive cell bodies by using adult cats pretreated with DFP. With a serial immunofluorescence (FITC)-AChE method, we found virtually every CAT-positive neuron in the caudate nucleus to be AChE-positive. We plotted 1892 of these neurons (mean surface areas: 300-320µm^{2} and 420-440µm^{2}) and found that 1486 (78.5%) lay outside the striosomes, 282 (14.9%) alongside them and 124 (6.6%) inside them. The striosomes comprised 14.3% of the area charted.

These findings demonstrate that the striosomes correspond to the islandic dopamine system and contain GAD-positive neuropil. A correspondence with cholinergic cell-body distributions seems unlikely unless the striosomal borders are rich in these cells.Funded by the Wills Foundation and Fogarty Fellowship No. 1505TW03204-01 to MFC.

8.5 THE DISTRIBUTION OF MUSCARINIC BINDING SITES IN THE FELINE STRIATUM AND ITS RELATIONSHIP TO OTHER HISTOCHEMICAL STAINING PATTERNS. M.A. Nastuk and A.M. Graybiel. Dept. of Psychology and Brain Sci., Mass. Institute of Technology, Cambridge, MA 02139

The distribution of muscarinic binding sites in the feline striatum was examined by applying the autoradiographic method of Rotter et al. '79 to tissue from 9 fetuses (estimated E45-E59), 14 kittens (5 hr-5 wk) and 1 adult cat. The ligand used was [^{3}H]-propylbenzilylcholine mustard (PrBCM), a specific irreversible muscarinic antagonist. Adjacent sections were stained for acetylcholinesterase (AChE) or Nissl substance, or processed by de la Torre's glyoxylic acid method for catecholamine (CA) fluorescence. Atropine controls for each case were negative.

The PrBCM autoradiograms show patchy non-uniform patterns of binding in fetuses and kittens. Typical late fetal binding distributions include ca. 200-300μm wide zones of high binding density (heavy labeling) as well as zones of low binding density (sparse labeling) at all levels of the caudate nucleus (CN) and putamen. There are also inhomogeneities in the nucleus accumbens. In the CN there is a mediolateral density gradient in the background matrix with more binding laterally than medially. The patches are also much more prominent laterally. Postnatal animals show a different characteristic binding pattern, though ventrally in the CN the prenatal pattern can persist for at least 5 weeks. Dorsolaterally the binding becomes nearly uniform except for small patches that are partly or totally surrounded by unlabeled capsules. The grain density within these patches is not much greater than that of the background matrix. In the adult, PrBCM binding is dense throughout the striatum and shows no mediolateral gradient. The binding is not fully homogeneous, however, because there occasionally are patches of labeling in the CN that are denser than the background.

In the fetuses and kittens there are striking correspondences between the PrBCM patch-patterns and those visible in serially adjoining AChE, CA fluorescence, and Nissl sections. AChE-rich, CA-rich patches (the "dopamine islands") are aligned with patches of dense PrBCM binding, and zones of low AChE and low CA fluorescence are aligned with zones of weak PrBCM binding. By contrast, the PrBCM and Nissl patterns are complementary, with PrBCM-dense patches matching cell-sparse zones, and PrBCM-weak zones matching cell-rich zones. In the adult cat, each of the rare PrBCM-dense patches was found to be in register with an AChE-poor striosome.

We conclude (a) that regions of high muscarinic receptor density develop in relation to zones containing early forming dopamine fibers, and (b) that this physical correspondence likely has a functional corollary.

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8.6 BUTYRYLCHOLINESTERASE IN THE DORSAL AND VENTRAL STRIATUM: OBSERVATIONS ON HISTOCHEMICAL DISTRIBUTIONS IN ADULT, FETAL AND NEONATAL CATS. C.W. Ragsdale, Jr. and A.M. Graybiel, Dept. of Psychol. & Brain Science, Mass. Inst. Tech., Cambridge, MA 02139

The striatum of the adult cat contains both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activity demonstrable by thiocholinesterase histochemistry. AChE activity in the caudate nucleus (CN) is characterized by 0.3-0.6mm wide enzyme-poor striosomes. Prolonged incubation of striatal tissue in BuChE solutions (with AChE inhibition) also results in patterned staining of striatal neuropil and staining of fiber bundles. In 5 of 6 cats studied, BuChE-positive areas in the CN largely fell outside of the AChE-poor striosomes, as though avoiding them, and there were sometimes BuChE rims around the striosomes. In a sixth cat, there were BuChE-rich patches which were found to coincide with the striosomes. In all animals there was a progressive decrease in BuChE staining from dorsal to ventral in the CN, but much of the ventral striatum had high levels of BuChE. In particular, a broad curved band of high BuChE activity extended ventrally from the medial n. accumbens and stretched over the olfactory tubercle to the ventral putamen. This band contained complexly arranged enzyme-poor and enzyme-rich zones which often coincided with, but were more prominent than, inhomogeneities seen in AChE staining. Striking 100-200μm wide BuChE-pale patches were distributed in an arc running through this BuChE-rich region. In the olfactory tubercle there were wedges of very dense BuChE activity (ca. 200μm across) which reached the surface of the brain and formed anterior-posterior strips in flattened tangentially sectioned material. These enzyme-rich zones lay in the hollows of, and at least partly overlapped, the cell-dense islands of Calleja. Traced serially, they seemed to merge with the ventral pallidum.

In the fetal material (E34-E61) there was a striking arrangement of BuChE activity in the ganglionic eminence (GE). Save for a dorsal BuChE-negative part, the GE had layers of staining parallel to the ventricular surface, large regions of heavy staining, and stained radial strands running from the ventricular face to the CN. This staining underwent reorganization and reduction during gestation. In the CN, BuChE-positive patches were visible at least by E43, replacing a previously more homogeneous distribution and remaining prominent in the perinatal period. These BuChE patches were aligned with the AChE-positive patches visible in the same brains, and also with circumscribed patches of cytochrome oxidase observed in a newborn kitten. In this neonate, the cytochrome oxidase patches were crisper and less interconnected than either the BuChE or the AChE patches. Finally, there were correspondences between all three sets of enzyme patches and patterns in adjoining Nissl sections.

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8.7 MULTIPLE PATTERNS OF CORTICOSTRIATAL PROJECTIONS AND THEIR RELATIONSHIP TO OPIATE RECEPTOR PATCHES IN RATS J.P. Donoghue and M. Herkenham Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

Although the general arrangement of corticostriatal projections is known in the rat, the precise projection pattern from major cortical fields and their relationship to other features of striatal organization have not been clearly established. We have examined corticostriatal projections from: (i) primary motor cortex (MI), (ii) primary somatic sensory cortex (SI), (iii) visual cortex, and (iv) the prelimbic cortex plus adjacent cortical fields (medial frontal cortex) using axonally transported [^{3}H]amino acids or lectin-peroxidase conjugates. In some cases mu opiate receptors were marked in adjacent sections with [^{3}H]naloxone or [^{125}I]DAGO-enkephalin to compare the location of corticostriatal projections with opiate receptor-rich patches.

Striatal projections from these four cortical regions differ from each other with respect to both terminal location and pattern. (i) MI corticostriatal fibers are distributed bilaterally and nearly uniformly in the lateral one-third of the striatum. These projections overlap both opiate receptor dense and sparse regions. (ii) SI projections are mainly ipsilateral and form patches that generally lie posterior to the MI projection zone, but may overlap in some regions. The patches of SI projections may abut, but do not overlap opiate receptor patches. (iii) Visual cortical projections are distributed ipsilaterally in a continuous band between the dorsal edge of the striatum and an inner, discontinuous strip of opiate patches. (iv) Medial frontal projections are distributed bilaterally over a large portion of the rostral striatum and form distinct patches that coincide with the opiate receptor patches. In the nucleus accumbens the patches also overlie cell clusters visible in Nissl-stained sections.

Thus, visual and somatic sensory areas of cortex project to striatal regions that are low in opiate receptors. In contrast, medial frontal cortex projects into receptor-dense regions, and motor cortex has a broad projection that overlaps both regions. These data suggest that certain cortical inputs to the striatum are rigidly segregated from each other and are functionally related to other aspects of striatal compartmentalization, such that shown by the opiate receptor patches. The data also suggest that dopamine may act indirectly on the opiate receptor-rich regions of the striatum through the prelimbic cortex because this area is the main cortical target of brainstem dopaminergic fibers.

8.8 PEPTIDE IMMUNOHISTOCHEMISTRY AND ACETYLCHOLINESTERASE STAINING DEFINE NUCLEAR BOUNDARIES OF THE RAT AND HUMAN BASAL FOREBRAIN. S.N. Haber[1] and W.J.H. Nauta[2]. [1]Mailman Research Center, McLean Hospital, Belmont, MA 02178, and [2]Dept. of Psychology and Brain Science, Mass. Inst. of Technology, Cambridge, MA 02139.

Recently, we have shown that enkephalin-like immunoreactivity (ELI), Substance P-like immunoreactivity (SPLI), and dynorphin-like immunoreactivity (DLI) appear in striatal efferent fibers projecting to the globus pallidus in a unique pattern called woolly fibers. These fibers have been shown in the rat and in human to extend the assumed boundaries of the pallidum to include: a massive ventral pallidum extending into the olfactory tubercle, regions of the amygdaloid complex, and parts of the bed nucleus of the stria terminalis (BNST). Acetylcholinesterase (ACHE) staining of both fibers and cell bodies, frequently used as a marker for the basal nucleus of Meynert, is also found in these regions. The purpose of this study was to describe the differential distribution of peptide immunoreactivity and ACHE staining on adjacent sections in both the rat and human basal forebrain area in an attempt to define the nuclear boundaries of this region.

After fixation, tissue was sectioned serially at 50 micra and processed for Met-enkephalin, Substance P (antibodies donated by Drs. Robert Elde and Ray Ho, respectively), dynorphin (antibodies donated by Dr. S.J. Watson), and ACHE. Peptide immunohistochemistry employed the PAP technique, and ACHE staining the Jensen-Blackstad technique. Some animals were treated with DFP to accentuate the ACHE-cell visibility, and then processed for both peptide and ACHE staining. Additional sections served as absorbtion controls or were stained with cresylviolet.

ACHE fiber staining does not take the unique form of woolly fibers and therefore can easily be distinguished from peptide-positive intrapallidal fibers. The results sharply contrast the distribution of ACHE-positive and woolly fibers. Both occupy large regions of the basal forebrain, but they overlap very little, and appear to avoid each other. While dense concentrations of ACHE-positive cell bodies also appear outside the boundaries of woolly fibers, scattered ACHE cell bodies do invade peptide-rich areas, particularly in the ventral pallidal - olfactory tubercle region. These cell bodies appear to constitute a group separate from the more densely packed ACHE-positive cells in woolly fiber-free regions. It is possible that the scattered ACHE-positive cells located within the peptide-rich areas, and thus lying in the path of striatal efferents, have a circuitry different from the densely packed ACHE-positive cells in peptide-poor regions of the basal forebrain. Supported by the Scottish Rite Foundation and NIH grant NS19170-01.

8.9 SUBDIVISIONS OF THE PALLIDUM AND THE SUBSTANTIA NIGRA DEMONSTRATED BY IMMUNOHISTOCHEMISTRY. M.-F. Chesselet and A.M. Graybiel, Dept. of Psychol. and Brain Sci., Mass. Inst. Tech., Cambridge,MA 02139

Using the Sternberger PAP method to characterize immunohistochemical patterns in the pallidum and the substantia nigra (SN),we have plotted the distribution of fibers containing somatostatin-like (SOM), substance P-like (SP), and enkephalin-like (ENK) immunoreactivity (antisera from R. Elde); dynorphin-like immunoreactivity (DYN) (antiserum from S.Watson); and glutamic acid decarboxylase immunoreactivity (GAD) (antiserum from J. Wu).

In the SN, there are discrete SOM-positive zones in rat, cat and monkey. In rat, SOM fibers lie in the pars compacta (defined by numerous tyrosine-hydroxylase immunoreactive cell bodies,TH-CB, detected with an antiserum from T. Joh), but in monkey, SOM fibers are mainly concentrated in a characteristic network in the pars lateralis. A similar distribution was found in the cat and so, other neurochemical markers in the pars lateralis were studied in this species. At rostral levels, the pars lateralis is separated from the pars compacta and reticulata by a fiber bundle and contains ENK, GAD, SP as well as SOM fibers but no TH-CB. It is heavily stained in butyrylcholinesterase (BuChE) sections and thus is distinct from the pars reticulata which contains no SOM, little ENK and BuChE, but dense DYN as well as GAD and SP fibers. Caudally, SOM fibers remain confined to the pars lateralis which contains TH-CB and dense DYN, GAD and SP fibers. To determine the origin of SOM fibers in the cat SN, we combined fluorescence immunohistochemistry and fast blue(FB) retrograde tracing after FB injections in SN. No neurons containing both FB and SOM were found in the caudate-putamen, amygdala, raphe or caudal midbrain tegmentum. However, doubly labeled cells appeared ventrally in the subthalamic region. Though en passage uptake of FB must be considered, these cells could constitute a source of SOM afferents to the SN.

Heterogenous peptide distribution have also been found in the pallidum and studied in detail in the cat. Rostrally, the globus pallidus (GP) contains dense ENK and GAD and moderate DYN, but SP fibers are present only in its external rim. Caudally, the GP contains SP and SOM fibers (and cells heavily stained for acetylcholoinesterase) in addition to ENK, GAD and DYN. In the entopeduncular nucleus (EN), GAD, DYN and SP fibers are dense throughout, whereas ENK is weaker and mainly confined to a curved lateral part including the rostral and caudal poles. No SOM fibers but a few SOM neurons were also present.

These results demonstrate neurochemical compartments within the EN, GP (rostral and caudal) and SN (the pars lateralis beeing one). This suggests that fiber projections arising from these intranuclear subdivisions are influenced by different neurotransmitters.

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8.10 DIFFERENTIAL PROJECTIONS FROM ACHE-POSITIVE AND ACHE-NEGATIVE VENTRAL-PALLIDUM CELLS IN THE RAT. E.A. Grove,* S.N. Haber, V.B. Domesick and W.J.H. Nauta. Dept. of Psychology and Brain Science, Mass. Inst. of Technology, Cambridge, MA 02139 and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

The term ventral pallidum (VP) originally denoted a dorsal part of the subcommissural forebrain region called substantia innominata, but later was expanded to include many of the large neurons of the olfactory tubercle (Heimer, L. in: Limbic Mechanisms,1978). The identification of this complex region as an extension of the globus pallidus (external segment) is based not only on cytomorphological likeness but also on histochemical similarities with the main body of the globus pallidus (dorsal pallidum, DP), e.g.: 1. high iron content, and 2. the dense plexus of enkephalin-positive striatal efferents pervading VP and DP alike. VP is, however, distinguished from DP by a second, substance P-positive striatal-fiber plexus that densely fills VP but abruptly dwindles at the VP-DP junction (Haber and Nauta, Soc. Neurosci. Abst. 7). Anterograde evidence indicates that VP receives its striatal innervation from a large antero-ventromedial striatal sector that includes the nucleus accumbens and is in turn innervated by several fiber systems originating from within limbic circuitry. Further autoradiographic findings suggest that VP, unlike DP, projects not only to the subthalamic nucleus but also to the nuclei basalis lateralis amygdalae (ABL), medialis dorsalis thalami (MD), and lateralis habenulae (HL) (Haber et al., Soc. Neurosci. Abst. 8).

The interpretation of VP efferents is complicated by the fact that VP contains among its majority of AChe-negative neurons scattered AChe-positive cells that could be non-pallidal elements of the AChe-positive basal magnocellular complex. To determine from which of the two cell types the various VP projections originate, experiments with the combined HRP-AChe procedure of Mesulam were undertaken. Preliminary experiments had shown cell labelling in VP by HRP placed in medial cerebral cortex (CC), ABL, MD, HL, ventral tegmental area (VTA) or dorsomedial midbrain tegmentum. Combination of the TMB procedure with the Jensen-Blackstad stain for AChe revealed that nearly all VP neurons labelled from either CC or ABL were AChe-positive, those labelled from MD, HL or VTA almost all AChe-negative. Findings concerning the apparent VP projection to (or through) the dorsomedial tegmentum thus far have been inconclusive.

These observations indicate that the two categories of VP cells have largely different projection targets. Further studies are in progress to determine whether both categories should be classified as sub-types of pallidal neurons, or as fundamentally different cell types.

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8.11 NON-TOPOGRAPHIC ORDER IN THE BASAL GANGLIA: EVIDENCE FOR A SECOND LEVEL OF ORGANIZATION SUPERIMPOSED UPON THE TOPOGRAPHICALLY ORDERED STRIATO-NIGRAL PROJECTION SYSTEM. C.R. Gerfen, The Salk Institute, La Jolla, CA 92037.

It is commonly believed that the topographic relationships that characterize cortical afferents to the striatum are maintained in the efferent projections of the striatum to the pallidum and substantia nigra. However, Yetterian and Van Hoesen (Brain Res., 139:43-63, 1978) have proposed that a second order organization may be seen in cortico-striatal projections. Thus, in addition to projecting heavily to a restricted topographically related portion of the caudate nucleus, a given cortical area also projects secondarily to regions within the rostral part of the caudate that also receive inputs from cortical areas with which it is reciprocally linked via associational connections. Evidence is presented in the present study to suggest that a similar kind of organization exists in the projection of the striatum onto the substantia nigra in the rat.

Discrete, nonoverlapping injections of the fluorescent retrograde tracers diamidino yellow (DY) and fast blue (FB) were placed into the medial or lateral parts of the pars reticulata of the substantia nigra (SNr) in the same animal. A large number of DY labeled striatal neurons was found in the medial part of the striatum, as is to be expected from the known topography of the striato-nigral projection. However, within the same region there were distinct areas which were devoid of DY labeled neurons but contained instead many FB labeled neurons. Conversely, within the lateral portion of the striatum clusters of DY labeled neurons were interspersed in a dense matrix of FB labeled neurons. Clusters of striatal neurons giving rise to non-topographic projections were distributed across the mediolateral and dorsoventral extent of the striatum such that there were no obvious discontinuities in the distribution of striatal neurons projecting to the SN as a whole. The numbers of striatal neurons projecting to non-topographically related regions of the SN was substantial, though clearly less than those projecting topographically. Interestingly, less than 10% of the striatal neurons that projected non-topographically were labeled with both dyes.

Iontophoretic injections of the anterogradely transported lectin PHA-L into various parts of the caudate-putamen (using the method detailed elsewhere - Soc. Neurosci. Abstr., 8:786, 1982) gave rise to labeled fibers with terminal-like swellings within the SNr that were concentrated in the topographically related zone of the SNr. For example medial striatal injections labeled terminals in the medial SNr. In addition, foci of labeled fibers and terminals were seen in non-topographically related parts of the SNr, and in many cases, areas devoid of terminal labeling separated the primary and secondary terminal zones. These results, indicate that the projection of the striatum upon the SNr is only partly topographically organized, and that there is a second level of organization superimposed upon the topographic striato-nigral system.

8.12 MOTOR RESPONSES TO MICROSTIMULATION OF THE PUTAMEN IN THE AWAKE MONKEY. G.E. Alexander and M.R. DeLong, Johns Hopkins Univ. Sch. of Med., Dept. of Neurology, Baltimore, Maryland 21205.

Microstimulation of the motor cortex has contributed importantly to our understanding of how this region influences the somatic musculature. This technique was therefore applied to the putamen, which receives a strong, topographically organized projection from motor cortex.

Three rhesus monkeys were trained to permit repeated examination of somatic musculature, joints, and skin surfaces. Neuronal activity was sampled at 250-500 um intervals and studied in relation to both the animals' active movements and the responses to passive manipulation of body parts. Once the response properties of each neuron had been characterized, microstimulation was delivered to the recording site through the microelectrode, and the threshold for evoking movements was determined. Currents were restricted to a maximum of 40 uamps (300 usec pulses, 400 pps, 100 msec trains). For comparison, microstimulation was also carried out in the motor cortex and in the capsular portion of the corticospinal tract.

A total of 814 neurons and corresponding microstimulation sites in the putamen were examined in 63 tracks from 4 hemispheres. The functional characterization of the neurons confirmed the somatotopic organization of the putamen described recently. Effective microstimulation sites also were grouped somatotopically with respect to the anatomic distributions of the evoked movements. Discrete movements of the contralateral arm, leg and orofacial structures were observed. Close correspondence between neuronal response properties and microstimulation effects was evident. Thresholds of effective microstimulation in the putamen were as low as 10 uamps, but the majority were in the range 15-25 uamps, while most values obtained in motor cortex were within the range 5-15 uamps. Chronaxie measurements within the putamen and motor cortex were in the range 250-350 usec, compared to a range of 125-225 usec for the capsular corticospinal fibers.

These results indicate that microstimulation of the putamen in the awake monkey produces motor responses comparable to those elicited from the motor cortex. We cannot dismiss the possibility that the responses arise from activation of motor cortex by antidromic conduction along corticostriatal axons. This possibility is diminished, however, not only by the results of the chronaxie measurements, but also by the observation that microstimulation of the corticostriatal fibers at the external border of the putamen did not elicit motor responses. Based on the available evidence it would appear that activation of output neurons of the putamen may result in discrete movements.

9.1 HETEROGENEITY IN SECRETORY RESPONSE TO GnRH WITHIN THE RAT PITUITARY GONADOTROPE POPULATION: QUANTITATIVE ANALYSIS AT THE SINGLE CELL LEVEL. P.F. Smith* and J.D. Neill*. Department of Physiology & Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294 (SPON:E. Geisert)

Heterogeneity in the amount of hormone secreted by pituitary cells of the same class in response to hypothalamic hormones has been reported, but its extent and functional significance remain unexplored. The recent development of a reverse hemolytic plaque assay (Neill and Frawley, Endocrinology 112:1135, 1983) has permitted us to analyze this heterogeneity at a single cell level. In the plaque assay, cells are cultured in the presence of protein A-coupled RBC, hormone antiserum, and complement, producing a zone of hemolysis (a plaque) surrounding individual hormone-secreting cells. In the present study, we have shown that the extent of hemolysis (size of the plaque in μm^2) is linearly related to the amount of hormone (expressed as pg) secreted by a cell.

Pituitary glands from adult female Holtzmann rats killed on the morning of proestrus or diestrus I were mono-dispersed with trypsin. The cells were incubated in Petri dishes for measurement of LH secretion by radioimmunoassay (NIADDK-rLH-RPI) or in Cunningham slide chambers for measurement of the fraction of cells forming plaques and the size of plaques formed. A wide range of secretory responses (>50 fold) was obtained by incubating 10 different batches of cells from the same dispersion in the presence of GnRH (0-100 nM) for varying lengths of time. For each batch, we derived mean plaque size vs. mean amount of hormone secreted per plaque-forming cell, and constructed a standard curve relating μm^2 of plaque area to pg of hormone secreted. Variations in secretory response among gonadotropes within a given treatment group was determined by measuring individual plaque areas and reading each from the standard curve as pg of hormone. Among pituitary gonadotropes derived from proestrus rats exposed to a maximally effective dose of GnRH (100 nM), the mean LH release/cell was 450 pg with a range of 10-2000 pg. Mean LH release by GnRH-treated (100 nM) cells from diestrus rats was 200 pg of LH per plaque-forming cell with a range of 2-2500 pg; however approximately half of the gonadotropes (as detected by PAP-ICC for LH) did not release detectable (<2 pg) amounts of hormone.

These results reveal surprisingly large differences (~1000 fold) in secretory activity among cells of one type from the same organ. Investigations of the morphologic and molecular basis of these differences will be required to understand regulation of the secretory process.

9.2 GASTRIN RELEASING PEPTIDE STIMULATES INSULIN SECRETION AND REDUCES FOOD INTAKE IN THE BABOON. Leslie J. Stein*, Dianne P. Figlewicz*, Daniel Porte, Jr.*, and Stephen C. Woods*. Dept. of Psychology and the Northwest Regional Primate Center, University of Washington, Seattle, WA 98195.

Intravenous (iv) administration of the frog skin-derived peptide bombesin reduces meal size and stimulates insulin secretion in the baboon. Gastrin releasing peptide (GRP), a mammalian peptide, is structurally similar to bombesin and mimics many of bombesin's physiological effects in mammalian systems, including the ability to reduce meal size in rats. In the present series of experiments, we evaluated the ability of GRP to affect food intake and insulin secretion in the baboon.

Four overnight-fasted baboons received a 5-minute iv infusion of either saline or GRP (1-8 µg/kg) and were then allowed to eat for 30 minutes. Blood samples were obtained before the beginning of the infusion (basal, t= -6 min); at the end of the infusion, immmediately prior to the presentation of food (t= 0 min); and at the end of the 30-minute meal (t= +30 min). Mean food intake following saline infusion was 366 ± 116 (SEM) kcal. Infusion of GRP at 1, 2, and 4 µg/kg suppressed 30-minute meal size slightly; food intake following infusion of 8 µg/kg GRP was reduced to 64 ± 27 kcal. Fasting immunoreactive insulin (IRI) concentrations were elevated by all doses of GRP. After the 30-minute meal, IRI was suppressed following 8 µg/kg GRP, presumably as a result of the large reduction of meal size observed after administration of this dose. The data are summarized in the following table.

IRI CONCENTRATIONS (µU/ml) FOLLOWING IV SALINE OR GRP

Time	Saline	1	2	4	8 µg/kg
-6	53±16	56±12	56±22	51±9	64±28
0	57±13	133±25	157±46	140±33	159±58
30	359±36	397±192	564±216	341±161	99±29

Fasting glucose levels were not affected by GRP infusion. After the meal, plasma glucose was suppressed compared to the saline control condition following infusion of 8 µg/kg.

These data indicate that GRP, like bombesin, is a potent stimulant of insulin secretion in the baboon. Additionally, GRP can reduce meal size in this species. It is not yet possible to compare the efficacy of GRP with that of bombesin because the doses of the peptides that have been tested thus far are not equivalent on a molar basis.

9.3 TRH AND EXPERIMENTAL SPINAL INJURY: EFFECTS OF TREATMENT DOSE AND TREATMENT TIME, A. I. Faden and T. P. Jacobs*. Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

We have previously demonstrated that early treatment post-trauma with thyrotropin-releasing hormone (TRH) significantly improves neurological recovery following experimental spinal injury in the cat (N. Eng. J. Med., 305: 1063, 1981). At high doses (2 mg/kg, i.v. bolus: 2 mg/kg/hr x 4 hr), TRH proved superior to treatment with either naloxone or high dose corticosteroids (Neurology, 1983). In the present studies we compared effects of early (beginning 1 hr post-trauma) high-dose TRH treatment against two lower doses of TRH (0.2 mg/kg and 0.02 mg/kg). Each dose was given as i.v. bolus followed by i.v. infusion over 4 hr (total doses: 10 mg/kg, 1 mg/kg and 0.1 mg/kg, respectively). Another group of cats received high dose TRH beginning 24 hr post-injury. Control animals were administered equal volume infusion of physiological saline. Neurological injury was produced by the Allen method in which a 30 gm weight was dropped 20 cm onto a 10 mm^2 impact plate resting on the dura mater. Animals were followed for 6 weeks post-trauma before sacrifice for histopathological examination. Neurological scores were graded by a neurologist unaware of treatment group utilizing a 5-point ordinal scale for forelimb and hindlimb function; summation of forelimb and hindlimb scores yielded a total functional neurological score ranging from 0 to 10. TRH treatment proved statistically superior to that of saline treatment with regard to neurological recovery at all three TRH doses, with the beneficial effects being dose-related. Median neurological scores were: saline = 5.0; TRH (0.02) = 7.0; TRH (0.2) = 8.5; TRH (2.0) = 9.0. Moreover, late treatment with high dose TRH proved nearly as effective as early treatment (median score = 8.0). These findings confirm the beneficial effects of TRH in experimental traumatic spinal injury and show that it has therapeutic effectiveness at 1% the dose previously demonstrated to be efficacious. Moreover, TRH improves neurological recovery even when given 24 hr post-injury. These findings provide further experimental support for human clinical trials and indicate that the classic hypotheses regarding the pathophysiology of spinal cord injury may need to be revised.

9.4 SUBSTANCE K: A NOVEL TACHYKININ IN MAMMALIAN CNS
J. E. Maggio*, J. C. Hunter*, B. E. B. Sandberg*, L. L. Iversen* and M. R. Hanley (SPON: C.-M. Lee). MRC Neurochemical Pharmacology Unit, MRC Centre, Hills Road, Cambridge CB2 2QH, UK.

The tachykinins are a family of naturally-occurring bioactive peptides sharing the carboxyl-terminal sequence -Phe-X-Gly-Leu-Met-NH_2, where X is an aromatic or a branched aliphatic amino acid (Erspamer, V., Trends Neurosci., 4:267, 1981). Until this year, Substance P was the only member of this group found in mammalian tissue; nevertheless, other (amphibian) tachykinins are more potent than Substance P in several peripheral bioassays and central behavioural effects in mammals (Erspamer, V., op. cit.; Sandberg, B. E. B. and L. L. Iversen, J. Med. Chem., 25: 1009, 1982). This could imply the existence in mammals of another endogenous tachykinin peptide with such activities.

Recently we reported the discovery of a novel endogenous mammalian tachykinin (Maggio, J. E., B. E. B. Sandberg, C. V. Bradley, L. L. Iversen, S. Santikarn, D. H. Williams, J. C. Hunter and M. R. Hanley, Irish J. Medical Sci. 152(Suppl. 1):45, 1983; Maggio, J. E. et al in Substance P - Dublin 1983, Boole Press, Dublin, 1983, in press). This new neuropeptide, named "Substance K" to reflect its kinship with both Substance P and the amphibian tachykinin kassinin, was isolated using a radioimmunoassay which crossreacts negligibly with Substance P. The biological activities of Substance K are noticeably different from those of Substance P, and it seems possible that Substance K is an endogenous ligand for mammalian receptors of the "SP-E" type (Lee, C.-M., L. L. Iversen, M. R. Hanley and B. E. B. Sandberg, Naunyn-Schmiedeberg's Arch. Pharmacol., 318: 281, 1982).

Some Substance P research should be re-evaluated in light of the fact that Substance P is not the only tachykinin neuropeptide in mammals.

9.5 SUBSTANCE P INTRATHECALLY AT SPINAL T_9 LEVEL INCREASES ADRENAL MEDULLARY OUTPUT OF FREE ADRENALINE AND NORADRENALINE IN THE RAT. Kiran Yashpal*, S.G. Gauthier and J.L. Henry, Depts. of Neurology and Neurosurgery, and of Psychiatry, McGill Univ., Montreal, Que.

Injection of neuroanatomical tracers into the adrenal medulla of the rat has demonstrated labelled cells in the intermediolateral nucleus (ILN) of segments T_7-T_{12}, with T_9 containing the largest number, and substance P-immunoreactive fibres can be observed surrounding both labelled and unlabelled neurones in the ILN. Substance P is found in cell bodies of brain stem structures projecting directly to sympathetic preganglionic neurones in the ILN of the thoracolumbar spinal cord and it may therefore be involved in supraspinal control of sympathetic output to the adrenals. In an attempt to identify the physiological role of substance P in spinal pathways controlling adrenal medullary output, the present experiments were done to determine the effects of the intrathecal administration of substance P on the release of catecholamines from the adrenal medullae.

Male Sprague-Dawley rats (approx. 500 g) were anaesthetized with urethane (800 μg) and implanted acutely with an intrathecal PE-10 catheter which was passed via the atlanto-occipital junction in the subarachnoid space to the T_9 level. A PE-60 catheter was inserted via the femoral vein so that the tip laid between the renal veins and the heart. After a stabilization period of 30 min, 1.5 ml of blood was withdrawn and replaced with warm (37°C) saline. After 5 min, substance P (10 μg in 10 μl of artificial cerebrospinal fluid) was injected intrathecally followed by 10 μl CSF to flush the catheter, and blood samples of 1.5 ml were taken at 1, 10 and 30 min later, each sample being replaced by an equal volume of saline. Plasma levels of free adrenaline and noradrenaline were measured by the method of Hallman (1978).

Substance P produced an increase in plasma concentrations of both catecholamines. Adrenaline levels increased by an average of 145.5% above baseline (n=6), and noradrenaline levels increased by an average of 181.5% (n=6). The effects were greatest at 1 min after administration and were almost completely reversed at 30 min. When vehicle solution alone replaced the substance P solution the rapid increase in catecholamine levels was not seen. In five additional cases, the analogue (D-Pro^2-D-Phe^7-D-Trp^9)-substance P (10 μg in 10 μl) was given intrathecally 15 min before substance P and the increases in catecholamine levels were effectively blocked.

These results demonstrate that intrathecal administration of substance P increases adrenal output of adrenaline and noradrenaline and suggests that this peptide may be an excitatory chemical mediator of synaptic transmission in brain stem-spinal pathways controlling adrenal medullary output.

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9.6 ELECTROPHYSIOLOGIC STUDIES OF NOCICEPTIVE CHOLECYSTOKININ-CONTAINING NEURONS IN THE MIDBRAIN CENTRAL GRAY. R.B. Innis and G.K. Aghajanian. Depts. Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Cholecystokinin (CCK) is a neuropeptide which has fulfilled almost all criteria for a neurotransmitter. The purpose of this study was to characterize the electrophysiologic properties of the anatomically well defined, densely packed neuronal cluster located in the midline periaqueductal gray of rat anterior midbrain (see Innis, et al., PNAS, 76:521, 1979). We recorded extracellularly in the chloral hydrate anesthetized rat.

A distinctive firing pattern was found in the region of this CCK neuronal cluster. The spike was typically biphasic with an initial positive deflection followed by a smaller negative deflection. The spontaneous firing rate was erratic. The cells sometimes fired in bursts and at other times fairly regularly (1-7 Hz) for up to an hour. Noxious stimuli such as toe pinch caused an increase in firing rate and a decrease in spike amplitude and duration. Continued painful stimuli led to a cessation of firing due to depolarization blockade.

Several lines of evidence indicate that these cells are CCK containing neurons. First, dye spots deposited at the end of a recording session have always been histologically located in the narrow region of the CCK neuronal group. Second, co-localization experiments have shown the electrode tip (marked by a small area of tissue damage) surrounded by a dense cluster of immunohistochemically stained CCK containing neurons.

Recent reports show that CCK blocks the analgesic effect of opiates. Thus, the increased firing rate induced by toe pinch is consistent with this anti-analgesic action. Furthermore, we found that intravenously administered morphine (0.1 mg/kg) potently inhibits the spontaneous and pain stimulated firing of these cells. This morphine induced inhibitioin can itself be reversed with the specific opiate receptor antagonist, naloxone. Preliminary exeriments with microiontophoretically applied morphine and naloxone suggest that each has the same effect as the systemically administered drug. Since these locally applied opiate agents are active, we examined the area carefully for endogenous opiate peptides. Immunohistochemical staining with antiserum to met-enkephalin shows a moderately dense distribution of apparent terminal fibers in the neuropil of this CCK neuronal cluster.

In summary, we have successfully recorded from CCK-containing neurons in the midbrain central gray. These neurons may be involved in pain perception and opiate analgesia.

9.7 LONG TERM ENHANCEMENT OF EXCITATORY AMINO ACID TRANSMISSION BY ARGININE^8-VASOPRESSIN (AVP): A FUNCTION OF AVP IN LATERAL SEPTUM OF RATS? I.J.A.Urban and M.Joels. Spons: European Neuroscience Association. Rudolf Magnus Institute for Pharmacology. Medical School, University of Utrecht, The Netherlands.

The lateral septum complex (LSC) of rats is rich in immunoreactive arginine^8-vasopressin (AVP). A great deal of neurons in dorsal part of the LSC are the neurons which are strongly orthodromically excited (SOE) by stimuli administered to fimbria-fornix (fi-fx). According to evidence obtained with selective agonists and antagonists, presumably glutamate or other closely related amino acid is the neurotransmitter mediating the synaptic excitations elicited in LSC neurons by fi-fx stimuli. AVP ejected microiontophoretically on SOE setal cells with currents at which it did not alter the spontaneous activity of these neurons, markedly increased the excitations elicited in SOE neurons with Glu, aspartate but also with quiscalate and N-methyl-D-aspartate, selective agonists for two kinds of excitatory amino acid receptors. In nearly 50% of these neurons, the AVP-elicited increase in responses to amino acids lasted for more than 10 min after termination of the AVP application. The inhibitory responses induced in SOE cells with GABA were not affected during the iontophoretic administration of AVP. In many SOE cells in which AVP enhanced the amino acid response, the number of times that they fired an action potential to a fixed series of fi-fx stimuli markedly increased during and after iontophoretical administration of AVP. This AVP-elicited increase in responsiveness of SOE cells to the trans-synaptic stimuli frequently persisted for more than 10 min after termination of the AVP administration. The synchronized excitation of the SOE septal neurons by fi-fx stimuli elicit a negative field potential (NFP)in LSC. Following topical administration of 10 μl 10^{-10} M AVP dissolved in artificial cerebrospinal fluid (ACF), amplitude of the NFP increased and remained increased after wash-out of the preparation with the control ACF. In more than 50% of the experiments the peptide-induced increase in magnitude of NFP lasted for more than 120 min after the wash-out of the peptide. Des-glycinamide AVP, and [pGlu^4,Cys^6]4-9 AVP, behaviorally acting fragments of AVP, also increased NFP in LSC. However, 10^{-7} M concentrations of the peptides were needed to obtain the effect. These experiments demonstrate for the first time that in LSC, one of the extra hypothalamic target structure for AVP, the exogenous peptide hormone can elicit a long-term enhancement of the excitatory amino acid neurotransmission and suggest that this presumably postsynaptic action of AVP may be one of the functions of this peptide in LSC. In addition, evidence accumulated so far suggests that for a full expression of these actions presumably the whole molecule of AVP is needed.

9.8 BOMBESIN DIRECTLY STIMULATES INSULIN SECRETION FROM A CLONAL LINE OF HAMSTER BETA CELLS. S. Swope* and A. Schonbrunn* (SPON: L. Levine). Dept. of Pharm., Harvard Med. School and Lab. of Toxicol., Harvard Sch. of Pub. Health, Boston, MA 02115.

Bombesin (BBS), a tetradecapeptide originally isolated from frog skin, stimulates insulin secretion both in vivo and in organ cultures. Furthermore, pancreatic neurones contain bombesin-like immunoreactivity. To determine whether BBS acts directly on pancreatic Beta cells, we have characterized its effects on insulin secretion by HIT cells, a clonal line derived by SV40 transformation of hamster islets (Santerre et al., 1981, PNAS, 78:4339). The addition of 100 nM BBS to HIT cells stimulated insulin release 20 fold by 30 seconds. The secretory rate returned to basal levels within 90 minutes. Treatment of HIT cells with 100 nM BBS elicited the secretion of only 1.04 ± .07 % (n = 2) of the total intracellular insulin during a one hour incubation, indicating that intracellular insulin stores were not depleted. The effect of BBS was dose-dependent with an ED_{50} of 0.54 ± .10 nM (n = 4). Structurally unrelated neuropeptides including secretin, VIP, TRH, substance P, neurotensin, CCK-octapeptide, and CRF did not alter insulin release, nor did they modulate the stimulatory effect of BBS. In contrast, maximal concentrations (100 nM) of 5 BBS analogs tested, including Gastrin Releasing Peptide, a mammalian BBS-like peptide, were as powerful as BBS in stimulating insulin secretion. The relative potencies of these analogs indicated that the C-terminal octapeptide sequence in BBS is sufficient for potent stimulation of insulin secretion. Maximal doses of glucagon (1 μM) stimulated insulin secretion 5.0 ± .8 fold (n = 3) in one hour and the effect of glucagon plus BBS was greater than the additive effect of the 2 peptides alone. Somatostatin, a recognized inhibitor of insulin secretion both in vitro and in vivo, blocked BBS stimulated release by 72 ± 2% (n = 3) with an ID_{50} of 2.6 nM. In conclusion, these data show that physiological concentrations of BBS rapidly and directly stimulate insulin release by a clonal line of hamster Beta cells. This system promises to be useful for investigating the mechanism by which Bombesin elicits insulin secretion.

9.9 INTRACELLULAR STIMULATION EVOKES PEPTIDE RELEASE FROM A SINGLE, IDENTIFIABLE PEPTIDERGIC NEURON IN AN INSECT. Nathan Tublitz and James W. Truman. Department of Zoology, University of Washington, Seattle, WA 98195.

We have been investigating two cardioacceleratory peptides (CAPs) isolated from the ventral nerve cord(VNC) of the tobacco hawkmoth, Manduca sexta. The two CAPs are co-released into the haemolymph during adult wing-spreading behavior, causing an increase in heart rate that facilitates wing inflation. We report on the identification of the CAP-containing neurons utilizing a variety of methods, most directly by evoking release of CAP-like bioactivity via intracellular stimulation of a single, identified neurosecretory cell.

The two CAPs are stored and released from the perivisceral organs, the classic VNC neurohaemal release site in insects, located on the segmentally arranged transverse nerve(TN). Previous work has established that each TN contains axons from two, morphologically distinct group of cells: four lateral cells with a single axon projecting to the ipsilateral TN, and five pairs of midline neurons, each with a bifurcating axon that terminates in both ipsilateral and contralateral branches of the TN. Based on microsurgical and electrophysiological experiments, the lateral cells, previously shown to contain another peptide hormone, bursicon(Taghert, P.H., J. Exptl. Biol., 98:305), do not contain the CAPs.

Several lines of evidence suggest that the CAP-containing neurons are restricted to three pairs of midline cells that differentiate post-embryonically during adult development. Neither CAP can be detected in the larval ventral nerve cord prior to the differentiation of these cells. Also, CAP accumulation in the ventral nerve cord closely parallels midline cell enlargement during adult development. Finally, intracellular stimulation of a single, identifiable midline neuron results in the release of detectable levels of protease-sensitive, CAP-like bioactivity. These data suggest that three pair of midline cells, which arise during adult development, contain and release the CAPs.

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9.10 A SUBSTANCE P-LIKE PEPTIDE MODULATES SENSORY AND CARDIAC ACTIVITY IN LIMULUS. Jorge R. Mancillas and A.I. Selverston. Depts. of Neurosciences and Biology, Univ. of Calif. at San Diego, La Jolla, CA 92093.

The central nervous system of the horseshoe crab (Limulus p.) contains substance P-like immunoreactive cells arranged in pairs of bilaterally-symmetrical clusters distributed regularly throughout all ganglia posterior to the protocerebrum. Their distribution was studied using indirect immunocytochemical techniques and a monoclonal antibody. Six bilaterally-symmetrical pairs of clusters are located in the circumesophageal connectives (CEC's) and the subesophageal mass (SEM), were some individual scattered cells can also be seen. Each of the first 5 abdominal ganglia contains an anterio-lateral, a medial longitudinal and a medial posterior pair of cell clusters. Substance P-like immunoreactive fibers leave through virtually all the ventral and dorsal nerves to innervate peripheral targets. An abundance of stained terminals can be seen in the CEC, SEM and abdominal ganglia.

Two of the substance P-like immunoreactive pairs of clusters in the CEC's seem to give rise to efferent fiber projections that leave through the 3rd and 4th dorsal nerves, enter the lateral optic nerve halfway between the brain and the lateral eye, and innervate several components of the ommatidia.

Synthetic substance P (10^{-9} to 10^{-7}m) injected subcorneally into the lateral eyes induces increases in the photoreceptors' sensitivity that are reversible, dose-dependent and mimic those that occur spontaneously in a circadian fashion (Barlow, J. Neurosci. 3:856). Substance P also alters the endogenous circadian rhythm in photosensitivity by increasing the amplitude of the nocturnal plateau, while injection of D-pro^2, D-phe^7, D-Trp^9 substance P in the afternoon retards the rise in sensitivity and reduces the nighttime levels. If the antagonist is applied after the nocturnal plateau has been reached, a short-term, reversible drop to diurnal levels is observed. Our data is consistent with an involvement of a substance P-like peptide in efferent-controlled circadian rhythms of photosensitivity. The 6 clusters in the CEC's and SEM may constitute a generalized, level-setting system with multiple targets, which can be driven by a circadian clock as well as by other systems responsible for integrated organismic responses.

A substance P-like peptide may have additional functions in Limulus, since: 1) Substance P-li fibers innervate arterial walls, where they may be involved in the regulation of blood flow, and 2) Substance P-li fibers, that may originate in the anterio-lateral clusters of the abdominal ganglia, innervate the heart, which is sensitive to the application of synthetic substance P.

9.11 ACUTE REGULATION OF GANGLIONIC TYROSINE HYDROXYLASE ACTIVITY BY SECRETIN, VASOACTIVE INTESTINAL PEPTIDE AND PHI. N.Y. Ip and R.E. Zigmond. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

We have previously shown that stimulation of the preganglionic cervical sympathetic trunk leads to an acute increase in tyrosine hydroxylase (TH) activity in the rat superior cervical ganglion (SCG). This increase in enzyme activity is mediated in part by a nicotinic and in part by a non-cholinergic mechanism (Ip et al., Proc. Natl. Acad. Sci. 80:2081, 1983). As a first step in an attempt to identify the transmitter responsible for this non-cholinergic effect, we screened thirteen neuropeptides at a concentration of 10 μM for their ability to increase TH activity. Of these, secretin and VIP were effective, while angiotensin II, bombesin, bradykinin, cholecystokinin octapeptide, insulin, luteinizing hormone-releasing hormone, [D-Ala^2, Met^5] enkephalinamide, motilin, neurotensin, somatostatin and substance P produced no effects.

Secretin produced a significant increase in TH activity at 1 nM and a maximal elevation (3-fold) at 0.1 μM. VIP produced a significant increase at 0.1 μM and a near maximal effect (4-fold) at 10 μM. The effects of these peptides were not altered by prior decentralization of the ganglia, by addition of hexamethonium (3 mM) and atropine (6 μM), or by lowering the concentration of calcium in the medium to 0.1 mM.

Several peptides with structural similarities to secretin and VIP were also examined for their ability to increase TH activity. Glucagon, gastric inhibitory peptide and human pancreatic tumor growth hormone-releasing factor produced no effect at a concentration of 10 μM, while PHI increased TH activity. A significant increase was obtained at 1 μM, and a near maximal effect (3-fold) was produced at 30 μM PHI.

Carbachol, a cholinergic agonist, also increases TH activity acutely (Ip et al., J. Pharmacol. Exp. Ther. 223:280, 1982). To investigate whether there is any interaction between the cholinergic and peptidergic regulation of TH, we examined the ability of secretin to increase enzyme activity in the presence of a low concentration of carbachol (3 μM). At this concentration, carbachol produced no significant effect on TH activity by itself; however, it potentiated the response to secretin at all doses of secretin tested (1 nM to 1 μM). The effect of VIP, examined only at 1 μM, was also enhanced by the addition of carbachol.

These data indicate that ganglionic tyrosine hydroxylase activity can be regulated by secretin and the closely related peptides, VIP and PHI. We are currently examining whether any of these peptides mediate the non-cholinergic increase in TH activity following nerve stimulation. (USPHS Grants NS12651 and MH00162)

9.12 EVIDENCE FOR THE INVOLVEMENT OF CYCLIC AMP IN THE ACUTE STIMULATION OF GANGLIONIC TYROSINE HYDROXYLASE ACTIVITY PRODUCED BY CERTAIN NEUROPEPTIDES. R.E. Zigmond, C. Baldwin* and N.Y. Ip. Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115.

Tyrosine hydroxylase (TH) activity can be increased acutely in adrenergic neurons by a variety of stimuli. One of the mechanisms which may account for these increases in enzyme activity is the stimulation of a cyclic AMP dependent protein kinase resulting in the phosphorylation and the consequent activation of TH. We have previously reported that 8-bromo-cyclic AMP can increase TH activity in adult rat superior cervical ganglion (Ip et al., Proc. Natl. Acad. Sci. USA 80: 2081, 1983). TH activity can also be increased by three neuropeptides--secretin, vasoactive intestinal peptide (VIP), and PHI. We have now further examined the involvement of cyclic AMP in regulating ganglionic TH activity and have investigated the possible role of this second messenger in mediating the effects of these neuropeptides.

8-Bromo-cyclic AMP produced a dose-dependent increase in TH activity in intact ganglia in vitro. A significant effect was found at 1 mM and a near maximal effect (4-fold) was found at 30 mM. Forskolin, a compound which stimulates adenylate cyclase activity, also increased TH activity. A significant increase was detected at 1 μM and a maximal effect (2.4-fold) was found at 30 μM. Isobutyl methylxanthine (0.5 mM), a phosphodiesterase inhibitor, increased TH activity by about 2-fold.

Volle and Patterson (J. Neurochem. 39:1195, 1982) recently reported that VIP can increase cyclic AMP levels in the rat superior cervical ganglion. We have investigated the ability of a number of peptides to alter cAMP levels in this tissue. The effect of each peptide was examined at a concentration of 10 μM during a 10 min incubation, and cyclic AMP in the tissue and medium was measured by a protein binding assay. VIP, secretin and PHI increased cAMP levels while glucagon, human pancreatic tumor growth hormone-releasing hormone, substance P, angiotensin II, bradykinin and neurotensin produced no significant effects. The latter group of peptides had no effect on TH activity.

The data indicate that ganglionic TH can be regulated by intracellular cyclic AMP levels and raise the possibility that secretin, VIP and PHI increase TH activity by elevating cyclic AMP levels in this ganglion.

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10.1 Monoclonal antibodies against sodium channel.

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The voltage dependent Na^+ channel plays an important role in the conduction of electrical impulses in various neurones.

In order to investigate the location, dynamic properties and chemical nature of Na^+ channel we have generated monoclonal antibodies against it.

Mice were immunized with electroplax eel membrane fragments which are rich with sodium channels. The spleens of the immunized mice were fused with NS 1 myeloma cells according to published procedures. The resulting hybridoma cells were cloned and screened for the properties of their secreted antibodies.

Several hybridomas secreting antibodies with binding properties associated with the sodium channel were selected and subsequently grown for further characterization.

One clone denoted SC-72-14 binds membrane from eel electroplax and from rat brain synaptosomes which are enriched with sodium channel. The binding of these antibodies is inhibited by Veratridine doubled by scorpion toxin but not effected by Tetrodotoxin. Moreover, these antibodies stain specifically nodes of Ranvier of single fibers in rat sciatic nerve when examined with Rhodamine labeled goat antimouse. Finally the antibodies block the action potential recorded from rat sciatic nerve and optic nerve. Preliminary voltage clamp studies imply that the antibodies block exclusively the inward sodium current of single axons without effecting potassium or leakage current or membrane potential.

The second clone denoted SC-72-23 binds to the same enriched preparation of the sodium channel and its binding is blocked by either Tetrodotoxin, Veratridine or Scorpion toxin. These antibodies stain nodes of Ranvier of rat sciatic nerve and block the action potential of rat optic and sciatic nerve.

The binding capacity of the third clone SC-79-17 is significantly decreased upon the addition of either Tetrodotoxin and veratridine to these samples. It also stains the nodes of Ranvier in rat sciatic nerve. It enlarged and prolonged the action potential of rat optic nerve.

These clones and new ones are further analysed. However, it is already clear that we have generated a repertoire of antibodies against different parts of the sodium channel. These reagents will be used to visualize the distribution and dynamic properties of the channel molecules on viable cells using the methods of image intensification microscopy and fluorescence photobleaching

10.2 MOLECULAR MORPHOLOGY OF THE TETRODOTOXIN BINDING SODIUM CHANNEL PROTEIN FROM ELECTROPHORUS ELECTRICUS IN SOLUBILIZED AND RECONSTITUTED VESICLE PREPARATIONS. M.H. Ellisman, J.A. Miller*, W.S. Agnew*, T.J. Deerinck*, D.B. Leong*, R.L. Rosenberg*, S.A. Tomiko*. Department of Neurosciences, Laboratory for Neurocytology, UCSD, La Jolla, CA 92093 and Department of Physiology, Yale University, New Haven, CT 06510.

Tetrodotoxin (TTX) has been used as a biochemical marker for following the purification of Na^+ channel protein solubilized from eel electric organ in Lubrol-PX and sodium cholate. The purified macromolecules were examined by electron microscopy both after reconstitution into liposomes, and solubilized form in the two detergents. Vesicles were visualized either by negative staining or by the replication techniques of freeze-fracture and freeze-etching. In the fractured membranes derived from either reconstitution system, incorporated proteins appeared as ~100 Å diameter particles. Exposure of the vesicle true surfaces by freeze-etching and replication by rotary-shadowing revealed particulate protrusions when examined in stereo-electron micrographs. Thus, the freeze-fracture analysis reveals a relatively large and symmetrical intramembranous particle projecting slightly beyond the bilayer. These structures, which we estimate to be roughly cylindrical of ~100 Å diameter x 80 Å length (min.) were difficult to correlate with the distinctive and regular rod-shaped (40 Å x 170 Å) particles delineated by negative staining of purified Lubrol-PX solubilized protein (Ellisman et al., PNAS 79, 1982). In an effort to reconcile this discrepancy we examined material solubilized in both cholate and Lubrol using a battery of negative staining techniques at various pH's. Examination of the cholate solubilized protein at pH 5 with uranyl acetate revealed numerous "doughnut-shaped" structures. No rods of the sort seen earlier in the Lubrol preparations stained at lower pH were seen. Rather, the irregularly sized doughnuts appeared similar to solubilized acetylcholine receptor when it is spread on carbon-filmed grids. Lower pH staining of the cholate preparations resulted in aggregation of material into grape-like clusters with doughnut structures still apparent. After longer exposure to lowered pH staining, rod-like structures were apparent in the same aggregates but fewer doughnut-like structures were seen. The rod-like structures in the cholate preparation were less uniform in width and length than those we observe in Lubrol. The highly ordered ribbon-like arrays seen in the Lubrol solubilized protein were not encountered in the cholate solubilized preparations. (Supported by NIH grants NS17928 to W.S.A. and NS14718 to M.H.E.; NMSS grants to W.S.A. and M.H.E.; MDA grant to M.H.E.; MDA Fellowship to J.A.M.)

10.3 THE NATURE OF THE PEPTIDE WHICH FORMS THE TETRODOTOXIN-BINDING SITE OF THE SODIUM CHANNEL FROM ELECTROPHORUS ELECTRICUS. J.A. Miller* and W.S. Agnew* (SPON: W.C. Wallace). Department of Physiology, Yale University School of Medicine, New Haven, CT 06510.

Tetrodotoxin (TTX) has been used as a biochemical marker for following the solubilization and purification of the voltage-regulated sodium channel protein from the electric organ of Electrophorus electricus. The native protein has been purified in the non-ionic detergent Lubrol-PX and in the bile salt detergent sodium cholate. In the latter detergent the extracted TTX binding site is intrinsically less stable than in comparable extracts in non-ionic detergents; however, by carefully maintaining low temperatures throughout, by providing adquate ratios of stabilizing phospholipid and by saturating the binding site with TTX, it is possible to isolate an active protein with a high degree of purity. The protein isolated contains only a large ($\geq$260,000 daltons) peptide; no smaller peptides, either covalently or non-covalently linked, have been detected. This large peptide displays a variable degree of glycosylation as evidenced by marked electrophoretic microheterogeneity. Enzymatic digestion with endoglycosidases results in a limit digest which electrophoreses as a single well-defined band of 30% lower apparent molecular weight. This observation is consistent with an earlier analysis which determined that the peptide was 29% by weight carbohydrate (Miller, Agnew and Levinson, Biochemistry 22:462, 1983). Precise determinations of the true molecular weight of either the complete molecule or the limit digest are not possible by sodium dodecyl sulfate (SDS) gel electrophoresis, as direct measurements reported here reveal binding of atypically high amounts of SDS.

Aside from producing a greater yield of product of high purity, the use of cholate as the solubilizing agent for the protein permits a choice of methods for reconstitution into vesicles. This has allowed us to reconstitute the molecule into well-defined, unilamellar vesicles. These vesicles are suitable for future analysis of the functional properties of this purified protein.

10.4 EFFECTS OF BATRACHOTOXIN, VERATRIDINE AND SCORPION TOXIN ON Na CHANNELS IN LIPID BILAYERS. B. Rudy* (SPON: J. Jacoby). Dept. Physiol., New York Univ. Med. Ctr., New York, NY 10016.

Sodium channels were incorporated into black lipid membranes (BLM) from whole brain membrane vesicles placed in one of the solutions bathing the membrane (CIS side). The membrane vesicles were obtained from a sucrose gradient fraction of rat brain homogenates enriched in Saxitoxin (^{3}H-STX) binding, i.e. 8-10 pmols/mg protein. The protein is incorporated into the BLM probably by fusion of the membrane vesicles with the bilayer. Single Na channels were recorded in this membrane in the presence of batrachotoxin (BTX), or Veratridine, with or without scorpion toxin. In the presence of BTX, the channels are similar to those described by Krueger, Worley & French (Biophys. J. 41: 142a, 1983) in PE:PS membranes except that the single channel conductance is slightly smaller (20 pS vs. 30 pS). This is probably the result of differences in membrane composition; negatively charged membranes will result in higher local Na concentrations. The channels are inserted always with the TTX binding site exposed to the CIS side. In the presence of veratridine the single channel conductance seems to be half that with BTX. The average open time for the channel is also smaller and its kinetics is slower than with BTX. These results give an insight of why veratridine is much less effective than BTX in increasing the permeability of Na channel containing cells or reconstituted vesicles. Addition of scorpion toxin to the solution induces a novel voltage and time dependent response. A much faster opening and closing of the channels appears minutes after the addition of the toxin but only when the membrane potential is positive on the CIS side. If the membrane potential is changed from +50 to -50 mV, the fast gating subsides after a short time to that observed in the absence of scorpion toxin. Returning the membrane potential to +50 mV allows the fast gating to reappear after a short delay. The time course of the appearance and vanishment of the gating induced by scorpion toxin are of the order of tens of seconds depending on the membrane potential. The sign and the time course of the voltage dependent effect suggest that it is related to the binding and unbinding of scorpion toxin to specific binding sites (Catterall, J. Biol. Chem. 252: 8660, 1977). Because the fast gating may reflect the opening and closing of the inactivation gate, the inactivation gate becomes accessible to study at the single channel level in membranes where molecular studies are possible. Supported by NIH Grant GM26976 and BSRG SO7RR05-399-21.

10.5 MODULATION OF PHARMACOLOGICALLY INDUCED DEPOLARIZATION IN FROG NERVE. T.A. Rando*, G.K. Wang* and G.R. Strichartz. Anesthesia Res. Labs., Brigham and Women's Hosp. and Harvard Medical School, Boston, MA 02132

The depolarizing actions of the sodium channel activators veratridine (VTD) and batrachotoxin (BTX) were measured in frog sciatic nerve by the sucrose-gap method. DC potentials were recorded in Ringer with 12mM TEA at pH 7.2, 20-22°C. VTD produced a rapid depolarization which was readily reversible upon washing with Ringer. The BTX-induced depolarization had a much slower onset and was irreversible. The extent of the depolarization was concentration-dependent for both drugs with a maximal depolarization being obtained at about 50 μM VTD or 5 μM BTX.

To test whether the actions of these activators were affected by the state of the sodium channel, their effects were studied in nerves treated with Leiurus scorpion toxin (LQ) or chloramine-T (CT). Both reagents inhibit Na^+ channel inactivation. LQ increased the rate and extent of depolarization by both activators. In contrast, CT had mixed effects. While CT did not increase the extent of depolarization by either activator, it did greatly increase the rate of BTX-induced depolarization. Little effect was seen on the rate of onset by VTD, but the reversal rate on washing was markedly decreased. A similar effect was seen when the nerve was stimulated at 1-10 Hz during activator treatment; the onset rates were increased, the extent of depolarization was unchanged, and the washout rate of VTD was decreased.

The interactions between local anesthetics and Na^+ channel activators were studied using lidocaine (LID). LID was a competitive inhibitor of VTD, reducing the potency of VTD in depolarizing the nerve. LID had no effect on the extent of the depolarization when present during BTX treatment or when applied to a nerve already depolarized by BTX. However, the presence of LID greatly slowed the rate of BTX-induced depolarization. We compared the potency of another local anesthetic, RAC 109I, to its stereoisomer, RAC 109II, in their ability to repolarize a nerve depolarized by VTD; RAC 109I was about 3 times as potent as RAC 109II (which was comparable to their relative potencies in reducing the compound action potential amplitude in this system).

These results are consistent with a scheme wherein activators react most rapidly with "open" channels to stabilize that channel configuration. Thus, treatment that increase the probability of the open channel configuration (e.g. LQ, CT, stimulation) speed activator action, whereas local anesthetics may inhibit activator action by decreasing the fraction of channels in the open configuration and/or (as previously suggested by Huang et al., Biophys. J., 23:219, 1978) by competing directly with activators at a common binding site. The prevention of inactivation per se does not potentiate activator-induced depolarization.

10.6 PHARMACOLOGIC EVIDENCE FOR SALTATORY CONDUCTION ALONG THE GOLDFISH MAUTHNER AXON. Paul G. Funch and Donald S. Faber. Div. Neurobiology; Dept. Physiology; SUNY at Buffalo; Buffalo, NY 14214.

The Mauthner (M-) axon of the goldfish apparently lacks the typical node of Ranvier structures which are found along other vertebrate myelinated axons. Nevertheless, active sites spaced about 2.4 mm apart were discovered using electrophysiological techniques (Funch & Faber, J. Neurophysiol., 47:1214-1231, 1982), and our morphological studies of the oligodendrocytic sheath (Wood, Funch & Faber, Soc. Neurosci. Abstr., 9, 1983) have demonstrated that single sheath segments span the internodal region between the active sites. The experiments described here were designed to test the hypothesis that the active sites are the only regions of the M-axon which produce the inward sodium current necessary for action potential propagation.

Localized extracellular pressure ejections of tetrodotoxin (TTX; 100 μg/ml) from a microelectrode were made immediately adjacent to the M-axon sheath; this position was determined electrophysiologically on the basis of the voltage produced across the myelin sheath by the M-axon action potential. The size and spread of the TTX ejection was monitored visually by using Fast Green in the TTX solution as a marker. The duration of the ejection was limited so that the initial droplet of TTX was roughly 100 μm in diameter. The TTX ejections were effective in rapidly blocking components of the M-axon's action potential only when they were made at the nodal regions (2.4 mm, 4.9 mm, and 7.0 mm caudal to the axon hillock-initial segment (AH-IS)). Beginning at the first active site caudal to the AH-IS, successive active sites were blocked with local TTX ejections and the effects on amplitude, maximal rate of rise (dV/dt), and spike duration (time from maximal dV/dt to minimal dV/dt) of the antidromic and orthodromic action potentials were assessed. The all-or-none changes in these parameters were consistent with the active site spacing, the space constant of the M-axon (5 mm), a safety factor of 6-7, and asymmetrical coupling of the M-axon to the soma-dendritic region (Funch & Faber, vide supra).

Thus, although the M-axon apparently lacks morphologically distinguishable nodes of Ranvier, it has clearly defined and functionally distinct nodes and internodes which support the saltatory conduction of impulses. (Supported by NIH Grant NS 17063.)

10.7 EVIDENCE FOR INCREASED G_{Na} IN THE SYNAPTIC REGION OF RAT SKELETAL MUSCLE. S.J. Kinnamon*, J.H. Caldwell, & W.J. Betz. Dept. of Physiology, Univ. of Colorado Med. School, & Dept. of Molec. & Cell. Biology, Nat'l. Jewish Hosp., Denver, CO.

Action potentials recorded near the synapse in skeletal muscle have a higher maximum rate of rise ($\dot{V}_{max}$) than those recorded in extrajunctional regions (Nastuk & Alexander, Fed. Proc. 32:333, 1973; Thesleff et al. Acta. Physiol. Scand. 91:196, 1974). Based partly on this observation, Thesleff et al. suggested that voltage gated Na channels are relatively concentrated at the end plate region. However, the results could alternatively be interpreted as reflecting nonuniform passive cable properties of the membrane. In fact, recent studies in our laboratory suggest that Cl conductance (G_{Cl}) is reduced in the end plate region, which alone could increase action potential $\dot{V}_{max}$ at that site.

We therefore repeated measurements of V_{max} in normal saline, and compared them to measurements in low [Cl] solution (Cl replaced by isethionate; all experiments on rat lumbrical muscle). In normal saline, $\dot{V}_{max}$ was 43% greater near the end plate than in extrajunctional regions. In low [Cl] solution, $\dot{V}_{max}$ increased at both sites, and the difference was reduced to 28%. Thus, a reduced G_{Cl} at the end plate can account for some, but not all of the difference in normal saline.

In addition, we used a vibrating, extracellular microelectrode (Jaffe & Nuccitelli, J. Cell. Biol. 63: 614, 1974) to record a steady membrane current generated by bath application of veratridine (plus alpha bungarotoxin). This treatment caused a large inward current at the synaptic region, which was abolished by tetrodotoxin. The inward current persisted in Cl-free solutions, consistent with the suggestion that Na channels are more concentrated at the end plate region. Finally, we measured the length of fiber over which an increased $\dot{V}_{max}$ could be recorded; the results suggest that the increase is restricted to a region within 150-200 um of the end plate.

10.8 Ca-ACTIVATED K CHANNELS IN RAT BRAIN SYNAPTOSOMES. D.K. Bartschat* and M.P. Blaustein. Dept. Physiol., Univ. Maryland Sch. Med., Baltimore, MD 21201

Modulation of potassium channel function has been implicated in the regulation of neurotransmitter release from nerve terminals in vivo, but the small size of mammalian presynaptic nerve terminals has precluded use of electrophysiological techniques to assess K channel activity. Accordingly, we used ^{86}Rb efflux (cf. Neurosci.Abstr.,6:572, 1980) as a probe for K permeability, P_K (Hille, J.Gen.Phys.,61:669, 1973) in rat forebrain synaptosomes (cf. J.Membr.Biol.,50:287, 1979). After loading with ^{86}Rb at 30°C for 30 min. in physiological salt solution (PSS) containing 5K and 145Na, 50 μl aliquots were placed on pre-washed glass fiber filters (Schleicher and Schuell #25), and extrasynaptosomal ^{86}Rb was removed with five 2 ml washes of PSS. Efflux was initiated with 2 ml of "reaction medium", frequently containing elevated K, decreased Na and/or drugs. Efflux was terminated after 1-20 sec by rapid addition of 2 ml "stopping solution" containing 145 mM TEA^+ and 1 mM TBA^+, agents known to block K channels. Suction was applied, and ^{86}Rb released into the filtrate was assayed by liquid scintillation methods. ^{86}Rb remaining in the synaptosomes was also measured, and ^{86}Rb release was expressed as % total ^{86}Rb released for each time point.

^{86}Rb efflux into 5K PSS was slow (0.1-0.2%/sec); increasing K markedly stimulated efflux. Two components of K-stimulated efflux were seen: a rapid component apparently inactivating in less than 1 sec (C_f), and a slower component that remained linear for 5 sec (C_s). Both were graded with increasing K_o, but C_f was much more dependent on external Ca (Ca_o) than was C_s. This suggested the presence of Ca-activated K^+ channels in the terminals.

Three lines of evidence supported this view; a) More than 50% of the K-stimulated ^{86}Rb efflux at short times was dependent on Ca_o in a concentration-dependent manner. La^{3+} abolished the stimulation by Ca_o. b) ^{86}Rb efflux could be stimulated in 5K by addition of Ca + A23187. Neither agent alone was effective in 5K. Ca + A23187 produced larger, more prolonged stimulation of ^{86}Rb efflux in 75K than Ca alone. A23187 alone was without effect in 75K. c) Membrane potential changes measured with the voltage sensitive dye CC_5 revealed that Ca + A23187 decreased fluorescent intensity (FI) in 5K, suggesting membrane hyperpolarization; this is expected if P_K is increased. Ca or A23187 alone produced little change of FI in 5K or 100K. Ca + A23187 produced little change of FI in 100K. The data are consistent with the presence of Ca-activated K channels in mammalian presynaptic nerve terminals. Tracer flux measurements may yield insights into physiological and pharmacological properties of these channels in vitro. Supported by NS-16106.

10.9 PATCH-CLAMP STUDY OF CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE IN PANCREATIC ISLET CELLS. D.L. Cook*, M. Ikeuchi* and W.Y Fujimoto* (SPON: J. Miller). Dept. of Medicine and Physiology and Biophysics, University of Washington and Seattle VA Med. Ctr., Seattle, WA 98108.

Glucose, the major physiological stimulus for insulin release, triggers a bursting pattern of membrane electrical activity in pancreatic islet cells similar to the pattern seen in molluscan pacemaker neurons. In islets, voltage-dependent Ca^{++} spiking is superimposed on a "plateau" depolarization due to a slower, voltage-dependent current (also likely to be a Ca^{++} current) which triggers the spikes. Pacemaker-like depolarization (during the silent phase) and repolarization (during the plateau phase) trigger the onset and offset of the plateaus. Increasing glucose changes the pacemaker to prolong plateaus and shorten silent phases so that a larger fraction of time is spent Ca^{++} spiking (the electrical correlate of glucose-dependent Ca^{++} uptake). A candidate pacemaker mechanism for which there is indirect evidence in islets is the Ca^{++} activated K^+ conductance. To test this, we have voltage-clamped excised membrane patches of identified, cultured rat beta cells bathed symmetrically with KCl (140 mM), $MgCl_2$ (2 mM), HEPES (10 mM), EGTA (1 mM) and varying levels of free Ca^{++}. We have identified a channel with a large unit conductance (216-235 pS at temp = 20-22C, n = 4) which is activated as Ca^{++} level on the cytoplasmic surface is raised from 1 μM to 100 μM. The response to Ca^{++} occurs immediately (within 100 msec) and does not accommodate. The channel conductance is linear (over a +/- 80 mV range), and is not, itself, affected by Ca^{++}. The channel is not activated by external Ca^{++}, but is activated by depolarization to positive potentials at low Ca^{++} and is less steeply voltage sensitive at high Ca^{++} levels where the channel is active even at negative potentials. CONCLUSION: Rat pancreatic beta cells possess K^+ channels which are activated by depolarization and by intracellular free Ca^{++} as have been recently described in rat muscle and salivary gland. These channels may serve as pacemakers for glucose-dependent, bursting electrical activity in pancreatic islet cells.

10.10 IN VITRO INNERVATION MODULATES A Ca-ACTIVATED K-CONDUCTANCE IN RAT MYOTUBES. B.A. Suarez-Isla and S.I. Rapoport. Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, NIH, Baltimore City Hospitals, Balto., Maryland 21010 USA

We have shown (Suarez-Isla et al., Neurosci. Abstr., 8: 125, that the incidence of slow hyperpolarizing after-potentials (slow HAPs) decreases significantly in rat myotubes which are innervated in vitro by chick spinal cord neurons that form stable neuromuscular synapses but not after innervation with chick retinal neurons, which form transient synapses. The slow HAP is associated with an increased Ca-dependent K-conductance (Barrett et al., Dev. Biol. 82:258, 1981) and probably maintains spontaneous contractile activity before innervation in vivo, being suppressed during maturation.

To investigate whether the decreased incidence of the slow HAP following innervation is due to modulation of a Ca-dependent K-conductance, we used an extracellular patch clamp technique to examine gating and kinetic properties of single Ca-activated K-channels ($I_{K(Ca)}$ channels) in intact membrane patches of control myotubes and myotubes cocultured with spinal cord neurons.

The $I_{K(Ca)}$ channels were active in control and cocultured myotubes, even in the absence of slow HAPs. They were in 80% of the patches tested. The single channel conductance equaled 110 pS. In a few cases, channels appeared in bursts followed by silent periods of seconds to minutes. Action potentials, evoked by a second intracellular electrode, transiently increased the probability of opening of the $I_{K(Ca)}$ channel. The fraction of time that a channel spent in the open state (f(V)) increased e-fold with 20 mV of depolarization for low levels of activity in control and cocultured myotubes. Shifts of about 30 mV along the voltage axis were observed in both cases. The voltage dependence of open and closed time-constants was similar in both types of cells. However, the current-voltage relation depended on innervation. $I_{K(Ca)}$ channels were blocked at lower holding potentials in cocultured myotubes and the I/V relation "rolled over" at about +40 mV, showing a clear negative conductance region. In control myotubes, "roll over" became apparent only beyond +75 mV of holding potential. A model of voltage dependent ion block of the channel (Woodhull, J. Gen. Physiol. 61, 687 1973) indicates that the voltage sensitivity of the block is significantly increased in cocultured cells. These results indicate that neurons that form stable neuromuscular synapses in vitro exert a neurotropic influence that is rapidly expressed as a change in the conductance properties of Ca-activated K-channels of muscle cells in culture. Further experiments with excised membrane patches are needed to test whether the neurotrophic effect has also affected the Ca sensitivity of the $I_{K(Ca)}$ conductances.

10.11 ACTIVATION OF A K^+ PERMEABILITY IN THE GH3 ANTERIOR PITUITARY CELL LINE BY THYROTROPIN RELEASING HORMONE. A.K. Ritchie*. (SPON: J.E. Blankenship). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Multinucleated cells (up to 50 μm in diameter) have been produced by fusion of cells from the GH3 anterior pituitary cell line with polyethylene glycol. These cells were used to study the electrophysiological response of this cell line to 50 nM thyrotropin-releasing hormone (TRH). One of the responses to TRH is a pronounced hyperpolarization accompanied by an increase in membrane conductance (Ozawa and Kimura, 1979, Proc. natn. Acad. Sci 76: 6017, 1979). The reversal potential of this response is −68mV and varies by 59 mV/decade change in external K^+ concentration, indicating that an increase in K^+ ion permeability is largely responsible for the hyperpolarization. The response is inhibited by 0.5 mM quinine, 0.1 μM apamin and 20mM TEA-HCl.

Single electrode voltage clamp studies of GH3 cells (< 20 μm in diameter) show that the TRH induced current flow is enhanced as the membrane potential is clamped to more positive levels. This voltage dependence and the observation that the hyperpolarization is sensitive to agents which are known inhibitors of the calcium-activated K channel suggests that the TRH response may be mediated by activation of such channels. Voltage clamp experiments show that the Ca activated K current (I_c) in these cells is partially inhibited by 10mM TEA-HCl and completely inhibited by 20mM TEA-HCl, however, the effects of quinine and apamin on I_c in these cells is still under investigation.

If the TRH induced hyperpolarization is due to Ic then the source of calcium ions must be intracellular since the response persists in Ca^{++} free solutions and under conditions in which the voltage dependent Ca^{++} channel is completely inhibited by 0.5 mM $CdCl_2$. Consistent with this hypothesis is the observation by others that TRH causes a dramatic increase in $^{45}Ca^{++}$ efflux (but not influx) suggestive of a mobilization of internal stores of Ca^{++}. Supported by NSF Grant BNS-7924466.

10.12 PURIFIED CATALYTIC SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE CLOSES THE SEROTONIN-SENSITIVE POTASSIUM CHANNEL OF APLYSIA SENSORY NEURONS IN CELL-FREE MEMBRANE PATCHES. J. S. Camardo*, M. Shuster*, S. A. Siegelbaum*, C. M. Eppler* and E. R. Kandel (SPON: L. Rowland). Center for Neurobiology & Behavior, Dept. of Pharmacology, Columbia Univ., College of P & S, and N. Y. State Psychiatric Institute, New York, NY 10032.

Serotonin induces a slow EPSP in the sensory neurons of Aplysia by closing a specific K^+ channel, which has been previously identified by single channel recording techniques (Siegelbaum et al., Nature, 229:413). The serotonin-sensitive (S) channel is selective for K^+ ions, open at the resting potential, voltage-independent and independent of intracellular calcium. Evidence from biochemical and electrophysiological experiments indicates that serotonin acts via cAMP-dependent protein kinase, but the molecular site of kinase action remains unknown. We now report preliminary experiments indicating that purified catalytic subunit of cAMP-dependent protein kinase (PKC) decreases the opening of the S channel in cell-free membrane patches.

Single channel currents were recorded from inside-out membrane patches with the cytoplasmic surface of the membrane exposed to a 360 mM KCl, low Ca_i^{++} bath solution. Two types of K^+ channel currents with similar but not identical single channel conductances were recorded in these patches: a Ca_i^{++}-dependent, voltage-dependent K^+ channel (I_C), and the Ca_i^{++}-independent, voltage-independent S channel. Purified catalytic subunit of protein kinase (kindly provided by E. G. Krebs, C. S. Rubin, and D. A. Walsh) was added to cell-free patches after precautions were taken to assure that the enzyme had ready access to the patch. In three out of three experiments under these optimal conditions with patches that contained only S channels, the kinase (1-7.5 uM), in the presence of MgATP, caused a rapid (within 2-10 sec.) and pronounced decrease in S channel activity. After addition of PKC, the time-averaged patch current, <I>, was reduced to 10 ± 4 % of its control value. The effects of PKC were reversed after addition of calf alkaline phosphatase (293 units/ml) to the patch: <I> increased to 74 ± 8 % of its control (pre PKC) level. In all three experiments subsequent application of PKC again reduced channel activity significantly.

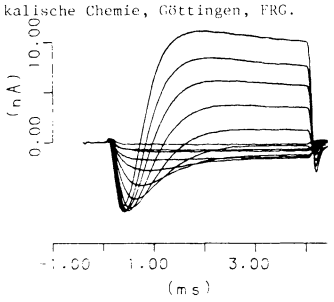
PKC shows some specificity for the S channel. In two experiments on patches containing only the Ca_i^{++}-dependent K^+ channel, PKC had no effect on channel activity, measured during exposure to either 100 uM Ca^{++} (where the channels were largely open) or 300-400 nM Ca^{++} (where the channels were largely closed).

Our results indicate that the activity of the S channel, a serotonin-sensitive K^+ channel, can be regulated by a cAMP-dependent protein kinase. The ability of this enzyme to modulate channel activity in a cell-free system suggests that the substrate for kinase action is either the S channel or a regulatory molecule which remains attached to the membrane patch when it is withdrawn from the cell.

10.13 VOLTAGE-DEPENDENT SODIUM CONDUCTANCE IN AN INVERTEBRATE STRIATED MUSCLE. W. Stühmer* and L.M. Schwartz. Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

Many neurons, from both vertebrates and invertebrates, have been shown to use an influx of sodium to generate action potentials. This is also a feature of vertebrate skeletal muscles. In contrast, all invertebrate muscles examined to date use predominantly an influx of calcium to generate action potentials. At some point during evolution, there must have been selective pressures which favored the expression of sodium channels in straited muscles. We report here that in planktonic arrow worm (Sagitta elegans, phylum: Cheatognatha) that sodium constitutes the predominant charge carrier during depolarization. Using the loose-patch voltage clamp (Stühmer & Almers, PNAS 79, 946-950, 1982) we characterized the inward current flowing in response to membrane depolarization. While technical constraints prevented us from performing ion substitution experiments, several lines of evidence suggested that this current flows through sodium channels. (1) These channels were voltage-dependent with peak transient currents flowing in response to depolarizations of 90 mV from a resting potential of approximately -70 mV. (2) The channels opened rapidly with maximal current flow occurring within 0.4 ms at 4°C. (3) This channel showed voltage-dependent inactivation with 50% of the channels being inactivated at +25 mV from rest. (4) This inward current was abolished by 500 nM TTX. These data suggest that Sagitta muscles use voltage-dependent sodium channels to generate action potentials. Thus sodium spikes in striated muscle can no longer be considered a purely chordate trait.

Supported by USPHS grant #AM17803 to Prof. Wolfhard Almers and an American Heart Assoc. of Washington fellowship to L.M.S. Present address for W.S. is Max-Planck-Institut für Biophysikalische Chemie, Göttingen, FRG.



Currents recorded through a 10 µm diameter patch electrode in responce to depolarizations from 10 to 120 mV in steps of 10 mV. Temp. 4°C.

DEVELOPMENT OF THE VISUAL SYSTEM: RETINOTHALAMIC PROJECTIONS

11.1 TOPOGRAPHY OF GANGLION CELL PRODUCTION IN THE CAT RETINA. C. Walsh, E.H. Polley, and T.L. Hickey. Comm. on Neurobiology, U. of Chicago, Chicago, IL 60637, Depts. of Anatomy, Ophthalmology and Neurosurgery, U. of Illinois School of Medicine, Chicago, IL 60680, and School of Optometry/The Medical Center, U. of Alabama, Birmingham, AL 35294.

Ganglion cells in the cat's retina are formed as several rough, central-peripheral waves which overlap one another as cells are produced first for central retina and then for peripheral retina. Medium-sized cells are produced before large cells, and the smallest cells seem to be produced throughout the period of ganglion cell addition (Walsh et al, Nature, 302: 611, 1983). The present report is based on further analysis of retinae from 24 cats which received ^{3}H-thymidine between the 21st and 36th days of gestation (E21-E36), and then were killed 2-10 months after birth. Our earlier work contains procedural details.

While each cell class shows the same general pattern of addition, it is best illustrated for alpha cells. The oldest alpha cells, labelled after ^{3}H-thymidine injections on E25, lie mainly above the optic disc, scattered over a wide area extending nasally and superiorly from area centralis (a.c.). After injections at later ages (E26,27,29), the area covered by heavily labelled alpha cells expands superiorly, nasally, and inferiorly, while spreading only slowly temporally across the a.c. itself. In superior retina the region containing heavily labelled alpha cells is bounded temporally by a line running superiorly and temporally from a.c. This corresponds roughly to the raphe line, which separates ganglion cells whose axons run inferior to a.c. from those with axons that run superior to a.c., towards the optic disc (Murakami et al, Br. Beh. Evol. 21: 67-113, 1982). Later injections (E31) labelled alpha cells near or below the temporal horizontal meridian (i.e., inferior and temporal to the raphe line) but no alpha cells nasal to the raphe line, or anywhere in the nasal retina except in the far periphery. Hence, retinal regions containing the oldest alpha cells are also the first regions to complete alpha cell production. Injections at later ages (E35, 36) did not label any alpha cells.

In the cat then, production of those ganglion cells which survive in the adult is in no way symmetrical about either the optic disc or area centralis. Nasal retina leads temporal retina, and superior leads inferior, in the production of each ganglion cell type. Cells in the area centralis are produced over an extended time period. Moreover, cells in the region near and below the horizontal meridian of temporal retina are produced quite late. (Supported by NIH grants EY-01338, GM-07281, and EY-02374).

11.2 GENESIS OF MORPHOLOGICALLY IDENTIFIED NEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS OF THE CAT. Peter F. Hitchcock*[1,2] and T. L. Hickey[1]. School of Optometry/The Medical Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294[1] and The Division of Biological Sciences, Univ. of Michigan, Ann Arbor, MI 48109[2].

Neurons in the dorsal lateral geniculate nucleus (dLGN) of the cat can be placed into five morphological classes (1-5) and three physiological classes (X,Y,W), based on a variety of structural and functional characteristics. Recently, Friedlander, Lin, Stanford, and Sherman (J. Neurophysiol., 46: 80-129, 1981) and Stanford, Friedlander, and Sherman (J. Neurosci., 1: 578-584, 1981) have shown how the three physiological classes of neurons distribute among the five morphological classes.

Utilizing ^{3}H-thymidine autoradiographic techniques, we earlier demonstrated the period of time during prenatal development when cat dLGN neurons, classified according to adult soma size and location, undergo their final cell division. Dorsal lateral geniculate nucleus neurons undergo their final cell division between gestational days 22 (E22) and E32. Neurons generated early in this period are ultimately distributed throughout the dLGN and exhibit a full range of soma sizes.

The purpose of the present work was to directly demonstrate the relationship between the time a dLGN neuron undergoes its final cell division and its adult morphology. This task was accomplished by combining ^{3}H-thymidine labelling of dividing neurons with Golgi impregnation procedures, using a technique developed in our laboratory.

In this study, 2138 Golgi impregnated neurons were classified in the dLGNs of seven cats, each of which had received a single injection of ^{3}H-thymidine on one of the following gestational days, E24-E28 or E30. Of the 2138 neurons classified, 1517 were successfully resectioned and recovered. Of these, 385 (25.4%) were found to contain the ^{3}H label. In animals that had received an injection of ^{3}H-thymidine between E24-E28, dLGN neurons from each of the five morphological classes were found to contain ^{3}H label. In a cat that had been injected with ^{3}H-thymidine on E30, only class 3 and class 5 neurons were found to contain ^{3}H label. These findings demonstrate that neurons from each of the five morphological classes undergo their final cell division between E24-28 and, when combined with the structure/function relationships described above, suggest that X, Y, and W cells in the cat's dLGN undergo their final cell division simultaneously, at least between E24 and E28. The morphological and physiological properties of dLGN neurons that do not undergo their final cell division until E30 may be more restricted.

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11.3 DEVELOPMENT OF THE CORTICOGENICULATE PATHWAY IN THE CAT: AN ELECTRON MICROSCOPIC AUTORADIOGRAPHIC STUDY. A. J. Weber and R. E. Kalil, Neurosciences Training Program and Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI, 53706.

Previous anatomical research has demonstrated a topographically organized projection from the visual cortex to the lateral geniculate nucleus (LGN) in the cat. Combined degeneration and electron microscopic studies suggest that corticogeniculate axon terminals are a specific morphological class. In brief, these synaptic endings are characterized by their 1) round synaptic vesicles, 2) small size and 3) dark mitochondria, and therefore these profiles have been classified as RSD terminals (Guillery, Z. Zellforsch., 96, 1969).

At present, it is not known whether all corticogeniculate axons terminate as RSD profiles since degeneration methods usually do not preserve the normal ultrastructure of terminals. In addition, our knowledge of the synaptic organization of the corticogeniculate system is limited to adult cats since this pathway has not been studied in younger animals with the electron microscope. In the present study, electron microscopic autoradiography (EMAR) has been used to investigate the corticogeniculate pathway in adult cats and to study its development.

Thirty-five cats, ranging in age from newborn to adult, received large injections of ^{3}H-proline into visual cortex near the representation of the intersection of the zero vertical and horizontal meridians. Following survival periods of 24 to 36 hours, the cats were perfused with paraformaldehyde and glutaraldehyde, the lateral geniculate nuclei were removed in 1mm parasagittal or coronal blocks, and embedded in epon-araldite. Five micron sections from each block, processed for light microscopic autoradiography, were used to localize the area of the LGN that was labeled anterogradely from visual cortex. Thin sections for EMAR were then cut from the most densely labeled region of lamina A.

At each age studied, labeled corticogeniculate terminals conform to the RSD classification. Furthermore, corticogeniculate endings make synaptic contacts at birth that do not appear to differ from those seen in older kittens or adults. Thus, at all ages corticogeniculate axons terminate exclusively on dendritic processes and are always presynaptic. This suggests that the essential pattern of corticogeniculate synapses does not change during development.

The number of labeled terminals increases steadily during the first postnatal month and rapidly during the second. In all animals, however, many unlabeled RSD profiles are seen in the immediate vicinity of labeled terminals. This result suggests that not all RSD profiles are cortical in origin.

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11.4 EFFECTS OF RETINAL GANGLION CELL BLOCKADE ON THE MORPHOLOGICAL DEVELOPMENT OF RETINOGENICULATE SYNAPSES IN THE CAT. R. E. Kalil, M. W. Dubin, G. L. Scott* and L. A. Stark*. Dept. of Ophthalmology, Univ. of Wisconsin, Madison, WI, 53706, and Dept. of Molecular, Cellular and Developmental Biology, Univ. of Colorado, Boulder, CO, 80309.

Synapses between retinal ganglion cells and neurons in the dorsal lateral geniculate nucleus (LGN) of the cat develop in an orderly sequence during the first two postnatal months (Kalil and Scott, Soc. Neurosci. Abstr., 1979), and this sequence is largely unaffected by partial or complete visual deprivation (Kalil and Scott, Soc. Neurosci. Abstr., 1981). Recently, it has been demonstrated that impulse blockade in the retinogeniculate pathway during development leads to severe physiological abnormalities in the LGN (Archer et al., Science, 217, 1982). In the present experiments we investigated whether blocking retinal ganglion cell activity also affects the morphological development of retinogeniculate synapses.

Intraocular injections of tetrodotoxin (TTX), at a concentration known to block ganglion cell activity (Archer et al., op. cit.), were administered unilaterally to three cats. The injections were begun shortly after birth, and continued on alternate days for the next 7 to 8 weeks. The cats were then perfused with mixed aldehydes, and blocks of tissue from the A laminae of the LGN ipsilateral and contralateral to the injected eye were prepared for electron microscopy. Control tissue from two cats that received intraocular injections of citrate buffer only was also available

In comparison with retinogeniculate (RLP) terminals from control cats or from the normal eye of experimental animals, RLP terminals from the TTX treated eye display several abnormalities. First, terminal cross-sectional area and perimeter are reduced in size by about 50%. Second, these terminals make approximately 30% fewer contacts than normal, however the length of individual contacts is increased on average by 60%. Third, RLP terminals from the TTX treated eye make simple axodendritic contacts almost exclusively, and are found only rarely in complex encapsulated zones. By contrast, about 33% of all contacts made by normal RLP terminals are with vesicle filled profiles (F profiles) and one-third of normal RLP terminals are encapsulated.

Taken together, these structural abnormalities show that retinogeniculate synapses in cats reared for two months with retinal ganglion cells silenced by TTX share many features in common with synapses in neonatal kittens. This result suggests that electrical activity plays an important role in the morphological development of synaptic patterns.

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11.5 PHYSIOLOGICAL SEGREGATION OF GENICULATE AFFERENTS IN THE VISUAL CORTEX OF DARK REARED CATS. N.V.Swindale* and M.S.Cynader (SPON:A.Fröhlich). Dept. of Psychol.,Dalhousie Univ.,Halifax,N.S. B3H 4J1, Canada

In cats dark reared from birth, normal segregation of geniculate afferents to layer IV of area 17, as revealed by trans-neuronal autoradiography, fails to occur (Swindale, Nature,290,332,1981). In area 18 on the other hand, segregation appears normal (Swindale,Soc.Neurosci.Abstr.,8,297,1982). We took advantage of the relative unresponsiveness of cortical neurones in the visual cortex of dark reared cats to record from the population of geniculate axons in layer IV of area 17 and 18, with the intention of comparing the anatomical distribution of geniculate inputs with their physiological activity.

In area 18 and parts of area 17, ocular dominance was measured by making numerous vertical penetrations and sampling at depths between 500 and 800 um; in area 17 tangential penetrations were also made down the medial bank of the marginal gyrus, and histological reconstructions used to verify that the recordings were from layer IV.

Identification of activity evoked by moving or flashed spots of light as being from geniculate afferents was based on several criteria: 1) the responses followed flashes without habituation up to several Hz; 2) there was no selectivity for orientation or direction and 3) responses appeared first at at depth of about 500 um below the cortical surface and, in vertical penetrations, grew less strong below about 800 um. The evoked activity (measured by listening to an audio-monitor) was probably multi-unit since 1) individual units could rarely be resolved either aurally, or by looking at the oscilloscope trace; 2) the measured ocular dominance remained the same when the electrode advanced vertically through layer IV. We used this method to record the ocular dominance distribution in area 17 and 18 of dark reared cats ranging in age from 4 months to 3 years.

In area 18, most responses were strongly dominated by one or the other eye, implying that the anatomical distribution of geniculate afferents is a good predictor of their physiological activity. In area 17 there were more binocular responses, but there were still many regions where responses could only be obtained from one eye. This has to be reconciled with the results of trans-neuronal autoradiography which show that inputs from both eyes are present in all regions of layer IV. A tentative explanation would be that in some regions, geniculate afferents are physiologically silent i.e. some axonal branches fail to conduct impulses. In normal segregation conduction failure might precede the physical removal of axons: development in dark reared cats may perhaps be arrested between these two stages.

11.6 PRENATAL DEVELOPMENT OF RETINOGENICULATE AXONAL ARBORIZATIONS IN THE CAT. D.W. Sretavan and C.J. Shatz. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

In the development of the cat's retinogeniculate pathway, afferents from both eyes initially overlap and then segregate into distinct contralateral and ipsilateral regions within the lateral geniculate nucleus (LGN). Segregation begins by embryonic day 47 (E47) and is complete at birth 3 weeks later. Here, we examine the morphology of individual retinogeniculate arborizations (LGN) during this period.

Nine fetuses (E47-E64) and a 2-day-old kitten have been examined. The diencephalon was removed from the cranium and small crystals of HRP were inserted into the exposed optic tract. The tissue was bathed in oxygenated Ringer's solution in a slice chamber for six hours at 37° C. The tissue was fixed, sectioned in the horizontal plane at 100 µm intervals, and reacted for HRP histochemistry. A total of 45 fibers have been reconstructed.

In the younger fetuses (E47-E53), most axons, upon entering the LGN, turn sharply (>90°) and course anteromedially in straight trajectories. The simplest fibers are unbranched and end in growth cones. Slightly more complex fibers have many fine, short processes (10-30 µm) arising from all portions of the axon. However, the distal (furthest from the optic tract) portion of some axons is smooth and unbranched. Often fibers of different degrees of complexity are found next to each other. This arrangement may reflect the differential arrival and maturation of axons from retinal ganglion cells born at different times.

In older fetuses (E53-E55), significant arborizations are first seen. Fibers end in fan-shaped arbors consisting of radially directed branches roughly equal in length (100 µm); proximally the axon is smooth and unbranched. More complex fibers have elaborate, elongated arborizations (500 µm) but are restricted in width to less than 100 µm. Close to birth (E63, E64), arbors are even more highly branched and have a columnar shape similar to that seen in the adult (Bowling, D.B. and Michael, C.R., J. Neurosci., in press.). Most occupy the full thickness of an LGN layer while others arborize chiefly in the top or bottom half. However, unlike the adult, clusters of boutons are not seen and the width of arborizations remains less than 100 µm.

These findings demonstrate that during the period of segregation, retinogeniculate axons are relatively simple in morphology and only begin to acquire complex, highly focused branching patterns towards the end of the period, shortly before birth. Furthermore, the absence of widespread branching throughout this period suggests that the retinogeniculate projection already possesses a significant degree of topography.

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11.7 PHYSIOLOGICAL AND MORPHOLOGICAL DEVELOPMENT OF RETINOGENICULATE AXONS IN KITTENS. M. Sur, R.E. Weller and S.M. Sherman, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY 11794.

We have studied the postnatal development of physiological and morphological properties of single retinal axons innervating the lateral geniculate nucleus (LGN) in kittens by using the intracellular HRP technique. At 3-4 weeks of age, most optic tract axons can be classified as either X or Y. Latencies to optic chiasm stimulation are shorter for Y-cell than for X-cell axons. However, nonlinear responses from Y-cells are often immature. Both X- and Y-cell receptive fields are large (often 4-5 times adult sizes) and lack antagonistic surrounds. Both X- and Y-cell terminal fields are morphologically different from their adult counterparts. The X-cell terminal fields in lamina A or A1 are slightly wider in 3-4 week old kittens than in adults (kitten: N=6, mean=167μm, range=120-240μm; adult: N=16, mean=140μm, range= 100-175μm; p=0.05). In contrast, the Y-cell terminal fields in the kittens are narrower than in adults (kitten: N=9, mean=188μm, range=160-260μm; adult: N=12, mean=293μm, range=220-410μm; p< 0.001). Most terminal boutons of both X- and Y-cell axons are small (<2μm), although some are medium-sized (2-5μm). Many boutons have filopodial extensions with growth cone-like appendages. By 8 weeks of age, receptive field sizes are comparable to adult X- and Y-cell fields, surrounds have developed, and Y-cell nonlinear responses are stronger and more stable. Four recovered X-cell axons have terminal widths of 120-180μm with a mean of 145μm. Two recovered Y-cell axons have widths of 160 and 330μm. Terminal boutons appear adult-like, with many small and medium-sized swellings. There are still a few boutons with filopodial extensions, and relatively few of the large, crenulated boutons that are common in adult axons. At 12 weeks, receptive field properties of X- and Y-cell axons are indistinguishable from adult axons. Two recovered X-cell axons have widths of 100 and 115μm, while 3 recovered Y-cell axons have widths of 180, 185 and 310μm. Terminal boutons appear adult-like in size and form.

Our conclusions must be qualified because of the possibility that we have sampled the most mature axons. Nonetheless, receptive field properties, terminal arbors, and individual boutons of X- and Y-cells appear to develop rapidly in kittens between 3 and 8 weeks of age. However, some X-cell axons at 3-4 weeks have terminal arbors that are broader than those seen in adults, whereas most Y-cell arbors are smaller at 3-4 weeks than in adults. This suggests that, during the critical period, a process of enlargement of Y-cell arbors is concomitant with the pruning of exuberant connections made by immature X-cell arbors.

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11.8 VISUAL RESPONSE LATENCIES AND CONTRAST SENSITIVITY FUNCTIONS IN PRIMATE LGN AFTER MONOCULAR DEPRIVATION. G. E. Irvin*, M. A. Sesma, T. K. Kuyk*, T. T. Norton and V. A. Casagrande. Dept. of Physiol. Optics, School of Optometry/The Medical Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294; Depts. of Anatomy and Psychology, Vanderbilt Univ. Nashville, TN 37232.

We previously reported (Sesma et al., Soc. Neurosci. Abstr. '82) that Y-like and X-like cells in the LGN of galago appear to be unaffected by monocular deprivation as judged by measurements of encounter rate, latency to chiasm and cortical stimulation, receptive-field size, response to targets of appropriate standing contrast and phasic-tonic index. We have now extended these investigations to include visual response latencies and contrast sensitivity functions (CSFs) of single cells in the LGN of 5 adult greater galagos which received monocular lid suture for more than 14 mos. Response latencies were measured for small visual stimuli (1.0 log unit above threshold) centered in the receptive field. The results are comparable to those in normal galagos in that response latencies to both light onset and peak response were shortest for Y-like (mean, 51 msec onset; 91 msec peak), intermediate for X-like (mean, 65 msec onset; 120 msec peak) and longest for W-like cells (mean, 85 msec onset; 144 msec peak). Monocular deprivation did not affect the response latencies. There were no significant differences either between the latencies of deprived and non-deprived cells in the deprived animals or between cells in deprived and normal animals. Thus, following monocular deprivation, information carried by deprived W-, X- and Y-like cells is capable of reaching the visual cortex at the same time as information carried by their non-deprived counterparts.

Limited CSF data showed that deprived Y-like and X-like cells responded similarly to non-deprived Y-like and X-like cells. For Y-like cells, the mean high spatial frequency cut-off (deprived vs. non-deprived) was 0.8 c/deg vs. 0.9 c/deg and the peak sensitivity occurred at a mean spatial frequency of 0.2 c/deg for both the deprived and non-deprived cells. For X-like cells, the mean high spatial frequency cut-off was 0.9 c/deg (D) vs. 1.2 c/deg (ND) and the peak sensitivity occurred at a mean spatial frequency of 0.3 c/deg (D) and 0.4 c/deg (ND). When differences in the eccentricity of the sampled cells were considered, the averaged CSF values for the deprived animals were not significantly different from those found in normal galagos (Kuyk et al., Invest. Ophthalmol. Vis. Sci. (Suppl.), '83). These data provide additional evidence that monocular deprivation has very little effect on either Y-like or X-like cells in primate LGN.

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11.9 THE RELATIONSHIP BETWEEN AFFERENT LAMINAR DEVELOPMENT AND CELL LAYER FORMATION IN THE LATERAL GENICULATE NUCLEUS (LGN). V. A. Casagrande and J. K. Brunso-Bechtold. Depts. of Anatomy and Psychology, Vanderbilt University, Nashville, TN 37232.

Our previous work in tree shrew has shown that early eye removal prevents the formation of interlaminar spaces (ILSs) between the six LGN cell layers indicating that retinal input plays a key role in cell layer formation. Yet, some species exhibit more layers of retinal input than cell layers suggesting that other factors such as the development of extraretinal input may be important. In order to investigate this further we studied the development of lamination of retinal input in relation to LGN ontogeny by injecting both eyes of 15 tree shrews with several combinations of tracers at different developmental stages.

In the adult, spacing between retinal input to all six layers is not equivalent. Uncrossed and crossed input to layers 1 and 2, respectively, extends into the ILS leaving only a small label-free gap. In contrast, crossed and uncrossed input to cell layers 4 and 5 is confined to the cell layers as is the crossed input to layers 3 and 6. Unlike retinal input, extraretinal input in adults is concentrated within all ILSs except that collicular fibers avoid layers 1 and 2 and the ILS in between. At birth (P 0) when no cell layers are present retinal input reveals a similar but less distinct pattern than in adult. For example, retinal input to future layers 1 and 2 shows little, if any, space between them while input to the remaining future layers is separated by zones of lighter label. At that time, cortical input has not yet entered the LGN and collicular input is present but unlaminated. By P 5 retinal input exhibits a more distinct laminar pattern. At that stage, ILSs between cell layers have begun to form and corticogeniculate fibers show evidence of concentration within these forming ILSs. Finally, at P 16 when all cell layers are apparent, label from the retina shows a pattern indistinguishable from that of the adult.

The timing of the above events suggests several conclusions: First, retinogeniculate input is clearly laminated prior to the formation of the ILSs and thus is in a position to influence their development. Second, cortical input begins concentrating in the ILSs at the time these are forming and thus may further this development. Finally, it is of interest that the unequal spacing of retinal input is weakest in the region (layers 1 and 2) lacking collicular input in the adult. (Supported by EY03881 and 1K04-EY00223).

11.10 NEONATAL DEVELOPMENT OF GAD IMMUNOREACTIVITY AND GAD+ SYNAPSES IN THE DORSAL LATERAL GENICULATE OF THE MONKEY: A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. M.P.Ogren, A.E.Hendrickson,& J-Y. Wu, Dept. Psych., MIT,Cambridge MA 02139; Dept. Ophth., Univ.Wash., Seattle WA 98195; Dept. Cell Biol., Baylor Sch. Med., Houston TX 77030.

Our previous study of the dorsal lateral geniculate (dLG) in the adult monkey (Ogren et al '82, Hendrickson et al '83) described the morphology and distribution of neurons and synapses that contain gamma-aminobutyric acid (GABA) by immunohistochemical staining of an antiserum to the GABA-synthesizing enzyme, glutamic acid decarboxylase (GAD). We now report on the development of GAD reactivity and GAD+ synapses in the dLG during the first postnatal month. The dLG of 3 normal macaque monkeys at 3 postnatal ages; 2 da, 2 wk, & 4 wk were prepared for light (LM) and electron (EM) microscopy. The synaptic development of the dLG was also examined with conventional EM in 3 other normal animals at similar ages.

GAD reactivity in the LM is remarkably pale in the dLG at 2 da, and gradually increases in intensity at 2 and 4 wk. The number of GAD+ structures does not appear to change with age. Rather, the labeling is more faint so that higher magnification is required to visualize it at 2 da, and to a lesser extent at 2 wk. This impression is supported by EM analysis in these animals in which the full adult-like complement of GAD+ neurons, dendrites and synaptic terminals is easily detected at all ages. Dendritic labeling, however, is relatively more pervasive in the youngest animals. This suggests that the pale LM appearance of GAD in the dLG may be due to its more diffuse distribution within the reactive neurons at early ages, although the concentration of the GAD enzyme may also increase with age. By 4 wk GAD reactivity in the LM is crisp and robust and is visable at low magnifications as in the adult. Higher concentrations of GAD+ labeling in the laminar, particularly the magnocellular zones is apparent at all ages.

EM analysis shows another age related difference in frequency of synapses made by GAD+ synaptic terminals within the glomeruli. These are likely to be presynaptic dendrites because of their intermediate position between retinal terminals and dLG dendrites in serial and triadic synapses. At 2 da the GAD+ terminals in glomeruli are postsynaptic to retinal terminals, but are rarely presynaptic to dLG dendrites, so that serial and triadic synapses are correspondingly rare. Outside the glomeruli, GAD+ terminals engage in numerous simple synaptic contacts of all types reported for the adult. At 2 and 4 wk the frequency of GAD+ synapses within the glomeruli increases dramatically. This corresponds to our quantitative findings from conventional EM and a previous finding by Hamori et al '75. This result indicates that retinally driven GABA-ergic transmission of presynaptic dendrites in monkey dLG is immature at birth. Supported by Grants EY-01208, NS-12116 and the Dolly Green Scholar fund of RPB, Inc.

11.11 DEVELOPMENT OF RETINAL PROJECTIONS TO THE AUDITORY AND SOMATOSENSORY SYSTEMS. D.O. Frost, Sect. of Neuroanatomy, Yale Med. Sch., New Haven, CT. 06511

When the superior colliculus (SC) and dorsal nucleus of the lateral geniculate body (LGd) are ablated in newborn hamsters and alternative terminal space is made available in the ventrobasal (VB) or medial geniculate (MG) nuclei by ablation of their ascending afferents, optic tract (ot) fibers form permanent, orderly connections to VB or MG. Here we report that during normal development retinofugal axons project transiently beyond the borders of the visual system, and that the abnormal adult projections to VB, but not MG, result from the stabilization of some of these exuberant juvenile connections.

On the day of birth (day 0) and postnatal days 1,2,3,4,6 & 8, normal hamster pups received intraocular injections (ca 1 µl) of 40% horseradish peroxidase or 2% HRP/wheatgerm agglutinin conjugate. 14±1 h after injection the pups were perfused and sections of their brains were reacted by the TML procedure to visualize anterogradely labeled ot axons. Additional pups operated on day 0 so as to induce permanent retinal projections to MG and VB received, at corresponding ages, similar injections contralateral to the operated side of the brain; they were processed as above.

The retinal projections of normal day 0 hamsters are more mature than previously reported. Large numbers of terminating crossed retinofugal axons extend into LGd, the ventral nucleus of the lateral geniculate body (LGv), the pretectum, SC and the accessory optic nuclei. Some ot axons pass across LGd to form an unequivocal projection to the neighboring dorsolateral region of VB, where ectopic projections are found in operated, but not in normal adults. The normal projections to VB persist on day 4, but not on day 8. In normal hamsters, no retinal projections to MG were seen at any age, although ot fibers pass over its surface. In operated hamsters, the normally transient retinal projection to VB persists indefinitely. In these animals, by day 2 the retina projects ectopicly to MG. Collectively, these results suggest that permanent retinal projections to VB are formed by the stabilization of a normally transient projection, whereas projections to MG are rapidly induced de novo, as a result of neonatal surgery. Thus, different mechanisms operate in the formation of abnormal retinal projections to VB and MG.

Additional new observations on normal day 0 pups include: 1) Uncrossed projections to LGd, LGv and the midbrain have already begun to form. 2) The stratum opticum and the stratum griseum superficiale of SC have not yet differentiated. 3) Fascicles of retinofugal fibers pass caudally superficially in SC; they descend into the subjacent tissue across the entire extent of SC. 4) The inferior colliculus receives a transient retinal projection which is present on day 0 but disappears by day 4.

11.12 DENDRITIC REACTIONS TO EARLY POSTNATAL LESIONS IN THE CAT RETINA. U.Th. Eysel, L. Peichl* and H. Wässle*. Institute of Physiology, University of Essen and Max-Planck-Institut für Hirnforschung, Frankfurt, F.R.G.

In the normal cat retina the α-ganglion cell dendritic fields respect each others territories (Wässle et al., Nature, 292:344, 1981). We investigated whether these territories remain fixed when the neighboring cells are destroyed by retrograde degeneration following retinal lesions. In adult cats neither α- nor β-cells change their dendritic territory after retinal lesions. This is shown in retinal whole mounts after retrograde filling with HRP. Following lesions in early postnatal life (3rd postnatal day) and several months survival α- and β-ganglion cells with asymmetric dendritic fields are found. They extend their dendrites into the parts of retina which are free of ganglion cells due to retrograde degeneration. Similar results were reported after retinal lesions on the day of birth in rats (Perry & Linden, Nature, 297:683, 1982). A quantitative evaluation of αcell changes was done in retinae stained with a neurofibrillar method where dendritic staining is superior to HRP material. Cells at the border of the region lacking ganglion cells have dendritic fields of normal size and ellipticity but they show conspicious morphological changes: dendrite diameters as well as dendritic density (number of dendrites per unit area) are larger in the part of the dendritic tree which lies in the ganglion cell-free region; many cells show skewed fields with dendrites pointing preferentially towards that region.

In contrast to the lesions in adulthood the early lesions are made during a period of ocular growth. Asymmetric dendritic fields could therefore result from active dendritic growth or from distortions due to inhomogeneous growth of the retina. The denervated parts of retina expand less than adjacent normal parts during growth. This is revealed by area measurements and an increased density of horizontal cells which are also stained by the neurofibrillar method. Thus passive stretch of dendrites into the denervated area is very unlikely. An active shift of dendritic volume to that part of the dendritic field not competing with other dendritic territories seems to be the most probable explanation. This may be an effective way to contact more of the abundantly available presynaptic sites there without enlarging the dendritic territory as such.

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LEARNING AND MEMORY: ANATOMY I

12.1 FORMATION OF VISUAL DISCRIMINATION HABITS IN RHESUS MONKEYS AFTER INFERIOR TEMPORAL NEOCORTICAL LESIONS DURING INFANCY AND ADULTHOOD. J. Bachevalier*, B. L. Malamut* and M. Mishkin (SPON: C. Duncan-Johnson), Lab. Neuropsychol., NIMH, Bethesda, MD 20205.

The storage and retention of experience depends on at least two qualitatively different processes served by two separate neural mechanisms: a memory system and a habit system (Mishkin et al, in The Neurobiology of Memory:1983). With regard to vision, the memory system appears to include both the anterior part of inferior temporal cortex (area TE) and limbic structures (Mishkin, Phil. Trans. R. Soc. Lond., B298:1982). In contrast, the habit system in vision does not depend on limbic structures, though it may involve area TE (Malamut et al, unpublished data). To examine this latter possibility, we assessed the effects of area TE lesions on a test thought to be selectively dependent on the habit system. In addition, since this system is functional early in infancy, we compared the effects of TE ablations made in infancy with those made in adulthood.

Area TE was removed bilaterally in four newborn monkeys and four adult animals. A two-stage surgical procedure was performed in all infants at 1 week and 3 weeks of age and in one adult animal; the remaining 3 adults sustained a one-stage procedure. Seven unoperated infants, matched for age, and ten unoperated adults served as control animals. Effects of surgery on the concurrent discrimination learning were assessed postoperatively at the age of 3 months in infant monkeys and after a 15 day recovery period in adults. In this task, a set of 20 pairs of easily discriminable objects (Set A) was presented once every 24 hours. The positive and negative objects within each pair, as well as the order of the pairs, remained constant across sessions. When the monkeys reached a criterion of 90 correct responses in 100 trials, they were trained on a second set of 20 new object pairs (Set B) in the same manner as before. In adult animals, unoperated monkeys learned each of the sets in an average of 10 sessions, whereas the operated animals required an average of 27 and 33 sessions to learn set A and set B, respectively. No difference was found between one- and two-stage operated adult animals. Like normal adults, the normal infants learned the two sets in an average of 16 and 7 sessions respectively. By contrast, unlike operated adults, the infants with TE lesions were only slightly impaired in the acquisition of set A and, furthermore, were unimpaired in learning set B, performing as well as normal infants and adults.

The findings support earlier data showing that habit formation proceeds at adult rates in early infancy and suggest that, although area TE is involved in the habit system in adults, neonatal ablation of this cortical area leads to significant functional sparing of the system.

12.2 RESPONSES OF NEURONS IN THE INFERIOR TEMPORAL VISUAL CORTEX IN SHORT AND LONG TERM MEMORY TASKS. G.C.Baylis* and E.T.Rolls (SPON: M.Calford). Dept. Exptl. Psychol., Oxford Univ., Oxford, England.

There is evidence that some neurons in the inferior temporal visual cortex (ITC) have activity related to the performance of a delayed matching to sample task. In this task the monkey must remember whether he has seen a particular stimulus in the preceding few seconds. To investigate the role of the ITC in memory, we have compared the activity of ITC neurons in the rhesus monkey in this relatively short term memory task with their activity in a task requiring memory over longer periods in which different, interfering, stimuli are seen. In the delayed matching to sample task, the stimuli were geometric shapes of one of four colors presented on a TV screen for 1s, separated by a delay of 2-5s. If the second stimulus matched the first, the monkey could lick to obtain fruit juice, and if not the monkey had to withold his lick to avoid obtaining saline. The longer term memory task was a serial recognition task in which 0-8 other stimuli intervened between the novel and familiar presentations of a stimulus, with each stimulus being shown only twice per day (Rolls et al, 1982). The monkey indicated which stimuli he recognized by licking on the familiar trials to obtain fruit juice.

ITC neurons did not respond differentially to novel and familiar visual stimuli if more than 1-2 stimuli intervened between the novel and familiar presentations of a given stimulus. Typically the neurons which responded to the novel and not the familiar stimuli in the recognition task only did so if there were no intervening stimuli. A number of these neurons had corresponding responses, on non-match trials, in the DMS task. Some other neurons responded to the familiar stimuli in the recognition task (and had corresponding responses on match trials in the DMS task), but only responded in this way if the familiar presentation of a given stimulus immediately followed its novel presentation. Thus it was found that the responses of ITC neurons which are related to the memory aspects of these tasks (26 neurons in the present sample of 421 neurons) occur in relation to relatively short term memory aspects of these tasks, so that the longer term aspects of the memory required for this type of recognition appear to be processed in structures beyond the ITC (see Rolls et al, 1982).

Of the total of 141 visually responsive neurons, including those with memory-related responses, 49 or 34.8% had activity which occurred selectively to some of the stimuli, often on the basis of color, shape or orientation but in some cases selectively to faces, and the remainder had responses which occurred to a broad range of the stimuli used in the tasks.

Rolls ET, Perrett DI, Caan AW, Wilson FAW (1982) Brain 105: 611.

12.3 A FURTHER EXAMINATION OF THE MEDIAL TEMPORAL-LOBE STRUCTURES INVOLVED IN RECOGNITION MEMORY IN THE MONKEY, E. A. Murray and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20205.

Monkeys with combined but not separate removal of the amygdaloid complex (A) and hippocampal formation (H) are severely impaired in visual recognition memory (Mishkin, Nature, 273: 279, 1978). Since neither the A nor H lesion alone included substantial portions of the entorhinal cortex (Ent), whereas the combined lesion included all of it, the results are also consistent with the possibility that either H+Ent or A+Ent lesions are sufficient to produce the severe memory deficit.

To test these possibilities 9 cynomolgus monkeys were trained on visual delayed nonmatching-to-sample (DNMS) with trial-unique objects. In this task, the monkey is trained to displace a baited sample overlying the central well of a three-well testing board and then, 10s later, to avoid this sample in favor of a completely novel object, both objects now overlying the lateral wells. On attaining the criterion of 90 correct responses in 100 trials, three animals each were given either H+Ent lesions or A+Ent lesions or kept as unoperated controls. After a two week rest period, the monkeys were retrained on DNMS to the same criterion as before or for a maximum of 2000 trials. They were then given a performance test in which: i) delays of 30s, 60s, and 120s were interposed between the sample presentation and test, in blocks of 100 trials each, and ii) lists of 3, 5, or 10 sample objects were presented successively prior to the choice tests, in blocks of 150 trials each.

The control monkeys showed perfect retention of the basic task and averaged 93% correct responses on the performance test. Monkeys with H+Ent lesions were mildly impaired, requiring a mean of 317 trials to relearn the task and averaging 84% correct responses on the performance test. Monkeys with A+Ent lesions, by contrast, failed to reattain criterion; they achieved 81%, 82%, and 83% correct responses, respectively, in the last 100 trials of the 2000-trial training period. On the performance test, the animals averaged 60.5% correct responses, or about the same as that obtained by the A+H lesion group (rhesus) in the original study (Avg.=59.5%).

Since addition of the Ent lesion did not augment the recognition deficits produced by hippocampectomy alone, but greatly exacerbated those of amygdalectomy alone, the Ent lesion appears to be equivalent to a hippocampectomy in this situation, presumably because it disconnects the hippocampus from the inferior temporal visual cortex. The results support the earlier conclusion that combined damage to the amygdaloid and hippocampal systems is necessary to produce a profound memory impairment in monkeys.

12.4 EFFECTS OF LIMBIC LESIONS ON PICTURE RECOGNITION VS. PICTURE DISCRIMINATION LEARNING IN MONKEYS. W. H. Overman, G. E. Ormsby* and M. Mishkin. Psychology Dept., Univ. N. C. at Wilmington, Wilmington, N. C. 28406.

Following combined ablation of amygdala and hippocampus, monkeys show abnormally rapid forgetting on one-trial tests of both object recognition and object-reward association (Mishkin, Phil. Trans. R. Soc., London, B-298, 1982) although they show normal rates of learning two-choice object discriminations even when successive trials are separated by 24 hours (Malamut, et al., Neurosci. Abstr. 6:191, 1980). In the present study we sought to determine whether the same findings would be obtained in a semi-automatic version of the task utilizing photographs of objects instead of the objects themselves.

Six rhesus monkeys with combined ablations of the amygdala and hippocampus (Group A+H) and unoperated controls (Group N) had been matched for performance on trial-unique delayed nonmatching-to-sample (DNMS). Postoperatively, Group A+H required significantly more trials than Group N to relearn up to a 10 sec DNMS. At longer delays of 30, 90, and 180 seconds Group A+H performed at 76%, 62% and 64% correct while Group N performed significantly better at 89%, 88% and 80% correct. A second experiment in which delay intervals of various lengths were intermixed in daily test sessions revealed the same results as Experiment I. In Experiment III the animals were trained on three consecutive two-choice picture discriminations for 35 trials/day with 20 sec intertrial intervals. Group A+H learned all discriminations as rapidly as Group N, both learning to criterion in 3-4 sessions. In Experiment IV, both groups were trained on a concurrent visual discrimination in which a set of 20 pairs of slides were presented only once every 24 hours. The positive and negative slide in each pair, as well as the order of the pairs remained constant across sessions. Groups A+H learned in an average of 14 sessions (92 errors) which was not statistically different from Group N which learned in an average of 18 sessions (94 errors). The last two experiments demonstrate that the impairments previously seen in Group A+H were not due to either perceptual or general learning deficits. These results provide additional support for the view that the limbic system is necessary for one-trial visual recognition memory but not for visual discrimination habits acquired through repetition (Mishkin, et al., Conf. Neurobiol. Learn. Memory, McGaugh, et al., (Eds.), Guilford Press, N.Y., in press.)

12.5 AMNESIC MONKEYS WITH CONJOINT HIPPOCAMPUS-AMYGDALA LESIONS DEMONSTRATE PRESERVED LEARNING AND RETENTION OF SKILLS. S. Zola-Morgan,* and L.R. Squire. V.A. Medical Center, San Diego, CA 92161 and Dept. of Psychiatry, UCSD Sch. of Med., La Jolla, CA.

The traditional view of the human amnesic syndrome has been that despite their impairments these patients can under some circumstances exhibit good learning and retention across long intervals. The best known examples of this observation come from the learning of perceptual-motor skills. During the last ten years, investigators have compiled a considerable group of tasks that are less clearly perceptual-motor, but that can elicit signs of retention in patients who by other indications are profoundly amnesic. A particularly good example is the demonstration that amnesic patients improved their skill at learning to read mirror-reversed words over a three-day period and then retained the skill at a normal level three months later. These findings, among others, have suggested a distinction between skills, which are spared in human amnesia, and information based on facts, or the outcomes of engaging in skills, which is impaired.

As part of efforts to develop an animal model of human amnesia, we have attempted to parallel the findings from human amnesia of preserved skill learning. Groups of monkeys with demonstrated amnesia were administered a series of tasks that appear to depend substantially on skill-learning.

We utilized two motor-skill tasks as well as two visual pattern discrimination tasks. The first motor-skill task required monkeys to learn to manipulate a fragile breadstick around a series of barriers without dropping or breaking it. The second required monkeys to learn to retrieve a candy lifesaver threaded onto a thin metal tube by moving the lifesaver around a right angle bend to the end of the tube. Monkeys with conjoint lesions of the hippocampus and the amygdala (HA=3) acquired each of the two motor skills over a period of several days at the same rate as normal control monkeys (N=3). Retention of the lifesaver motor-skill, after a one month interval, was the same for the HA and N groups. On acquisition and retention of the pattern discrimination task, HA monkeys were impaired only mildly if at all. Moreover, monkeys with lesions of the medial thalamus, an area that has been implicated in human amnesia also performed pattern discrimination tasks successfully. The relationship of pattern discrimination learning to skill learning will be discussed.

This demonstration of preserved learning and retention of skills in monkeys, who by other measures are amnesic, brings into correspondence the behavioral data from human amnesic patients and operated monkeys, and sets the stage for identifying precisely what brain structures must be damaged to produce amnesia.

12.6 DISSOCIATIONS BETWEEN SKILL LEARNING AND VERBAL RECOGNITION IN AMNESIA AND DEMENTIA. M. Martone*, J.T. Becker, M. Payne* and N. Butters. Psychology Service, Boston VA Medical Center, Boston, MA. 02130.

Several previous studies (e.g., Cohen & Squire, Science, 210: 207, 1980) have shown that amnesic patients with hippocampal or diencephalic lesions are impaired on tests of recognition memory (i.e. declarative knowledge) while retaining the capacity to acquire general skills (i.e. procedural knowledge). The purpose of the present study was to evaluate this separation of skill and recognition memory in a group of demented patients (Huntington's Disease patients) with known memory deficits due to basal ganglia lesions (Butters, et al., Adv Neurol., 23: 203, 1981).

Ten patients with Huntington's Disease (HD), 8 amnesic patients with alcoholic Korsakoff's syndrome (AK) and 10 normal control subjects (NC) were trained to read word triads which appeared as mirror images of themselves over 3 consecutive test days. Sixty triads, arranged in 3 blocks of 20 triads, were presented on each day. Half of the triads in each block occurred only once during the 3 test days (unique words) and half were repeated from block to block (repeated words). The time needed to read each triad was recorded. Following the third day of testing, a recognition test consisting of 15 unique words, 15 repeated words and 30 distractor items was administered to each subject. Skill learning was assessed by analyzing the time needed to read the unique words. Verbal recognition was assessed in two ways: 1)by determining the difference in reading time between the unique and repeated words and 2)by performance on the recognition test.

Consistent with the previous results, both the NC and AK groups readily acquired the mirror reading skill, showing a steady decrease in the time needed to read the unique words both within and between test days. However, unlike the NC subjects, AK patients were impaired on recognition; that is, they did not read the repeated words significantly faster than the unique words nor were they able to identify the repeated words on the recognition test.

In contrast to the AK patients, HD patients were impaired on the acquisition of the mirror reading skill: they did not show a steady decrease in reading time of the unique words over the 3 days and showed less improvement on the unique words from day 1 to day 3 than did the NC subjects. However their performance on both measures of recognition memory was equivalent to that of the NC subjects.

This finding of a double dissociation between skill learning and recognition memory in HD and AK patients suggests that these two abilities are separable and may depend upon the integrity of the basal ganglia and limbic systems respectively. Supported by the VA Medical Research Service and NIAAA grant AA00187.

12.7 MEMORY FOR REMOTE PERSONAL AND PUBLIC EVENTS AFTER BILATERAL MEDIAL TEMPORAL LOBECTOMY. Suzanne Corkin, Neal J. Cohen,* and Harvey J. Sagar,* Dept. Psychol., MIT, Cambridge, MA 02139.

In characterizing differences between amnesias of different etiologies, one tack is to compare the duration of remote memory impairment. H.M., who underwent bilateral medial temporal lobectomy in 1953 for intractable seizures, is of particular interest because his retrograde amnesia appears to be temporally limited; it has been distinguished from the less restricted remote memory dysfunction documented in patients with Korsakoff's syndrome. Previous studies indicated that H.M.'s retrograde loss, though difficult to delineate precisely, was restricted to the 2 to 3 years immediately preceding the operation. New attempts to test the limits of his recall and recognition of both personal and public remote information suggest that the remote memory impairment, though still temporally limited, may be more extensive than had previously been reported. The results confirm a marked disorder from around the time of operation, and in addition reveal a milder deficit dating back to the time of onset of generalized convulsions in 1942.

In tests of knowledge of public events, H.M.'s performance was within the normal range for events from the 1940s and 1950s but inferior for the 1960s and 1970s. He was also normal in his ability to produce specific episodic memories, but the median age of these episodes (44 years ago) was greater than that of any healthy age-matched control subject and derived exclusively from the period before his 17th year of life. When constrained to the period after his 16th year, he performed poorly and was inconsistent in dating his memories. In 1982, H.M. could still draw an accurate floor plan of the house in which he had lived for many years after his operation until 1974. He also recognized someone else's drawing of that floor plan when it was presented with foils. His recognition of photographs of relatives and family friends was generally good, except that he did not recognize a photograph of his grandfather as an old man, of himself and his mother on his 50th birthday in 1976, or of the woman who took care of him for several years in the late 1970s. H.M.'s memory for old tunes that had been hits during the period from 1920 to 1967 was poor. The few that he could name had been popular before 1955, except the "Children's Marching Song," which was a hit in 1959. Not only does H.M. have no recollection of his high school graduation in 1947, he did not recognize his classmates when reunited with them at their 35th reunion. A number of them, however, recognized H.M. and recalled that he had had epilepsy.

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12.8 ACQUISITION OF SEMANTIC AND LEXICAL KNOWLEDGE IN AMNESIA. John D. E. Gabrieli,* Neal J. Cohen,* and Suzanne Corkin (SPON: Michael Kuperstein). Dept. Psychol., M.I.T., Cambridge, MA 02139

The noted patient H.M. has exhibited global amnesia since undergoing bilateral resection of medial temporal-lobe structures in 1953. The present series of experiments demonstrates the role of these structures in the acquisition of new semantic and lexical knowledge. Several tests assessed H.M.'s knowledge of 184 words added to the Merriam-Webster Dictionary in dictionary supplements published every five years since 1954. First, H.M. was asked to judge whether a string of letters constituted legitimate English words. Then, he was asked to recall or recognize from among four choices the definitions of the new words. H.M.'s recall (74 pts.; max=368) and recognition (51%) for the meanings of new words were far below normal, but still above chance. He performed especially poorly on words added to the dictionary since 1966. H.M. correctly judged 67% of the new words to be legitimate English words, which was inferior to his near-perfect judgement of old words, but in marked contrast to his low (10%) rate of false judgements of nonwords as words. Lexical decision performance was at chance for items missed on the recognition test, suggesting that he does not have an autonomous lexical representation of new words whose meaning he does not know, despite his continued exposure to these words over many years.

We also demonstrated H.M.'s inability to learn new vocabulary words in a controlled laboratory setting. Subjects were presented with uncommon words whose definitions they could not produce. Each word was presented with a one-line definition for study, followed immediately by a test in which each word was to be matched with the appropriate definition. The test was administered repeatedly until subjects reached a criterion of correct matches for all words within a trial. Each word was then presented with a common synonym for study, followed immediately by a matching test; this procedure was repeated until subjects reached criterion. A final test required each word to be matched with incomplete sentence frames, until criterion was met. Twelve college students tested with 20 to-be-learned words required only 2.0, 1.1, and 1.2 trials to reach criterion on the definition, synonym, and sentence-completion tests, respectively. H.M. was tested with only 8 to-be-learned words. Still, he never reached criterion on any test despite 15 trials for each test on each of 4 consecutive days. The number of words matched correctly on 60 trials per test revealed no savings across tests; H.M. averaged 3.8/8, 3.8/8, and 4.4/8 words correct on the definition, synonym, and sentence-completion tests, respectively.

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12.9 IMPAIRMENT IN RECOGNITION MEMORY AFTER MAMMILLARY BODY LESIONS IN MONKEYS. R.C. Saunders* (SPON: C. Leonard), Dept. Exp. Psych., Univ. Of Oxford, Oxford, England.

Despite a long history of clinico-neuropathological studies of patients with Korsakoff's syndrome, the critical lesion responsible for the amnesia has remained uncertain. Mammillary body (MB) pathology has been a consistent finding; however, it occurs usually in conjunction with pathology in the medial thalamus. The present study investigates whether lesions restricted to the MB produce a severe memory impairment in monkeys analogous to that described in amnesic patients.

Amnesic patients are impaired in yes-no recognition and forced-choice recognition memory tasks. The present study tested cynomologus monkeys in two analogous visual recognition memory tasks. First, they were trained pre-operatively on a running recognition memory paradigm, in which a series of complex pictures were presented in succession. The monkey had to make an instrumental response (yes) when a picture appeared for the second time or withhold the response (no) during its first presentation. The task was made more difficult by increasing the number of intervening stimuli between the first and second presentation of a picture. Pre-operatively monkeys reached a high level of performance at the most difficult stage, with up to 14 intervening stimuli. They were then given a bilateral MB lesion; bilateral fornix (Fx) transection or a combined bilateral MB lesion plus Fx transection or remained as unoperated control subjects (NC). The MB lesions and/or Fx transections had a substantial effect on the monkeys' performance in this task. They took significantly longer to relearn the task and in the final stage their performance was significantly worse than control animals. There were no differences in performance between lesions groups. Average performance scores for each group were: $\overline{NC}$=91%; $\overline{MB}$=76%; $\overline{Fx}$=75%; $\overline{MB+Fx}$=77%.

The same monkeys were then trained on a delayed non-matching to sample task similar to forced-choice recognition memory tests used with patients. All monkeys obtained a 90% criterion level of performance with a 15 sec delay between sample presentation and the retention test. The list of objects was then increased in two stages, to a list of 3 and next to 10. In stage 1, with a list of 3 objects, the retention tests of an object were presented in the reverse order to their initial presentation. Therefore, the retention interval varied systematically from 0 to 2 to 4 intervening items. In stage 2, with the list of 10 objects, the retention tests appeared in the same order as originally presented. The retention interval remained a constant 9 items. The performance of monkeys with MB lesions or Fx transections or both was again significantly worse than control animals at the longer retention intervals. Performance in stage 2 of monkeys with: $\overline{MB}$ lesions alone was 69% correct; $\overline{Fx}$ transection 83%; $\overline{MB}$ lesion+$\overline{Fx}$ transection 75% and $\overline{NC}$ 86%.

The results indicate that lesions restricted to the MB can produce a severe recognition memory impairment in monkeys and suggests further that MB pathology in man may be sufficient to produce the severe amnesia seen in Korsakoff patients.

12.10 RECOGNITION MEMORY DEFICIT IN MONKEYS EXACERBATED BY ADDITION OF A MEDIAL THALAMIC LESION TO A PRE-EXISTING HIPPOCAMPAL LESION. H. Mahut, L. Rehbein* and M.Moss. Dept. of Psychol., Northeastern Univer., and Boston Univer. Sch. of Med., Boston, MA.

Clinical evidence associates amnesia with damage to either limbic or diencephalic structures. In monkeys, hippocampal ablations impair memory assessed with a trial-unique delayed non-matching to sample (DNMS) recognition task (Mahut et al., J. Neurosci., 2: 1214, 1982). However, combined amygdalo-hippocampal ablations result in a more severe deficit (Mishkin, Nature, 273:297, 1978; Mahut et al., op. cit.). One way to account for the synergistic effect of the combined lesion is to consider that 1. Amygdala project to medial dorsal and midline thalamic nuclei in the monkey (Porrino et al., J. comp. Neurol., 198:121, 1981) and 2. Damage to medial thalamus is implicated in clinical cases of amnesia (Teuber et al., Neuropsychologia, 6:267, 1968; McEntee et al., J. Neurol. Neurosurg. Psychiat., 39:436, 1976). The present experiment was designed to see whether the addition of a thalamic lesion to a pre-existing hippocampal lesion would result in as severe a memory loss as that which follows an amygdalo-hippocampal lesion.

Four rhesus macaques that had served as a normal control group from 3 mos to 6 yrs of age were trained on the DNMS recognition task 1 yr before surgery and re-tested pre-operatively. Three received bilateral hippocampectomies and H1 and H2, but not H3, had also moderate bilateral damage of inferotemporal cortex. Their post-operative performance declined from 90% to 62%-80% correct responses with 130 sec delays and to 61%-78% with 10-item lists, with H3 the least impaired of the three monkeys. The fourth monkey (MD) received a thalamic lesion which included near-total damage of mid and posterior parts of nMD, bilateral interruption of the fornix with degeneration of MMB, and unilateral damage of cingulate bundle, stria medullaris, midline and anterior nuclei: This monkey made 69% correct responses with 130 sec delays and 61% with 10-item lists. A similar decline in post-operative performance after an equivalent lesion was reported by Aggleton and Mishkin (Neurosci. Abstr., 8, 1982). Hippocampectomized monkeys then received a thalamic lesion and were re-tested with the same task. In all three, nMD was partially spared in its anterior and mid portions, with radical removals of the posterior extent. In all other respects, the lesion resembled that sustained by monkey MD. This time, performance declined to chance: 54%-58% correct responses with 130 sec delays and 47%-56% with 10-item lists.

These preliminary results suggest that the severe memory loss after amygdalo-hippocampal lesions may be attributed to the combined effects of direct damage to the hippocampus and a functional disruption, as a result of amygdalar damage, of a parallel memory system located in the medial dorsal thalamus.

12.11 THALAMIC AMNESIA: MATERIAL SPECIFIC DEFECTS ASSOCIATED WITH DORSOMEDIAL NUCLEUS INFARCTION. F. Morrell, L. de Toledo-Morrell,* L.R. Squire, L. Bieliauskas,* E. Russell* and J. Messer,* Departments of Neurological Sciences, Psychology, Radiology and Cardiology, Rush Medical College, Chicago, Il. 60612 and Department of Psychiatry, UCSD, La Jolla, Ca. 92093

Although there are now several definitive reports of amnesia due to dorsomedial thalamic nucleus lesions, all previously reported cases have been studied long after establishment of the lesion. The present case allowed examination of the evolution of the amnesic syndrome correlated with serial CT scan changes.

The patient is a 53 yr old right handed woman who was studied intensively beginning 3 days after acute onset of a devastating amnesic syndrome. Initial CT scans revealed an area of infarction in the left dorsomedial nucleus of the thalamus. Prominent surrounding edema resulted in elevation of the frontal horn of the lateral ventricle and compression of the right dorsomedial nucleus. At that time, neurological and neuropsychological examination revealed frontal "release" signs, poor performance on the Wisconsin Card Sorting Test, decrease in word fluency, anomic aphasia and a severe amnesia for verbal, non-verbal and spatial material.

Six days after the infarct, all deficits for non-verbal and spatial material had cleared, frontal "release" signs had disappeared and card sorting performance was normal. A second CT scan 7 days after the infarct revealed complete resolution of the surrounding edema and persistence of a discrete lucency entirely replacing the left dorsomedial nucleus. The severe verbal memory deficit has remained. Remote memory tests also revealed generally poor performance.

The persistent deficits in this case are similar to those described in N.A. (Squire and Moore, Ann. Neurol., 6: 503, 1979). Our findings indicate that more widespread disturbance occurs if both thalamic nuclei are disabled as was the case during the early edematous phase. Our observations predict that a right sided thalamic lesion would show visual-spatial rather than verbal deficits (as recently shown by Speedie & Heilman, Arch. Neurol., 40: 183, 1983) and emphasizes the material specific nature of memory deficits in general.

12.12 ACUTE LOSS OF AUTOBIOGRAPHICAL MEMORIES IN AN AMNESIC PATIENT WITH ALCOHOLIC KORSAKOFF'S SYNDROME. N.Butters and L.S. Cermak.* Psychology Service, Boston VA Medical Center, Boston, MA 02130.

Recent studies have suggested that the retrograde of amnesia (RA) of alcoholic Korsakoff patients is related to two possible factors: (1)The alcoholics' failure to learn new information during their years of intoxification, and (2)An acute loss of (or access to) remote memories with the onset of Korsakoff's syndrome. To demonstrate that acute, extended losses of remote memories do occur in some amnesias, a single-case study of a scientist (Patient P.Z.) who developed alcoholic Korsakoff's syndrome three years following the publication of his autobiography was conducted. Since P.Z. must have had intimate knowledge of the information in his autobiography prior to his illness, any failure to recall this material could not be attributed to a deficit in original learning. In addition, P.Z. was administered tests of famous faces, public events and famous scientists from all decades between 1930 and 1980.

The results showed that P.Z., like other alcoholic Korsakoff patients, was severely impaired in his recall of public events and famous faces, with some sparing of very remote memories. On the test of autobiographical information, P.Z. could only recall facts from the first 25 years of his life. A similar temporally-graded RA was found in his recall of famous scientists, most of whom P.Z. had known on a professional and/or personal basis. These results suggest that P.Z.'s, and perhaps all Korsakoff patients', RA is due primarily to an acute and extended loss of old memories with the onset of Wernicke's encephalopathy. The fact that P.Z.'s loss of remote memories is temporally graded may indicate that information acquired during the past 20 years was less stable than facts learned 40 years previously. This weakness, and thus greater vulnerability, of recent memories may reflect the neurotoxic effects of long-term alcoholism. Supported by the VA Medical Research Service and NIAAA grant AA-00187.

12.13 OBJECT RECOGNITION IMPAIRED BY VENTROMEDIAL BUT NOT DORSOLATERAL PREFRONTAL CORTICAL LESIONS IN MONKEYS. Mortimer Mishkin and Jocelyne Bachevalier*. Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Within the prefrontal region of the monkey, least is known functionally about the ventromedial cortex, an area to which the magnocellular division of nucleus medialis dorsalis (MDmc) and the anterior thalamic nuclei (AntN) project. Since MDmc and AntN are major thalamic targets of the amygdala and hippocampus, and since these limbo-thalamic pathways appear to be involved in memory functions (Mishkin, Phil. Trans. R. Soc. Lond., B298:1982), we tested whether the prefrontal targets of these two limbo-thalamic pathways might also be involved in memory.

Nine cynomolgus monkeys were trained preoperatively in a trial-unique recognition task in which they were required to distinguish a completely novel object from a sample object presented 10 sec previously (delayed nonmatching-to-sample). After reaching a criterion of 90 correct responses in 100 trials, three monkeys each underwent bilateral lesions of either dorsolateral prefrontal cortex (DL), ventromedial prefrontal cortex (VM), or neither. DL lesions extended from the frontal pole to the depth of the arcuate sulcus, and from the ventral lip of the principal sulcus to the midline. VM lesions included middle and medial orbital cortex (targets of MDmc) as well as subcallosal and anterior cingulate cortex (targets of AntN). Postoperatively, all animals were retrained on the basic recognition task to a 90% criterion. Animals that did not reattain the criterion in 1500 trials were given 500 additional trials with a correction procedure.

Monkeys with DL lesions were slightly impaired, reattaining the 90% criterion in an average of 230 trials as compared with 0 trials for the unoperated controls. In sharp contrast, the monkeys with VM lesions were severely impaired, failing to relearn the recognition task within the limits of testing and achieving an average of only 75 correct responses in the last 100 trials prior to correction training. With correction training, their performance improved to an average of 85% correct in the final 100 trials.

Comparison of these results in recognition memory with those obtained earlier on spatial delayed response (Pribram et al, J. Comp. Physiol. Psychol., 45:1952) suggests that: (i) presumably because of its connections with the limbo-thalamic pathways, ventromedial prefrontal cortex may be more important than dorsolateral prefrontal cortex in general memory processes and (ii) the classical delayed-response deficit after dorsolateral prefrontal lesions may represent a perceptual-mnemonic impairment in spatial functions rather than a strictly mnemonic one.

12.14 AMNESIA FOLLOWING VENTROMEDIAL FRONTAL LOBE LESIONS. A.R. Damasio,* N.R. Graff-Radford,* P.J. Eslinger and N. Kassell,*Depts. of Neurology (Div. Behavioral Neurology) and Surgery (Div. Neurosurgery), University of Iowa College of Medicine, Iowa City, IA 52242.

Although patients with rupture of anterior communicating artery (ACoA) aneurysms have been known to develop behavioral disturbances, the precise nature and anatomical underpinnings of the syndrome have not been elucidated. Here we describe a series of 5 patients with damage to the ventromedial frontal lobe as a result of ACoA aneurysm rupture. On the basis of CT scan analysis and of intraoperative reports and photographs, we localized damage to the orbitofrontal and basal forebrain regions, i.e. the gyrus rectus, the septal nuclei, the nucleus accumbens, the substantia innominata and related pathways, i.e. the precommissural fornix.

The initial stages of the disturbance were hallmarked by bizarre fabrications and an inability to distinguish between real and confabulated events (e.g. one patient thought he was a space commander at the time of the Columbia space shuttle; another described how she shopped in the Falklands Islands at the time of the war). As the early phase resolved, a more circumscribed memory disorder emerged. Impairment was especially prominent for anterograde and retrograde contextual memory. The beneficial effect of cuing suggested that retrieval rather than encoding was the major defect. The amnesia was accompanied by personality changes in the form of social disinhibition and mild elevation of mood. The syndrome was markedly worse in the bilateral cases but in both unilateral and bilateral varieties, the characteristics and magnitude of the syndrome make it distinguishable from that seen in patients HM, NA, DRB (herpes encephalitis) as well as in the alcoholic patients.

We hypothesize that damage to basal forebrain (a) disrupts function in the hippocampus, a structure with which it is reciprocally connected, and (b) blocks cholinergic innervation of widespread cortical regions. (Supported by a University of Iowa Faculty Scholar Award to ARD and Grant NS 06720-02 to PJE).

12.15 EFFECTS OF FOCAL BRAIN LESIONS ON SENSITIVITY TO FREQUENCY OF OCCURRENCE. M.L. Smith* and B. Milner. Dept. of Psychology and the Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Automatic, as opposed to effortful, memory processes are those that draw minimal attentional capacity, occur without intention to remember and do not interfere with other ongoing activity (Hasher & Zacks, 1979). Studies with normal subjects have identified three types of information that are automatically encoded: spatial location, temporal order and frequency of occurrence. Smith and Milner (1981) have demonstrated an impairment in recall of spatial location after right temporal lobectomy that invaded the hippocampal region. Patients with right frontal lesions performed normally on this task but have marked deficits in the temporal ordering of recent events (Milner, 1982). The finding of different critical areas within the right hemisphere for the recall of these two types of automatically encoded information led us to explore the effect of such lesions on the recall of frequency of occurrence.

The study included patients with excisions of the left temporal (LT), right temporal (RT) or right frontal lobe (RF), and normal control subjects (NC). Lists of words or abstract designs, with the stimuli occurring 1,3,5,7 or 9 times, were presented. Each list was followed immediately by a frequency-estimation test comprising one example of each stimulus from the original list interspersed with four new stimuli. The subject was required to estimate the number of times each item had appeared in the first list.

There were no differences between the groups on the verbal task. With the designs, all patient groups were impaired at frequency 9; however, the impairment appeared first and was most pronounced in the RF patients, who not only underestimated the number of times they had seen the designs, but also did not distinguish between the frequencies 5, 7 and 9. Although few RT patients had extensive hippocampal lesions, it was evident that such patients did not differ from those with little or no hippocampal removal. Thus, the right frontal lobe appears to be involved in the automatic encoding of frequency of occurrence as well as in recency discrimination. The negative findings for the right hippocampal region on these two tasks contrast with the demonstrated role of this region in the automatic processing of spatial location.

CATECHOLAMINES: DOPAMINE RECEPTORS I

13.1 PARTIAL PURIFICATION OF D_2 DOPAMINE RECEPTORS BY AFFINITY CHROMATOGRAPHY. B. Chan* and B.K. Madras. (SPON: R.H. Ackerman), Psychopharmacology Section, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, canada M5T 1R8, and Department of Pharmacology, University of Toronto.

Partial purification of the D_2 dopamine receptor has been achieved with isoelectric focussing (Davis et al, Lilly et al, Madras et al, Soc. Neurosci. Abstr. 7, 10, 1981).

To further advance dopamine receptor purification, affinity chromatography of the receptor was performed using a derivative of haloperidol, dehydroxyamino-haloperidol (P. Seeman, H. Pieper, H. Machleidt) linked to a Sepharose gel. The compound has a lower affinity for the receptor than haloperidol.

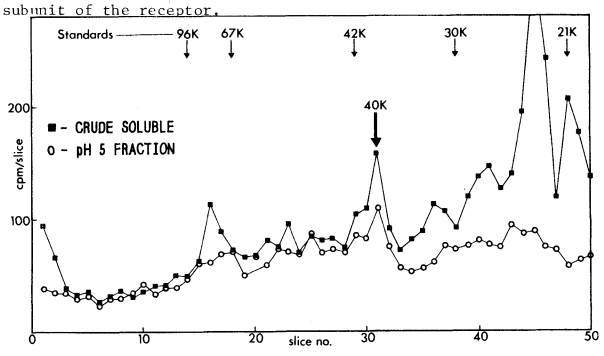
Canine striatum membranes were solubilized with digitonin and incubated with either butyrophenone-linked or unlinked gel. With prolonged exposure to either gel preparation, (16h at 4°C) the soluble material was depleted (95%) of receptor sites. After washing with buffer to remove non-specifically adsorbed proteins, the receptor was desorbed with a NaCl-spiperone solution. The supernatant of the centrifuged gel was dialyzed overnight and assayed for receptor binding. Specific binding to the receptor was detected using ^{3}H-spiperone, 4 nM, and (+)-butaclamol, 1 µM. Under optimal conditions 50-60% of the receptor and 10% or less of the protein was recovered from the gel. Unlinked gel, treated in an identical manner, yielded about 10% of the receptor after desorption. Preliminary data indicate that higher purification can be attained if the partially purified receptor is recycled through the gel. Affinity chromatography may prove to be a useful method for dopamine receptor purification.

Supported by grants from the Ontario Mental Health Foundation and the Bickell foundation.

13.2 D_2 DOPAMINE RECEPTOR PURIFICATION USING ^{3}H-NCA: IDENTIFICATION OF A 40,000 DALTON BINDING PROTEIN. L.B. Lilly*& P. Seeman (Spon: Y. Israel). Dept. Pharmacol., Univ. of Toronto, Toronto.

Since ^{3}H-NCA (N-chlorethylnorapomorphine; Dr. C. Filer, NEN, Boston) binds to more than one site, we have here used the isoelectric point of the D_2 receptor (5.06; Lilly et al., Soc. Neurosci. Abstr. 7: 10, 1981) to isolate a single protein which binds ^{3}H-NCA.

Canine striatal membranes prepared in Mg^{2+}-containing Tris-ion buffers were labelled with 50 nM ^{3}H-NCA for 30 min at 30°C. The membranes were washed 3-5x until all free ligand had been removed, and solubilized with 1% digitonin; about 15% of the counts appeared in the soluble fraction. This material was introduced into a vertical isoelectric focusing column containing a pH 3-10 gradient. Two binding peaks resulted from focusing, at pH 5 and pH 7.5. To determine if a single protein has been focused at pH 5, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed. Crude soluble fraction and the pH 5 fraction were each incubated in 50 mM Tris, 2% SDS and 5% mercaptoethanol prior to their application to a 10% gel. The gel was subsequently sliced and counted. In the soluble fraction and under denaturing conditions, ^{3}H-NCA labelled several proteins, including a prominent peak with a molecular weight of 40,000 daltons (see Fig.). In contrast, the column fraction appears to contain only a single 40,000 dalton peak. This peak may be the D_2 receptor; however, recent experiments using radiation inactivation have shown the intact receptor to have a molecular size of 123,000 daltons (Lilly et al., Mol. Pharm., in press, 1983); thus, the 40,000 dalton protein may be only a ligand-binding submit of the receptor.



13.4 DOPAMINE RECEPTOR TURNOVER RATES IN RAT STRIATUM ARE AGE-DEPENDENT. S.E. Leff, R. Gariano* and Ian Creese. Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

Aging in rodents and primates is accompanied by non-uniform alterations in CNS structure and biochemical function which affect behavioral and neurochemical responses to pharmacological treatment. We now report that after irreversible blockade with a dopamine receptor alkylating agent, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), CNS D-2 dopamine receptor turnover/recovery rates show an age-dependent decline. Male Sprague-Dawley rats (22-30 days or 9-12 months old) were injected with 8 mg/kg i.p. EEDQ eliciting 80-90% inhibition of D-2 receptor specific [^{3}H]spiperone binding in membrane homogenates of striatum 4-6 hours postinjection. The recovery of [^{3}H]spiperone binding activity after EEDQ blockade was dramatically faster for young rats (30 days old; $t_{1/2}$=2.5 days) compared to mid-life/mature rats (9-12 month old; $t_{1/2}$=4.5 days). Receptor levels were determined by saturation analyses which indicated that the changes in binding produced by alkylation and during recovery were the results of alterations in Bmax values. Prolonged intracerebral treatment (11 hours) with the protein synthesis inhibitor, cycloheximide, indicates that receptor recovery rates are dependent upon protein synthesis. Radioreceptor assays of EEDQ activity from sera of injected rats indicate that EEDQ is rapidly catabolized in vivo. Therefore different rates of EEDQ metabolism could not account for the age-differences in receptor recovery rates. The slower recovery rate we have observed in older rats may be consistent with a reported aging-related deficit in dopamine receptor up-regulation after chronic dopamine antagonist treatment in mice (Severson et al., Soc. Neurosci. Abstr. 5:11, 1979). These findings are consistent with clinical observations that older patients generally require lower doses of antipsychotic drugs for therapeutic effect and present a higher risk for the development of parkinsonian-like extrapyramidal side-effects to antipsychotic drugs. In preliminary, related experiments we find that chronic reserpine which leads to a dopamine receptor up-regulation markedly accelerates receptor recovery. In summary we suggest that theories of CNS neurotransmitter receptor changes produced by pharmacological treatments, or in aging or diseased brains should now take receptor turnover rates into consideration and not rely solely on absolute level determination for functional correlations. Supported by PHS MH32990 (I.C.), and NIMH Predoctoral Fellowship MH08918 (S.L.).

13.5 ELEVATION OF D_2 DOPAMINE RECEPTORS IN SCHIZOPHRENIA BRAIN. P. Seeman, C. Ulpian* and W.W. Tourtellotte*. Pharmacology Dept., Univ. of Toronto, Canada; Wadsworth Hospital, Los Angeles.

Although it had been originally shown that the binding of ^{3}H-neuroleptics to dopamine receptors in schizophrenic brain was elevated by 30% to 100% (Refs. 1-3), we have recently found that both the K_D and the density (B_{max}) of binding sites for ^{3}H-spiperone depend on the final concentration of tissue protein (Ref. 4). Thus, we re-studied the K_D and density of D_2 receptors in schizophrenic putamen (P), caudate nucleus (C) and nucleus accumbens (A), using a final tissue concentration of less than 1 mg of original tissue per final ml of incubate. For all schizophrenic tissues (medicated and non-medicated patients), the density of D_2 dopamine receptors was approximately double the control value. The Fig. shows data for tissues from Toronto (squares) and Los Angeles (circles). It is now essential to examine a larger series of post-mortem tissues, particularly from patients who had been actively schizophrenic but who had not been medicated with neuroleptic drugs.

(We thank Mr. Jim Kelly for his assistance. Supported by the Friends of Schizophrenics, the Ontario Mental Health Fdn., and The Medical Research Council.)

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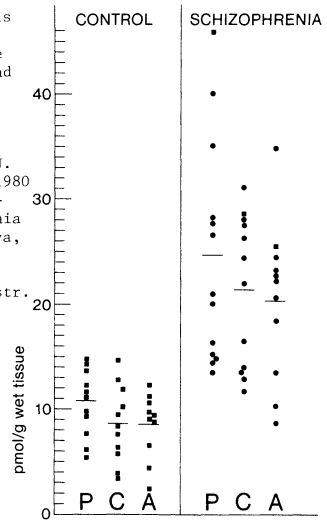
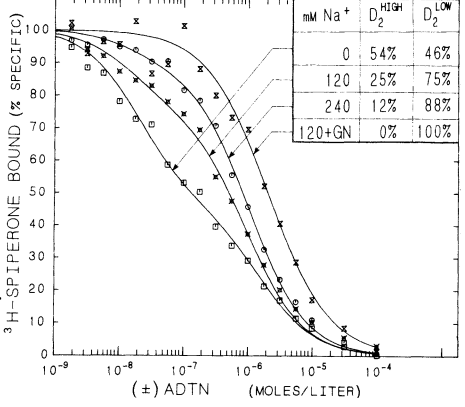
2. T. Lee & P. Seeman, Amer. J. Psychiat. 137: 191-197, 1980
3. P. Seeman & T. Lee, in Psychobiology of Schizophrenia ed. by M. Namba & H. Kaiya, Pergamon, 1982, 241-247.
4. P. Seeman, C. Ulpian & J. Wells. Soc. Neurosci. Abstr. 8: 718, 1982.



13.6 SODIUM IONS CONVERT BRAIN D_2 DOPAMINE RECEPTORS FROM HIGH TO LOW AFFINITY FOR DOPAMINE AGONISTS. D. Grigoriadis* & P. Seeman (Spon: N. Wiener). Pharmacol. Dept., Univ. of Toronto, Toronto.

Although it is known that guanine nucleotides lower the affinity of brain dopamine receptors for dopamine, it has not yet been possible to obtain a single homogeneous population of low-affinity dopamine receptors. Huff & Molinoff, as well as Wreggett et al., for example, found that even in the presence of 300 µM GTP there still remained about 23% of the ^{3}H-spiperone binding sites which had high affinity for dopamine. We now report that 300 µM Gpp(NH)p in the presence of Na^+ converts the high-affinity receptors (D_2^{High}) into a single population of sites with low affinity for dopamine agonists when done in the presence of ketanserin to preclude ^{3}H-spiperone from binding to serotonergic sites.

Rat brain striata were homogenized in sodium-free buffer (50 mM Tris-HCl, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 1 mM EDTA, 0.1% ascorbic acid, 12 µM nialamide). The tissue was pre-incubated for 10 min at 37°C, chilled, and added to incubation tubes containing 80 pM ^{3}H-spiperone and 50 nM ketanserin (final conc.); the contents were filtered after 45 min at 37°C. Specific binding was that inhibited by 1 µM (+)-butaclamol. The Fig. illustrates that in the absence of Na^+ the population of dopamine receptors consisted of 54% in the high-affinity state (K_D=20 nM) and 46% in the low affinity form (K_D=2 µM) for the aminotetralin ADTN. However, in the presence of 120 mM NaCl 75% of the receptors were D_2^{Low}. With high sodium (240 mM) 88% of the receptors were D_2^{Low}, and in the presence of 300 µM Gpp(NH)p and 120 mM NaCl, all the sites were completely converted to a single population of D_2^{Low} receptors. (We thank Dr. Susan George & Dr. J. Wells for advice).

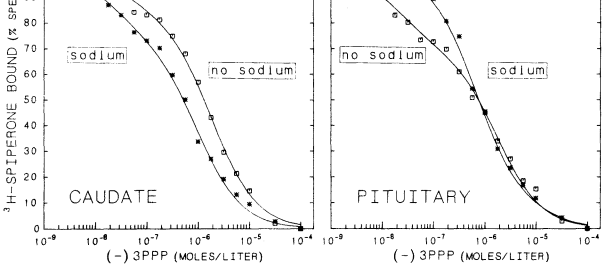


1) Huff & Molinoff, P.N.A.S., 79: 7561 (1982).
2) K. Wreggett, S. George & P. Seeman, Soc. Neurosci.Abstr. 8: 719 (1982).

13.7 THE BRAIN D_2 DOPAMINE RECEPTOR DIFFERS FROM THE PITUITARY D_2 RECEPTOR, AS DETECTED BY (-)3PPP. Susan R. George, Masayuki Watanabe* & Philip Seeman. Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada, M5S 1A8.

Although D_2 dopamine receptors in brain and pituitary have hitherto been considered identical, we now report that it is possible to differentiate them by means of (-)N,n-propyl,3-(3-hydroxy-phenyl)-piperidine or (-)3PPP.

In the complete absence of Na^+, (-)3PPP inhibited the binding of 200 pM ^{3}H-spiperone to porcine anterior pituitary in two phases, 30% with high affinity (D_2^{High}), and 70% with low affinity (D_2^{Low}) as is typical of dopamine agonists. In the presence of 100 mM Na^+, (-)3PPP detected only a single population of D_2^{Low} sites, indicating conversion of D_2^{High} to D_2^{Low} by Na^+; guanine nucleotides (GN) also did the same. In calf caudate, using excess ketanserin to occlude ^{3}H-spiperone binding to serotonergic receptors, (-)3PPP detected only a single population of D_2 sites, whether in the absence or presence of Na^+. The affinity of (-)3PPP for the D_2 sites was increased by Na^+, but unaffected by GN, both effects, we found, being typical of antagonists. Data for (+)3PPP in brain and pituitary were similar to that described for (-)3PPP in pituitary. Thus, as shown in the fig., the addition of Na^+ to pituitary results in the conversion of D_2^{High} sites detected by (-)3PPP into D_2^{Low} sites, and an enhancement of the affinity of (-)3PPP for D_2 sites in brain, suggesting that both enantiomers of 3PPP are agonists in pituitary, while in caudate, (+)3PPP is an agonist and (-)3PPP an antagonist. The opposite effects of Na^+ and GN on (-)3PPP provide the first evidence that the molecular properties of brain and pituitary receptors may differ.



13.8 NEUROLEPTICS EXHIBIT NONCOMPETITIVE EFFECTS AT DOPAMINE RECEPTORS. Jonathan E. Freedman[1], Robert J. Gould[2] and Solomon H. Snyder[2]. [1]Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06508, and [2]Depts. of Neuroscience, Pharmacology and Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

We have studied the kinetics of radioligand binding to various classes of dopamine receptor binding sites in rat corpus striatum membrane homogenates. D_1 receptor sites were labeled with the novel ligand ^{3}H-fluphenazine, which binds about 80°/° to D_1 sites and 20°/° to D_2 sites, with a pharmacologic profile resembling that of ^{3}H-cis-flupenthixol, but with an improved signal-to-noise ratio (2000 cpm specific binding, specific binding 80°/° of total binding). ^{3}H-Spiperone was used for D_2 receptor sites, while D_3 and D_4 high affinity agonist sites were labeled with ^{3}H-apomorphine.

The rate of dissociation of ^{3}H-ligands was accelerated in the presence of certain nonradioactive drugs, suggesting more than one class of binding sites. The dissociation of ^{3}H-apomorphine was markedly enhanced by butyrophenones, while ^{3}H-spiperone dissociated faster in the presence of dopamine. This indicates the existence of two distinct sites on dopamine receptors: a butyrophenone site and a dopamine recognition site which interact allosterically. Phenothiazines also accelerated ^{3}H-apomorphine dissociation, but dopamine did not accelerate ^{3}H-fluphenazine dissociation. Butyrophenones accelerated ^{3}H-fluphenazine dissociation, but required concentrations (100 nM) consistent with D_1 and not D_2 receptor-mediated effects, suggesting different mechanisms for phenothiazines and butyrophenones at D_1 receptors. Preliminary evidence suggests similar effects with ergots and sulpiride.

In contrast to the long-standing assumption that neuroleptics inhibit dopamine receptors competitively, our results indicate that butyrophenone neuroleptics do not interact with the dopamine recognition site. Phenothiazine neuroleptics also show some allosteric effects relative to dopamine, at least at D_2 receptors. Since D_1 and D_3 binding sites display a 1:1 stoichiometry, as do D_2 and D_4 sites, our results raise the possibility that D_3 sites may actually be the dopamine recognition sites of D_1 receptor molecules, while D_4 sites may be the dopamine recognition sites of D_2 receptor molecules.

13.9 NEUROLEPTIC RECEPTOR LABELING IN VIVO WITH [^{11}C]SPIROPERIDOL. C. D. Arnett*, J. S. Fowler*, A. P. Wolf*, J. Logan* and R. R. MacGregor* (SPON: L. Giron). Department of Chemistry, Brookhaven National Laboratory, Upton, NY 11973.

A model was developed and evaluated for the external detection of specific neuroleptic receptors in living brain using positron emission transaxial tomography (PETT) and the neuroleptic spiroperidol radiolabeled with the positron-emitting nuclide carbon-11. Pretreatment with nonradioactive butaclamol was used to differentiate the specifically bound pool of radioactivity from nonspecifically bound and free pools. The time course of each of these three pools over 60 min was determined in rats by an ex vivo experiment requiring killing of the animals at various times after injection and homogenization and rapid filtration of brain regions to separate bound pools from the free pool (Arnett, C. D., et al,. J. Neurochem. 40: 455-459, 1983). The proper choice of dose and timing for in vivo PETT studies was made on the basis of the results of this study.

Taking advantage of the short half-life of carbon-11 (20 min), this model was applied to PETT studies in baboons by using a double injection paradigm of [^{11}C]spiroperidol with an intervening injection of a high dose of a nonradioactive neuroleptic to block specific receptors. Pretreatment with the pharmacologically inactive (-)-butaclamol served as an injection control and allowed the normal distribution of [^{11}C]spiroperidol among the three brain pools. A high dose of (+)-butaclamol given after the decay of most of the radioactivity from the first [^{11}C]spiroperidol injection effectively blocked the specific receptors and allowed the subsequently administered second dose of [^{11}C]spiroperidol to distribute only to the nonspecifically bound and free pools. PETT scans taken after the second dose were subtracted from those taken after the first dose (normalizing for dose differences) to produce an accurate map of neuroleptic receptors in vivo.

These results demonstrate the successful application of this approach to the PETT mapping of neuroleptic receptors in live baboons. The proper choice of a highly selective drug for the intervening blocking dose can allow differentiation of [^{11}C]-spiroperidol binding to D-2 dopamine receptors, serotonin receptors, etc. Using different radioligands, this paradigm should be applicable to PETT studies of other types of receptors as well. This model demonstrates the potential utility of [^{11}C]-spiroperidol and PETT for mapping neuroleptic receptors in the human brain.

This research was supported by the U. S. Department of Energy, Office of Health and Environmental Research and by NIH Grant No. NS-15638.

13.10 THE D-2 DOPAMINE RECEPTOR IN THE INTERMEDIATE LOBE OF THE RAT PITUITARY GLAND REGULATES THE SYNTHESIS OF PROOPIOMELANOCORTIN AND MELANOTROPHIC HORMONES. M. Beaulieu, M.E. Goldman, T.E. Cote* K. Miyazaki* and J.W. Kebabian*. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

The intermediate lobe of the rat pituitary gland is innervated by dopaminergic neurons originating in the brain. A post-junctional D-2 dopamine receptor occurs upon the melanotrophs, the cells synthesizing the prohormone, proopiomelanocortin (POMC) and processing it into several molecules ressembling α-melanocyte stimulating hormone (α-MSH). This D-2 receptor is stimulated by bromocriptine (CB 154) and blocked by spiroperidol (SPIRO).

In vivo treatment of male Sprague Dawley rats with CB 154 (10 mg/kg/d; 2 d) lowers the content of messenger RNA (mRNA) directing the synthesis of POMC by 60%, diminishes the capacity of the IL to synthesize POMC by 60%, decreases the capacity of the IL to synthesize α-MSH-like molecules by 65%, and blocks the secretion of α-MSH-like peptides from the IL. Furthermore, prolonged treatment with CB 154 causes the IL to atrophy and diminishes the IL content of α-MSH-like molecules by 70%. Because a similar number of IL cells are retrieved from enzymatic dispersions of vehicle- and CB 154-treated animals, it seems probable that the CB 154 treatment is decreasing the capacity of each melanotroph to synthesize α-MSH-like molecules.

In vivo treatment of male Sprague Dawley rats with SPIRO (10 mg/kg b.i.d; 1 d) increases the IL content of mRNA directing POMC synthesis by 75%, increases the capacity of the IL to synthesize both POMC and α-MSH-like peptides by 250% and enhances the release of α-MSH-like molecules from the IL. Furthermore, treatment with SPIRO prevents the inhibitory effects of CB 154 upon POMC and α-MSH synthesis.

The present data demonstrate that the capacity of the IL to synthesize POMC and α-MSH-like peptides is variable and is regulated by the D-2 dopamine receptor. Dopamine released from nerve terminals in the IL stimulates the D-2 receptor and thereby keeps the synthetic capacity of the IL submaximal. CB 154 stimulates the D-2 receptor and further decreases the synthetic capacity of the IL. SPIRO blocks the D-2 receptor, thereby removing this inhibitory constraint; this accounts for the observed stimulatory effects of SPIRO, as well as the ability of SPIRO to block the effects of CB 154.

13.11 COMPARISON OF ESTROGEN AND HALOPERIDOL ON THE ELEVATION OF PROLACTIN SERUM LEVELS AND THE INCREASE IN DENSITY OF THE STRIATAL DOPAMINE RECEPTORS. Robert E. Hruska. Dept. of Biochemical Pharmacology, State University of New York, Buffalo, NY 14260.

Prolactin (PRL) serum levels are elevated by the administration of either estrogen or haloperidol (HAL). Several groups have also reported that either estrogen or HAL administration will increase the density of the striatal dopamine (DA) receptors. This suggested a link between the two treatments. Interestingly, it appears that each of these treatments has diminished effects on striatal DA receptor density in rats which are hypophysectomized (Hypox), and hence have no PRL available. This led to the postulation that a pituitary factor, namely PRL, was involved in the regulation of striatal DA receptors. When PRL was chronically administered to intact and Hypox male rats, it was able to increase the density of the striatal DA receptors in both cases, although Hypox rats required a larger dose of PRL. Importantly, the pituitary does not appear to be necessary for the maintenance of the striatal DA receptors, since Hypox rats have a normal density and affinity of these receptors.

Further studies have indicated that the regulation of striatal DA receptors by PRL is complex. Male, adult rats were injected s.c. with 17β-estradiol valerate at doses from 15-500 µg/rat. At various times afterward the rats were decapitated, trunk blood collected for PRL measurements, and the striatal tissue prepared for DA receptor measurements. There was a time-dependent relationship between the elevation in serum PRL levels and the elevation in the striatal DA receptor density. While PRL serum levels were maximally elevated at two days, the receptors were maximally elevated at four to six days. By ten days the elevated density of receptors had subsided to non-significant levels, while the serum levels remained significantly elevated above control levels. Therefore, the increase in striatal DA receptor density produced by estrogen administration may depend on the novelty of the increase in PRL levels or on the rise of serum PRL levels. These observations also suggest that the regulation of striatal DA receptors appears to be different after chronic HAL treatment compared to chronic estrogen treatment. After estrogen, PRL levels remain elevated while the receptor density returns to control levels. After HAL the levels of PRL return to control levels while the receptor density remains elevated. HAL also acts directly on striatal DA receptors and therefore may act via more than one mechanism. The elevation in serum PRL levels and striatal DA receptor density also appeared to be related to the dose of estrogen used. Maximal effects on both parameters, at six days after injection, were obtained by the 60 µg/rat dose of estrogen. Therefore, the modification of striatal DA receptors by PRL is multifaceted. (Supported by PMAF Res. Starter Grant.)

13.12 THE POSSIBLE ROLE OF 2-HYDROXYESTRADIOL IN THE DEVELOPMENT OF ESTROGEN-INDUCED STRIATAL DOPAMINE RECEPTOR HYPERSENSITIVITY. J.K. Clopton* and J.H. Gordon. Dept Pharmacology, Univ Hlth Scis/The Chicago Med Sch, N. Chicago, IL 60064.

Several reports have indicated that the administration of estrogen will result in the development of a striatal dopamine receptor hypersensitivity. However, all of these reports use non-physiologic (i.e. pharmacologic) doses and/or paradigms, thus posing the question of the physiologic relevance of these observations. In the first experiment we confirmed our previous observations of a biphasic response in dopamine receptor sensitivity following estradiol benzoate (EB) in doses of 10, 30 and 100 µg/kg. The first phase, seen at 24 hrs after the last dose of EB, was a dose related suppression of dopamine receptor sensitivity. The second phase was the development of a dopamine receptor hypersensitivity which was quantitated 72 hrs after the last dose of EB, and was only seen following the two higher doses of EB. These data thus confirm our previous work and support the hypothesis that the development of a dopamine receptor hypersensitivity following EB may be a pharmacologic phenomenon.

In the second experiment 2-hydroxyestradiol (2-OHE) was administered to ovariectomized rats in doses of 0.3, 1.0, 3.0, 10.0, 30.0 and 100.0 µg/kg for 3 days and apomorphine-induced stereotypy was quantitated at 24 and 72 hrs after the last dose. Doses of 2-OHE of 1.0 µg/kg or greater produced a striatal dopamine receptor hypersensitivity at both 24 and 72 hrs after the last injection. The threshold dose for 2-OHE was between 0.3 and 1.0 µg/kg, while the threshold dose for EB was between 10 and 30 µg/kg. Thus only 3% or less of the injected EB would need to be converted to 2-OHE for this metabolite to mediate the development of the hypersensitivity.

Some reports have suggested that the enzyme responsible for the conversion of estrogen to catecholestrogen is a microsomal enzyme. In the third experiment we administered piperonyl butoxide (PBO) a microsomal enzyme inhibitor in an attempt to block the development of the estrogen-induced dopamine receptor hypersensitivity. PBO, 400 mg/kg, was administered 4 hrs prior to the administration of a single dose of estradiol (3, 10, 30 and 100 µg/kg). Animals were then tested for apomorphine-induced stereotypy at 24 and 72 hrs after the injection of the estradiol. The administration of PBO shifted the dose response curve to the right, with the PBO treated animals showing about a 100 fold decrease in sensitivity to estradiol. Studies of the in vitro metabolism of ^{3}H-estradiol indicated a significant reduction in radioactivity in the catechol fraction following the administration of PBO.

These studies indicate that the two responses seen in striatal dopamine receptor sensitivity following estrogen maybe mediated by seperate molecular mechanisms. The association of the hypersensitive phase with pharmacological doses or paradigms, the development of a similar hypersensitiviy following the administration of the 2-OHE metabolite and the attenuation of the estradiol-induced response in PBO treated animals all suggest that the estrogen-induced striatal dopamine receptor hypersensitivity may be mediated by the catechol metabolites.

13.PO POSSIBLE ROLE OF THE DA-2 RECEPTOR IN PHENCYCLIDINE PSYCHOSIS. A.J. Giannini, S.M. Kalavsky, R.H. Loiselle,* M.C. Giannini,* and W.A. Price.* Psychiatry Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44222.

Previous treatment of phencyclidine (PCP) psychosis in our department has demonstrated the greater efficacy of dopaminergic blockers relative to opiates, benzodiazepenes, physostigmine and hydroxyzine (Castellani & Giannini a,b; Giannini & Castellani; Giannini & Price). The role of DA-1 and DA-2 receptors in this type of psychosis was tested as described below.

Twelve patients were seen in acute PCP psychosis. On a random basis six were treated with chlorpromazine (CPZ), a predominantly DA-1 blocker, and the remainder with haloperidol, a predominantly DA-2 blocker. Two equivalent dosages of either CPZ or haloperidol were injected at 20-minute intervals. Patients were then rated on a BPRS by a blind observer. All twelve responded equally well on anxiety, excitement and tension scales. Haloperidol-treated patients responded in a significantly better fashion on conceptual disorganization ($p > .05$) and hallucination scales ($p > .05$). These preliminary results suggest a role for the DA-2 receptor in PCP psychosis.

13.PO ROLE OF AMBIENT ANIONS IN RELIEVING SEROTONERGIC ANXIETY IN A NONCATIONIZED ENVIRONMENT. A.J. Giannini, R.L. Gilliland, R.H. Loiselle* and M.C. Giannini.* Psychiatry Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44222.

Twelve male students were placed in a highly stressed learning situation. During this sixty-minute period heart rate rose from 70.5/min. to 80.1/min. No differences were seen in platelet serotonin levels measured before and after the learning situation. Repetition of this paradigm with the same students in a highly anionized environment resulted in a change in heart rate from 71.6/min. to 74.2/min. Again no differences were seen in platelet serotonin levels measured before and after one hour of ionization.

These findings are different than those reported by Kreuger (19) who found stress to be associated with a rise in serotonin. Olivier's (19) work also noted that atmospheric anions would lower serotonin levels and that these levels would be inversely related to physiological parameters of anxiety. The above results would suggest, therefore, that the role of anions in relieving anxiety may be independent of serotonin.

13.PO DIFFERENTIAL CHANGES IN RAT BRAIN DOPAMINE AND SEROTONIN RECEPTORS AFTER CHRONIC TREATMENT WITH TYPICAL AND ATYPICAL ANTI-PSYCHOTIC DRUGS. Tyrone Lee and Siu Wa Tang. Psychopharmacology Unit, Clarke Institute of Psyciatry, Toronto, Canada M5T 1R8.

The blockade of central dopamine receptors has been proposed to be the major action of antipsychotic drugs such as haloperidol or chlorpromazine in controlling psychotic symptoms. Chronic administration of typical antipsychotic drugs is known to induce dopamine receptor supersensitivity in the central nervous system. In order to demonstrate if the atypical antipsychotic drugs share this same action, brain dopamine receptor numbers were measured in rats chronically treated with haloperidol, loxapine, clozapine or sulpiride. Serotonin receptor numbers were also measured for comparison.

Adult male Wistar rats (175-200 gm) were treated with once daily i.p. with haloperidol (5 mg/kg), loxapine (5 mg/kg), clozapine (30 mg/kg) or sulpiride (100 mg/kg) for 28 days. Control rats received equivalent volume of vehicle. Forty-eight hours after the last injection animals were sacrificed and striatal and frontal cortical homogenates were prepared for receptor binding assays using ^{3}H-spiperone. Dopamine receptor (D_2) was defined by 10 uM sulpiride and serotonin receptor (S_2) was defined by 50 nM mianserin. Receptor density (B_{max}, in femtomoles/mg protein) and affinity (K_D, in nM) were determined by Scatchard analysis. The results are as follows:

DRUG	DOPAMINE (D_2) RECEPTOR			SEROTONIN (S_2) RECEPTOR		
	B_{max}	%control	N	B_{max}	%control	N
Control	460±49*	100	4	412±33	100	3
Haloperidol	657±31	143**	3	464±12	113	3
Loxapine	484±43	105	6	167±15	41***	5
Clozapine	476±38	104	4	218±64	53	3
Sulpiride	547±16	119	6	346±31	84	5

*Mean ± S.E.; **$P<0.02$, ***$P<0.01$, Student's t-test.

Chronic exposure to haloperidol in the rat increased the density of dopamine (D_2) receptors (43%) but caused no significant change in the number of serotonin (S_2) receptors. Loxapine, clozapine and sulpiride did not cause any significant change in dopamine receptors but loxapine alone induced a significant reduction in serotonin receptor number (59%). No significant difference was observed in receptor affinities among the different drug groups.

Our present data suggest that not all neuroleptics induce an increase in dopamine receptor number on chronic administration. Loxapine, on the other hand, appeared to potently affect the serotonin system and this unique property so far is known to occur only with antidepressant drugs.

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13.PO MULTIPLE ^{3}H-SPIPERONE BINDING SITES DEFINED BY KETANSERIN IN RAT FRONTAL CORTEX: REGULATION BY ENDOGENOUS SEROTONIN, NEUROLEPTICS AND ANTIDEPRESSANTS. Daiga M. Helmeste and Siu Wa Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ont., CANADA M5T 1R8.

Ketanserin, a putative S_2-serotonin antagonist, has recently been used to specifically define the S_2-serotonin component of ^{3}H-spiperone binding and as a specific tritiated label for S_2-serotonergic sites. We have discovered that a ketanserin baseline (100 nM) defines more than one ^{3}H-spiperone site in the rat frontal cortex [K_{d_1} (nM)=0.3, B_{max_1} (fmol/mg.protein)=300; K_{d_2}=5, B_{max_2}=600]. [Examination of ^{3}H-ketanserin binding to rat frontal cortex (1 µM methysergide baseline) also revealed multiple binding sites]. To determine which of the ^{3}H-spiperone labelled sites are under serotonergic control, changes in binding sites were examined after chronic drug treatments which enhance (d-fenfluramine; 2.5 mg/kg i.p., twice daily for 28 days) or decrease serotonin release (PCPA 300 mg/kg, i.p. for first and last 3 days, 100 mg/kg every second day for days 4 to 18). These were compared to other treatment groups consisting of acute [single or double (2 days) injs., 48 hr before] administration of the antidepressants mianserin, amoxapine and imipramine or the neuroleptics haloperidol, thiothixene, perphenazine, thioridazine and loxapine.

Marked changes in receptor density (B_{max}) were observed in the 0.3nM K_d ^{3}H-spiperone site, as follows (expressed as percentage of controls): chronic d-fenfluramine (50% of control); chronic PCPA (155%); acute mianserin, 10 mg/kg, (57%); acute loxapine, 5 mg/kg, (69%); acute amoxapine, 10 mg/kg, (78%). Other groups showed no significant changes. This 0.3nM K_d ^{3}H-spiperone site can also be preferentially labelled using a 50nM mianserin baseline.

The ability of mianserin to acutely down-regulate the 0.3nM K_d site does not seem to be dependent on intact serotonin stores since chronic PCPA pretreatment did not prevent the mianserin induced down-regulation. The ability of mianserin, loxapine and amoxapine to acutely down-regulate the 0.3nM K_d site did not seem to be related to their high affinities for this site, since S_2-serotonin antagonists with similar affinities [cinanserin or ketanserin 10 mg/kg] had no effects on this site, either in terms of acutely blocking the mianserin effect or in changing the numbers of this site by themselves.

The above data suggests that the 0.3nM K_d ^{3}H-spiperone site is a serotonergic receptor and that its density can be acurely altered by certain compounds used in the treatment of the major psychoses.

13.PO AGONIST INDUCED DECREASES IN AGONIST AFFINITY AT DOPAMINE RECEPTORS. J. L. Klein*, A. C. Andorn*, K. R. Kohegan* (SPON: V. Rowland). Dept. of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

The IC_{50} of dopamine at ^{3}H-spiroperidol receptors in rat striatum is 2840 +/- 357.0 nM (N=5) when obtained in competition studies using a 15 min incubation and 0.2 nM ^{3}H-spiroperidol, 0.01 mM ascorbate, 34.4 mM Na-Hepes, and 0.20 +/- 0.05 mg protein. However, at an incubation time of 60 min the IC_{50} of dopamine is shifted to the right by 2 orders of magnitued to 100,000 +/- 0.0 nM (N=5). Preincubating the tissue for 60 min in the presence of 10 µM dopamine and using this tissue in a 15 min study results in an IC_{50} of 180,000 +/- 50,000 nM (N=2). (The contribution of the dopamine in the preincubate was corrected for). Preincubation of tissue in buffer alone does not change the IC_{50} at 15 min. The addition of pargyline to the preincubation step does not change the results observed at either 15 or 60 min. These findings suggest that agonist can induce decreases in the affinity of the receptor for agonist while sparing antagonist affinity. Such findings are not inconsistent with physiologically relevant receptors, as agonist induced desensitization is well known in other receptor systems (Harden, T. K., Su, S. Y., Perkins, J. P., J. Cyc. Nuc. Res. 5:99, 1979).

Interestingly, the IC_{50} of dopamine at a 60 min incubation is the same as its IC_{50} using a 15 min incubation, but having 100 µM Gpp(NH)p present in the assay. Further study will determine whether agonist induced changes in agonist affinity procede by mechanisms similar to or distinct from guanyl nucleotide induced decreases in ^{3}H-spiroperidol binding in rat striatum.

13.PO GUANYL NUCLEOTIDE INDUCED LOSSES OF ANTAGONIST BINDING AT HIGH AFFINITY DOPAMINE RECEPTORS. A. C. Andorn*, K. R. Kohegan*, J. L. Klein* (SPON: M.E. Maguire). Dept. of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Guanyl nucleotides induce a loss of high affinity ^{3}H-spiroperidol binding in rat striatum as seen in rate association studies in the presence of 100 µM Gpp(NH)p. The loss in binding is also seen in rate dissociation studies. These are biphasic in the absence of Gpp(NH)p ($t_{½\ rapid}$ = 0.1 min; $t_{½\ slow}$=31.5 +/- 9.4 min N=3). In the presence of Gpp(NH)p these studies are monophasic with $t_{½}$= 0.1 min (N=3). Saturation studies show that in the absence of Gpp(NH)p, at least two affinity states for ^{3}H-spiroperidol are present with K_{D_1}=8.7+/-1.7pM, B_{max_1}=45.0+/-17.3 fmol/mg, K_{D_2}=0.25+/-0.14nM, B_{max_2}= 183.3+/-23.0 fmol/mg protein (N=4). The same studies in the presence of 100 µM Gpp(NH)p indicate that only one affinity state is present with an apparent K_D of 0.16 +/- 0.02nM and B_{max} of 223.0 +/- 62.8 fmol/mg protein (N=3), corresponding to the lowest affinity state observed in the absence of nucleotide. The effects of guanyl nucleotide are blocked by the addition of 3.4 mM Mg^{++}, are reversible, and are not produced by 100 µM ATP. Some of these results are similar to the effects of guanyl nucleotide on ^{3}H-agonist binding in the opiate receptor system (Blume, A. J., Proc. Nat. Acad. Sci. 75: 1713, 1978). However, the results presented here demonstrate clear effects of guanyl nucleotides on ^{3}H-antagonist binding.

14.1 AFFERENT AND EFFERENT CONNECTIONS OF THE CLAUSTRUM OF TREE SHREW AND RAT. Teresa L. Neal* and Russell G. Carey. Div. of Neurobiol., Barrow Neurol. Inst., Phoenix, AZ 85013.

In both tree shrew and cat the dorsal claustrum is reciprocally connected with the visual cortex and, in particular, with area 17 (Carey et al., 1979; LeVay & Sherk, 1981). Consensus concerning subcortical connections of the claustrum in these species, however, has been highly elusive. Studies in the cat have tended to doubt the existence of such projections whereas results in the tree shrew (Carey & Bear, 1979) indicate their existence. The purpose of the present study was two-fold: first, to verify the existence of these projections by making injections directly into the tree shrew thalamus as well as in the claustrum; and second, to determine whether the pattern of connections observed in cat or tree shrew is representative of the pattern found in a third mammal, the Long-Evans rat.

After injections of WGA-HRP into the tree shrew claustrum, labeled cells and terminals in the thalamus are primarily localized within the lateral intermediate nucleus (Li) and the nuclear region embedded within the external medullary lamina (EML). Small electrophoretic injections into the same thalamic regions verify the existence of these reciprocal connections. After these injections a focus of moderate terminal activity is found in the same general area of the claustrum that projects to area 17. However, the pattern of retrogradely labeled cells from these injections is dissimilar. These labeled cells predominently lie on the outside margin of the claustrum and tend to encapsulate the nucleus for considerable distance. Thus it appears that a separate zone exists that projects to the thalamus that does not receive a projection from visual cortex.

The pattern observed in the rat however is distinct from that of either the tree shrew or the cat. First, as reported for the cat, there is only minimal evidence for subcortical connections. More important, however, is the finding of only limited connections with area 17. Only the cortical region that lies medial to area 17 (area 18M) has dense reciprocal connections with the claustrum. Cortical regions lateral to 17 do appear to receive a moderate projection from the claustrum but do not appear to project to the claustrum. Furthermore, after injections into the claustrum of the rat, the terminal activity is found maximally in the infragranular layers of 18M and not in the granular layers as is the case for tree shrew and cat. Similar to that of the other species, the reciprocal projection originates from cells of layer VI.

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14.2 THE CORTICO-CLAUSTRAL LOOP IN MONOCULARLY DEPRIVED AND STRABISMIC CATS. David J. Perkel* and Simon LeVay, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The dorsocaudal (visual) claustrum receives a convergent input from several visual cortical areas and projects back to them. In the normal cat most claustral neurons are about equally responsive to stimulation of either eye (Sherk & LeVay, J.Neurosci. 1:993). We have examined the effects on the visual claustrum of two conditions that are known to affect the eye preference of cortical neurons.

In 2 monocularly deprived (MD) cats (lid-sutured at 3 weeks) all claustral units responded only to stimulation of the experienced eye. Presumably this reflected the change in the ocular dominance of the cortical cells projecting to the claustrum. In one MD cat an injection of ^{3}H-proline was made into the visual claustrum. Autoradiography showed a normal return projection to visual cortex, including area 17: the terminals were distributed in an even, non-columnar fashion. This implies that cortical ocular dominance columns for the deprived and experienced eyes both receive a claustral input that is driven only by the experienced eye. To test whether the cortico-claustral loop might be involved in suppressing cortical responses to the deprived eye, the ocular dominance of neurons in area 17 of an MD cat was studied before and after ablation of the visual claustrum with an injection of kainic acid. There was no change: both before and after the ablation responses to the deprived eye were confined to small regions in or just above layer 4. Thus the cortico-claustral loop does not suppress deprived-eye responses, at least in long-term deprivation.

One strabismic cat was studied (both lateral recti cut at 3 weeks). When paralyzed it was cross-eyed by 17°. In the cortex (area 17) most cells were monocular and were grouped into sharply-defined columns. In the claustrum most neurons were still binocular, but the proportion of cells driven strongly or exclusively by one eye (OD groups 1,2,6 & 7) was much higher (46%) than in normal cats (15%). Cells dominated by one or the other eye were randomly mixed - strabismus did not cause the appearance of ocular dominance columns in the claustrum. As in normal cats, binocular claustral cells generally had a similar preferred orientation in the 2 eyes, and cells with similar orientations were grouped together. Although there was some breakdown of binocularity in the claustrum, no doubt reflecting that seen in the cortex, our results suggest that binocular convergence at the claustral level is comparatively resistant to strabismus. The results also indicate that the development of matching orientations in the left- and right-eye fields of claustral neurons does not require normal binocular vision, at least after 3 weeks of age.

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14.3 COURSE AND STRUCTURAL ORGANIZATION OF GENICULOCORTICAL AND CORTICOFUGAL PATHWAYS IN WHITE MATTER OF THE RAT VISUAL SYSTEM. W. R. Woodward and B. M. Coull*. Dept of Neurology, Oregon Health Sciences Univ, Portland, OR 97201.

We previously reported that geniculocortical and corticofugal fiber pathways are segregated within white matter of the rat visual system (Woodward & Coull, 1982). Geniculocortical fibers are localized in the external stratum, whereas corticofugal fibers run in the internal sagittal stratum of the medullary substance. These observations were extended, and the course and organizational structure of these fiber tracts within white matter were examined.

The entire geniculocortical and corticofugal fiber tracts were visualized by autoradiography 24 hours after focal ^{3}H proline and ^{3}H fucose injections into dorsal lateral geniculate nucleus (dLGN) or into striate cortex. Subcortical white matter in rat is approximately 300 µm wide. Geniculocortical fibers coalesce into a 100 µm wide band lateral to the ventricle and project to cortical targets in the external stratum, whereas corticofugal fibers course diagonally across the white matter and project to subcortical targets as a 150 µm wide band in the internal sagittal stratum. The two fiber tracts are separated from near the visual cortex to adjacent to the lateral ventricle. Neuronal degeneration studies following kainic acid lesions of dLGN or visual cortex confirm these findings.

Geniculocortical and corticofugal fiber tracts appear quite different in white matter in both orthograde transport and neuronal degeneration studies. When sectioned in the coronal plane geniculocortical fibers appear as a continuous, unbroken band, whereas corticofugal fibers appear as a series of ellipsoid fascicles. We considered the possibility that the fascicles represent cylindrical bundles of corticofugal fibers projecting through the coronal plane at an oblique angle. Two consequences of this assumption are (1) the minimum diameter of a fascicle is equal to the cross-sectional diameter of that bundle and (2) horizontal and vertical components of the angle that bundles form with the coronal plane can be calculated. The bundles are estimated to be 22.5 µm in diameter, and the horizontal angle increases and the vertical angle decreases for progressively more lateral bundles in the white matter. The latter prediction is consistent with the course of corticofugal fibers which initially project laterally and downward before turning rostrad.

Geniculocortical and corticofugal fiber organization within white matter has important implications for neurophysiology and for neurodevelopment.

This work has been supported by the Oregon Medical Research Foundation and the Roy L. Swank Foundation.

14.4 BRANCHING OF SINGLE AXONS FROM THE LATERAL GENICULATE NUCLEUS AND LATERALIS POSTERIOR TO THE VISUAL CORTEX IN THE NEONATAL RAT. L.K. Laemle and S.C. Sharma. Dept. of Anatomy, UMDNJ-N.J. Med. Sch., Newark, N.J. 07103 and Dept. of Ophthalmology, N.Y. Md. Sch., Valhalla, N.Y. 10595

The advent of techniques which permit double labeling of individual CNS neurons has demonstrated that a single axon may bifurcate thus innervating both halves of the brain. This phenomenon has been demonstrated in the visual pathway of the adult rat, where some ganglion cells in the temporal quadrant of the retina project to both ipsilateral and contralateral lateral geniculate nucleus (LGN) (Jeffrey et al, 1981).

In the present study we ask whether axonal branching is restricted to retinal ganglion cells, or whether this is a more general phenomenon in the visual pathway. The problem was approached by the use of double retrograde labelling with fluorescent dyes. Twenty-seven neonatal rats, ages 3 days to 7 days were used in this study. Injections of 5% true blue or 5% fast blue were placed in the right visual cortex (areas 17, 18, and 18a); 30 minutes to 72 hours later 1% nuclear yellow was injected into the homologous area of the left hemisphere. Animals were sacrificed 8 hours to 24 hours following injection with nuclear yellow. Total survival from the time of injection with true blue or fast blue was 24 to 96 hours.

Retrogradely labeled neurons were located in the LGN and in the nuclues lateralis posterior (LP). Small injections in area 17 resulted in restricted areas of labeled neurons in the LGN (dorsalis) and restricted label in LP. Large injections resulted in labeling of the entire LGN and LP. Examination of these nuclei revealed a predominance of single-labeled neurons which were labeled retrogradely from the ipsilateral cortex. Scattered among ipsilaterally labeled cells were occasional neurons single-labeled from the contralateral cortex. Double labeled neurons were observed in both the LGN and LP, and were more prevalent in the latter nucleus.

The present study demonstrates that single cells in the LGN and LP of the neonatal rat project bilaterally upon the visual cortex. This provides another mechanism for duplication of visual information at the level of the visual cortex. Supported by NIH grant 1 R01 EY04107-01A1 (LKL) and EY01426 (SCS).

14.5 CONTRALATERAL CORTICAL PROJECTIONS TO VISUAL THALAMUS IN THE CAT. N. Berman and B. R. Payne. Depts. of Anatomy and Physiology/Biochemistry, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Efferent pathways from visual cortical areas to subcortical structures are mainly ipsilateral. However, in recent years a number of studies have shown that, in the cat, there are projections from visual cortical areas to a number of contralateral subcortical structures, including the claustrum, caudate-putamen, thalamic intralaminar nuclei, pretectum, superior colliculus and pontine nuclei. Many of these contralateral projections, such as to the superior colliculus, are retinotopically organized. In our continuing studies of crossed corticofugal projections, we now describe a substantial projection from visual cortical areas to certain nuclei of the contralateral visual thalamus.

Cortical projections to subcortical regions were traced with tritiated amino acids injected into extrastriate visual cortical areas of cats. After survival times of two or seven days, sections of the brain were cut and prepared for autoradiography. Silver grains were observed, as expected, over the targets of visual cortical projections in the ipsilateral midbrain and thalamus. In addition, silver grains were seen overlying the tectal and posterior commissures and a number of contralateral thalamic and midbrain structures. In the thalamus, the label was heaviest over the lateral-posterior nuclear complex with the greatest density of silver grains over the presumed border between the lateral and interjacent subdivisions of this complex. Less dense label was observed over the posterior nuclear complex and rostral pulvinar.

All regions of the thalamus, which receive projections from contralateral visual cortical areas contain neurons which respond to visual stimuli and express well ordered maps of visual space. Comparison of the crossed corticothalamic terminal regions with published maps of the thalamus suggests that the crossed cortical projections are to parts of the thalamus representing visual space in the vicinity of the vertical meridian. This suggests that the contralateral cortical projections to the thalamus and superior colliculus are both organized in a retinotopic fashion. The presence of retinotopically organized crossed corticothalamic projections indicates that one hemisphere influences the response properties of cells in contralateral thalamus. This influence, however, is likely to be confined to representations of visual space close to the division of the two hemifields. In addition, the crossed corticothalamic pathway may provide a non-callosal route by which visual information can be transferred from one hemisphere to another.

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14.6 EXCITATORY NATURE OF CORTICOFUGAL PROJECTION UPON LATERAL GENICULATE AND THALAMIC RETICULAR NUCLEI, AS EVIDENCED BY CRYOGENIC BLOCKADE OF RAT VISUAL CORTEX. Yukihiko Kayama, Akira Shosaku* and Robert W. Doty[1]. Institute of Higher Nervous Activity, Osaka University Medical School, Osaka 530, Japan.

The function of the corticofugal projection to the dorsal lateral geniculate nucleus (LGNd) has frequently been studied using inactivation of the visual cortex (VC) by cooling. However, the reported effects have been complex and/or equivocal. Thus we re-examined the question in a lissencephalic species where VC can readily be fully and reversibly inactivated. The skull overlying visual areas 17,18 and 18a was removed in urethane-anesthetized rats, which were immobilized and artificially ventilated. Inlet and outlet tubes in an acrylic wall surrounding this opening permitted rapid application and removal of chilled or body temperature saline to the intact dura mater within this opening. Unit recording showed drastic curtailment of VC activity within 20 sec of cooling onset, and complete recovery within similar time upon start of rewarming. With 1-min cooling of VC, temperature at LGNd was unaltered, but background activity was significantly reduced in about half the neurons sampled. The effects were somewhat less striking on activity evoked by electrical stimulation of the optic chiasm (OX) or photic stimulation of plotted receptive fields. Although an occasional unit might become unresponsive to such stimuli consequent to VC cooling, only about one third were affected in any way. On the other hand, the effects upon units in the adjacent, visual portion of the thalamic reticular nucleus (vTRN), identified by their characteristic discharge to single electrical pulses applied to OX, were much more consistent and profound. Background activity was depressed in all 32 units observed, versus 12 of 22 units comparably studied in LGNd. VC cooling diminished responses to electrical or photic stimulation in about 80% of vTRN units. Thus, the continuous control which the VC seems to exert upon vTRN is significantly more powerful than that upon LGNd. In no case, for either LGNd or vTRN, were excitatory effects obtained by inactivation of VC, and it is therefore concluded that the normal influence of the latter upon these nuclei is uniformly excitatory. The projection of vTRN into the LGNd has repeatedly been demonstrated, anatomically and physiologically, to be inhibitory. Thus, in removing a tonic excitatory input to vTRN, inactivation of VC causes a disinhibition of LGNd which, to some degree, may counterbalance the disfacilitation which the latter also suffers from the loss of VC input.

[1]Fellow of the Japan Society for the Promotion of Science.

14.7 Spatial contrast sensitivity: effects of peripheral field stimulation during monocular and dichoptic viewing. R.T. Marrocco and M. Carpenter*. Institute of Neuroscience, Univ. of Oregon, Eugene, OR. 97403.

We have used a radial grating as a peripheral field stimulus surrounding a 2 deg target. The grating was seen in the same eye or the eye opposite to that seeing the central target. A central 2 deg. aperture assured that the radial grating did not encroach on the 2 deg stimulus. The task of 3 experimentally naive subjects with normal vision was to adjust the contrast of a foveally viewed, 2 deg, counterphasing sine wave grating target to threshold. In half the trials the radial grating was rotating at 5 deg/sec, while in the other half it was stationary. In each condition the contrast threshold change, if any, was taken as the difference between contrast settings for the stationary minus the moving radial grating conditions.

Our results showed that movement of the peripheral radial grating caused a decrease in the contrast thresholds for monocular and dichoptic viewing conditions. A shift of 0.6 to 0.8 log units was seen for monocular conditions and a shift of 0.4 to 0.6 log units was seen for dichoptic viewing. The size of the shift was dependent on the target grating spatial frequency. Low spatial frequencies were affected to a greater extent for the dichoptic paradigm than the monocular. The reverse was true for high spatial frequencies. Thus, the curve for contrast sensitivity appeared to be shifted to lower peak spatial frequencies for the dichoptic paradigm.

The changes in contrast thresholds for monocular viewing can be explained by lateral spatial interactions in the retina or lateral geniculate nucleus. However, since the effective visual stimulation of the visual pathway is equivalent for both paradigms at or beyond the point of binocular convergence (i.e., striate cortex), the residual effect in the dichoptic paradigm is thought to reflect the action of a feedback pathway to subcortical visual centers. Supported by NSF grant BNS 82-07531.

14.8 CONNECTIONS OF THE TECTORECIPIENT ZONE OF THE CAT'S LATERAL POSTERIOR-PULVINAR COMPLEX. Bruce P. Abramson and Leo M. Chalupa. Department of Psychology and The Physiology Graduate Group, University of California, Davis, CA 95616.

The medial portion of the cat's lateral posterior-pulvinar complex (LPm) receives dense ascending projections from the superior colliculus (SC). The aim of the present study was to determine the afferent and efferent connections of this thalamic tectorecipient region. For this purpose a small iontophoretic deposit of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was placed in the LPm. Survival periods ranged from 18-24 hrs. Brain sections were reacted for the peroxidase enzyme (Mesulam 1978) and alternate sections through the thalamus were prepared for acetylthiocholine histochemistry. The latter processing revealed an oblique, dorso-ventrally oriented wedge of stain which extended from the anterior third of the lateral geniculate body to the brachium of the SC. Only WGA-HRP deposits within the cholinesterase rich region labeled neurons in the SC, whereas deposits of peroxidase just outside this area did not label tectal cells. These observations confirm the findings of Graybiel and Berson (1980) that the tectorecipient zone of the lateral posterior-pulvinar complex can be identified by cholinesterase histochemistry.

Virtually all labeled neurons in the ipsilateral SC were restricted to the stratum griseum superficiale (99% of 879 cells). This tecto-thalamic pathway was derived from four types of cell: i.) 7.5% of these neurons were multipolar, 17.0±3.4 μm in diameter, with 4 or more radially extending dendrites; ii.) bipolar type cells with small fusiform somata 6.8±1.9 μm in diameter made up 19.8% of the population; iii.) 32.6% were Martinotti-like, having round somas 10.0±2.2 μm in diameter, with a long extending process at one pole and several processes extending radially from the opposite surface; iv) 40% of the sample were pyramidal cells, 10.4±1.7 μm in diameter.

The only other subcortical structure which contained labeled cells following an injection of WGA-HRP in the LPm was the reticular nucleus. These neurons formed a heterogeneous population confined to the dorso-caudal portions of this thalamic nucleus.

In every case anterograde and retrograde label was found in six visual cortical areas. Four of these were within the suprasylvian sulcus, including: the posteromedial, posterolateral, dorsal and ventral lateral suprasylvian areas (PMLS, PLLS, DLS, VLS of Palmer et al. 1978). The other two were: the posterior suprasylvian area and area 21a. In all areas of the cortex the anterograde label appeared to be confined to layer IV. The majority of labeled perikarya were within layer VI. In coronal section these formed a continuous sheet of small pyramidal cells 9.3±1.8 μm in diameter. Fewer labeled cells were found in layer V (<10% of filled cortical neurons) and these were comprised of medium sized pyramidal cells (17.4±4.1 μm in diameter) which were distributed in uneven patches in each visual area.

The reciprocal connections of the LPm with at least six visual cortical areas and the discovery of a projection from the reticular nucleus emphasize the integrative role of this portion of the cat's lateral posterior-pulvinar complex. The extensive extra-collicular visual inputs to the LPm could provide an explanation for the differences in visual response properties which have recently been noted between SC and LPm neurons (Chalupa et al. 1981).

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14.9 CELLS OF ORIGIN OF THE CORTICOCOLLICULAR PROJECTION IN THE CAT. R. Hartwich-Young, B. Hutchins and J.T. Weber. Department of Anatomy, Tulane University Medical School, New Orleans, La. 70112.

The laminar distribution and the specific cell type(s) of the corticotectal projection were examined by placing injections of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) within the superior colliculus of the cat and processing the tissue for tetramethylbenzidine reaction product (Mesulam '78). WGA-HRP positive neurons are found primarily within visual cortical areas 17, 18, 19, 21A, the ectosylvian visual area (EVA), the splenial visual area (SVA) and the suprasylvian areas (i.e., AMLS, ALLS, PMLS, PLLS, DLS and VLS). Labeled neurons are also found within area 7, the insular cortex and the cingulate cortex. The majority of labeled neurons are located within layer V; however, a substantial number of labeled neurons are observed within layer IV, with a few scattered cells located within layer VI. Labeled neurons restricted to layer V are found only within area 7 and the EVA. Within the insular and cingulate cortices, labeled neurons are also present within the supragranular layers; i.e., layers II and III. The shape, size and number of labeled neurons vary considerably among different cortical areas. For example, while the predominant labeled cell type is pyramidal within all areas examined, labeled stellate-like cells are also seen primarily within areas 17, 18, DLS and VLS. The lateral suprasylvian areas contain the most heterogeneous population of labeled pyramidal cells, ranging from the smallest to the largest and exhibiting a great variety of different cell shapes. The insular and cingulate cortices contain only small to medium-sized labeled pyramidal neurons within the supragranular as well as the infragranular layers; within area 7, only medium-sized pyramidal cells are labeled. These data are not in agreement with the traditional view that the cortical projection to the superior colliculus originates only from pyramidal neurons located in layer V. In fact, these results clearly demonstrate that the cells of origin of the corticocollicular projection are not confined to any single cortical lamina or cell type.

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14.10 RECEPTIVE FIELD PROPERTIES OF CORTICOTECTAL CELLS IN VISUAL AREAS 1 AND 2 OF THE RABBIT. T.G. Weyand and H.A. Swadlow, Dept. Psychology, U-20, University of Connecticut, Storrs, CT 06268.

The receptive field properties of neurons which project to the superior colliculus (CT cells) from visual cortical areas 1 and 2 (V1, V2), were studied in the awake, unparalyzed rabbit. CT cells were identified by antidromic activation following electrical stimulation of the superior colliculus. Antidromic latencies in V1 (median = 2.29 msec) and V2 (median = 2.14 msec) were quite similar. Receptive field properties of CT cells in V1 (27 cells) and V2 (12 cells) were classified according to the scheme of Murphy and Berman (J. Comp. Neurol., 188: 401, 1979). The primary feature distinguishing CT cells in V1 from those in V2 was receptive field size; the mean receptive field diameter (major axis + minor axis/2) of CT cells was 4.6° in V1 and 25.0° in V2 ($p < .001$). In other respects the receptive field properties were remarkably similar. Twenty-four CT cells (16 in V1 and 8 in V2) were classified as complex, and 5 of these displayed end-stopping. Thirteen neurons (10 in V1 and 3 in V2) were movement neurons and 6 of these displayed end-stopping. Two neurons (1 in V1 and 1 in V2) were obviously directional but were not orientation selective. Sharp orientation tuning was not observed among any complex CT cells and 9 of 13 of these cells displayed little, if any, spatial summation. While most CT cells would respond to stimuli over a broad range of velocities, some cells would respond poorly to stimuli moving $<$ 10 /sec while other cells responded poorly to stimuli moving $>$ 30 /sec.

Ninety-three additional cells were classified in V1 (78 cells) and V2 (15 cells). Although 42 simple cells were observed in V1, none were CT cells. On several occasions, a simple cell was found between two CT cells in a single penetration. With the exception of CT cells and a special type of movement cell, neurons in V2 were very difficult to reliably drive with visual stimulation.

These results suggest that the CT cells in each representation project information about the identical visual events; the only difference being that the individual V2 CT cell has access to a larger region of the visual field than each V1 CT cell.

14.11 THE LATERAL SUPRASYLVIAN CORTICOTECTAL PROJECTION IN CATS. R.L. SEGAL* & R.M. BECKSTEAD (Spon: D.A. Keefer), Dept. of Anat., Univ. of Virginia Schl. of Med., Charlottesville, VA 22908.

The lateral suprasylvian area (LS) of the cat cortex has a significant influence on the visual responsiveness of neurons in the deep layers of the superior colliculus (SC) and appears to be necessary for the normal performance of some visually guided tasks. In an earlier abstract (Segal et al., Neurosci. Abstr., 8:191.3, 1982), we showed by the retrograde transport of wheat germ-HRP (WGA-HRP), that all portions of the LS project to the deep layers of SC. To confirm and extend these findings, we used the anterograde transport of either ^{3}H-amino acids (for autoradiography) or WGA-HRP from single zones of LS, or a combination of these two tracers from various LS zones. Examination of terminal distributions in SC in several such cases indicates both an areal and a laminar organization of the LS projection to SC.

Tracer deposits within any zone of the LS (anterior and posterior subdivisions of the medial and lateral banks and the dorsal and ventral subdivisions of the caudal suprasylvian sulcus) result in an intracollicular distribution of labeled axons that is typically most concentrated in the stratum opticum (SO) with an irregular and progressively diminishing amount of label both dorsally in the superficial gray layer (SG) and ventrally in the intermediate gray layer (IG). The densest concentration of terminal labeling occurs in the dorsal SO after injections along the medial bank of the LS and is located more ventrally in SO after injections along the lateral bank. Similarly, the lateral bank projection tends to be biased preferentially toward IG and the medial bank towards SG. An outstanding exception to this general feature, is that labeled axons from the approximate border region of the anterior and posterior subdivisions of the lateral bank of the LS reach IG and the deep gray and white layers of SC as well as the lower portion of SO.

The areal distribution within SC presents a more complex pattern than the laminar organization. The anterior subdivision of the medial bank projects primarily to the rostral two thirds of the lateral SC. The posterior subdivision can be further subdivided into rostral and caudal portions, which project respectively to the caudal and the rostral SC. The posterior subdivision of the lateral bank can be further subdivided into rostral, intermediate and caudal portions with the rostral and caudal portions projecting to the rostral SC and the intermediate portion projecting to the caudal SC. In a single case involving the caudal LS (dorsal and ventral subdivisions), the label is confined to the rostral SC. Curiously, all regions of the LS project to the lateral two thirds of the SC, but only the posterior subdivision of the lateral bank appears to project to the medial SC.

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14.12 Y-CELL INPUTS TO THE LATERAL SUPRASYLVIAN AREA: A SUBSTRATE FOR Y-INDIRECT INFLUENCE ON THE DEEP SUPERIOR COLLICULUS. David M. Berson* (SPON: J.T. McIlwain), Section of Neurobiology, Div. of Biol. and Medicine, Brown University, Providence, RI 02912

Retinal Y-cells provide excitatory input to most cells of the cat's deep collicular layers by way of a polysynaptic pathway involving the visual cortex (Berson and McIlwain, J. Neurophysiol. 47, 1982; Berson, Soc. Neurosci. Abstr. 8, 1982). Results of the present study indicate that the posteromedial lateral suprasylvian area (PMLS) may relay some of this indirect Y-cell influence.

Cats were anesthetized with ketamine, nitrous oxide and/or barbiturates and, in some instances, also paralyzed and artificially ventilated. Units recorded in the medial interlaminar nucleus (MIN) of the lateral geniculate complex could be activated antidromically by electrical stimulation in ipsilateral PMLS (latencies 0.4 - 1.4 ms; n=12). The same cells could also be driven orthodromically from the optic disk (OD) and optic chiasm (OX) at latencies comparable to those previously reported for geniculate Y-cells (OD: 1.2 - 2.1 ms, $\bar{X}$= 1.7 ms, n=8; OX: 0.9 - 1.4 ms, $\bar{X}$= 1.2 ms, n=10).

Units in PMLS could be driven by OD and OX shocks, often at latencies short enough to suggest monosynaptic input from geniculate Y-cells (OD: 1.9 - 7.2 ms, $\bar{X}$= 4.2 ms, n=42; OX: 1.6 - 6.3 ms, $\bar{X}$= 3.8 ms, n=42). For 95% of cells (40/42), responses following OD and OX shock differed in latency by less than 0.9 ms, indicating involvement of rapidly conducting (>25 m/s) Y-cell axons. Units in PMLS could be activated antidromically from the superior colliculus (latency 0.5 - 6.4 ms, n=32), and some of these could also be driven orthodromically with OD-OX latency differences characteristic of Y-cell influence.

These results indicate that some cells in PMLS projecting to the superior colliculus receive Y-cell input, probably via MIN. Since deep tectal cells are activated at short latencies by electrical stimulation in PMLS (Berson and McIlwain, submitted), it is proposed that the prominent indirect Y-cell influence observed previously in the deep tectum is mediated, in part at least, by a pathway through MIN and PMLS.

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14.13 EXCITATORY AND INHIBITORY INFLUENCES OF STRIATE AND EXTRASTRIATE CORTEX ON SUPERIOR COLLICULUS CELLS. K. Ogasawara*, J.G. McHaffie* and B.E. Stein. (Spon: R. Clemo) Dept. Physiol & Biophys, Medical College of Virginia, Richmond, VA 23298.

Visual corticotectal projections originate from areas 17-18 and the posterior suprasylvian cortex (PSSC). The present experiments were initiated to determine whether their influences on superior colliculus (SC) cells were similar. Thirty adult cats were used in these studies. Each was anesthetized with a single dose of sodium pentobarbital (35 mg/kg), then paralyzed and artificially respired with N_2O (75%) and O_2 (25%).

Once a cell in the SC was isolated, its receptive field was mapped and its response properties were determined. The optimal stimulus was then presented 16 times successively, and evoked discharges were counted and displayed as rasters and histograms. One of the cortical areas (e.g. PSSC) was then deactivated by cooling it to 12°C with a cooling probe, and the same tests were repeated. The area was then rewarmed and the tests were run once again. The entire series was repeated with the second area (eg 17-18) cooled and then rewarmed. The majority of the cells studied had receptive fields within the central 30° of the visual field.

Regardless of the cortical area cooled, binocular cells (81/83) were affected more frequently than monocular cells (2/22). The loss of normal cortical input to SC cells was apparent as a depression of the responsiveness of these cells during cortical cooling, and was most dramatic on SC cells that were both binocular and directionally selective. Deactivation of area 17-18 profoundly depressed all (n=41) the superficial lamina (stratum opticum and above) cells studied. However, cooling PSSC affected only 60% of these cells and did so by producing only a slight response decrement. On the other hand, all deeper layer cells (n=29) were profoundly depressed by cooling PSSC, but none was obviously affected by cooling 17-18.

In a second series of experiments, area 17-18 or PSSC remained cooled while visually responsive SC cells were sought in 62 electrical penetrations. Comparatively few responsive superficial layer cells were encountered when 17-18 remainted cooled (n=2) and few deeper layer cells were found when PSSC remained cooled (n=4). Yet the cells that were visually responsive during cortical cooling exhibited response depression when cortex was rewarmed. This confirms earlier speculations that at least some SC cells normally receive suppressive corticotectal influences.

From a functional standpoint, the present study suggests that superficial layer cells depend upon excitatory and/or inhibitory inputs primarily from 17-18, whereas deeper layer visual cells depend upon corticotectal inputs from PSSC but not from 17-18.

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14.15 ALTERATIONS OF RESPONSE PROPERTIES IN THE CAT ACCESSORY OPTIC SYSTEM FOLLOWING VISUAL CORTEX LESIONS. Keith L. Grasse and Max S. Cynader. Dept of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

The response properties of cells in the lateral (LTN) and dorsal (DTN) terminal nuclei of the accessory optic system (AOS) were examined in 14 cats which underwent unilateral visual cortex ablation. Following decortication, single units in the LTN and DTN ipsilateral to the cortical lesion no longer showed the high degree of binocular convergence characteristic of the intact animal. Instead LTN and DTN units became almost completely dominated by the contralateral eye. In addition, responsivity of LTN and DTN cells to high stimulus velocities was abolished by removal of cortical input. This decrement in high velocity response was evident in both the excitatory and the inhibitory components of the velocity response profile. While decortication did not reduce the incidence of direction selective units in either the LTN or the DTN, the distribution of preferred and non-preferred directions was dramatically altered in the LTN, and to a lesser extent in the DTN. In the LTN, this change in distribution of preferred and non-preferred directions was expressed as a reduction in the incidence of LTN cells which displayed maximal excitation for upward stimulus motion. In contrast to the distribution of direction selective LTN cells observed in normal cats, where approximately equal numbers of upward and downward preferring units were found, most LTN units recorded in the decorticate animals preferred downward-directed stimulus motion. In the DTN of the decorticate cat, the majority of direction selective units still preferred horizontal (temporal-nasal) stimulus motion as in the intact preparation, but the overall distribution of preferred directions displayed a significant downward-going vertical component when compared to normal. Other response properties, such as receptive field size and position in visual space, or preference for large textured stimuli, appeared unaffected by cortical lesions.

The results of these experiments, together with anatomical investigations which have demonstrated that the visual cortex provides a large and diffuse afferent projection to the cat AOS (Berson and Graybiel Neurosci., 5:2203, 1980, Marcotte and Updyke Brain Res., 242:205, 1982), strongly suggest that cortical input to the cat AOS plays a highly significant role in the formation of response properties of cells in the LTN and DTN. In particular, the visual cortex provides a major source of ipsilateral eye input, high velocity responses, and upward direction selectivity for the AOS nuclei examined in these experiments.

CORTEX AND CORTICO-SUBCORTICAL RELATIONSHIPS I

15.1 DIFFERENCES IN THE SPATIAL ORGANIZATION OF MITRAL AND TUFTED CELL INTRINSIC AND EFFERENT CONNECTIONS IN THE HAMSTER. T.A. Schoenfeld and F. Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Classes of output neurons in the main olfactory bulb (MOB) are known to differ morphologically in several ways, including in (a) the length, laminar distribution, symmetry and degree of branching of dendrites, (b) the degree and distribution of intrinsic axon collaterals, and (c) the distribution of axonal projections to different regions of the olfactory cortex. We recently discovered some additional morphological distinctions among MOB output neurons and their intrinsic and central circuits, using axonal transport of wheat germ agglutinin-HRP. External and middle tufted (Te and Tm) cells were found to make intrinsic axonal connections that project topographically and reciprocally between medial and lateral sectors of the MOB. To our knowledge, this is the first report of extensive intersector connections intrinsic to the MOB. The deeper-lying, internal tufted (Ti) and mitral cells do not appear to make such intersector connections; their intrinsic interactions are dominated by intrasector lateral and reciprocal synaptic contacts between their dendrites and the processes of interneurons. The Te cells were found to be the only class of output neuron to project no further caudally than pars externa (pE) of the anterior olfactory nucleus. Moreover, efferent projections to pE by Te cells, and possibly by the other output neurons as well, are topographically organized. A topographic pattern in turn is seen in the efferent projections of pE to homotopic sectors of the contralateral MOB. In contrast, the long-projecting axons of mitral and Ti cells appear to collateralize in widely dispersed regions of olfactory cortex without a clear sector-to-sector topography. Thus, superficial (Te and Tm) cells engage in extensive intrabulbar and, through projections to pE, interbulbar axonal projections that appear roughly analogous to associational and commissural projection systems in neocortex arising principally from superficial pyramidal cells, whereas the axonal connections of deep (mitral and Ti) cells are dominated by long projections to extrabulbar olfactory structures, roughly analogous to the long neocorticofugal systems that arise principally from deep pyramidal cells. Such similarities to neocortical organization belie the characterization of the olfactory bulb as a "simple" cortical system. We propose that the mitral and tufted cells and their intrinsic and central circuits may be organized as functionally defined parallel pathways.

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15.2 GRID PATTERN OF LAYER V EFFERENT NEURONS OF MOUSE BARREL FIELD CORTEX. J.E. Crandall, M. Korde* and V.S. Caviness, Jr. Dept. Neurol. and Dept. Neuropath., M.G.H. and E.K. Shriver Ctr., Boston, MA 02114

Topologically ordered efferent systems with multiple, widely separated targets arise from layer V of parietal and frontal fields, corresponding to SI and MI representations of rodent neocortex. The present study is an analysis in the adult mouse of the relative distribution of cell bodies of origin of multiple systems efferent from these cortical regions. The analysis is based upon serial 50 um sections in the tangential plane following injections of HRP into selected target regions (superior colliculus, pons and cervico-medullary junction). For each system, efferent neurons labelled retrogradely by transport of HRP are mapped as registered with outer, middle and inner areal thirds for each of barrels 2-4 in rows C and D of the posterior medial barrel subfield (PMBSF) or SI representation of the parietal cortex and within an equivalent area of the MI representation of the frontal cortex.

Neurons are distributed uniformly throughout layer V in these areas of cortex. Efferent neurons labelled retrogradely with HRP in both fields represent no more than 5-20% of the total population of the layer. In general nearest neighbors of labelled cells are not labelled by this method. In MI cortex labelled neurons are distributed diffusely among the nonlabelled neurons. In the PMBSF cortex, by contrast, labelled cells efferent to each subcortical target area are in register preferentially with the periphery or wall not the center or hollow of the barrel.

The set of observations suggests that layer V is an efferent mosaic where the mosaic units are single rather than groups of cells. In the barrel field, neurons of subdiencephalic projection are distributed in a grid pattern which appears to be the complement to the specific thalamic afferents relaying sensory information from the contralateral mystacial vibrissal pad.

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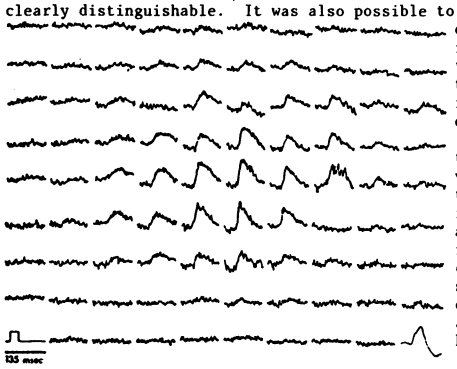
15.3 OPTICAL MONITORING OF NEURON ACTIVITY IN RAT SOMATOSENSORY AND VISUAL CORTEX. H.S. Orbach, L.B. Cohen, A. Grinvald, and R. Hildesheim*. Dept. of Physiology, Yale Univ. New Haven, CT 06510, and Dept. of Neurobiology, Weizmann Inst., Israel.

We investigated the use of voltage sensitive dyes to monitor neuron activity in rat cortex. The fluorescence from 96-124 areas of cortex stained with styryl or oxonol dyes was measured simultaneously by projecting an image of the cortex onto a diode array. To reduce noise from movements related to the heart beat the trials were triggered by the electrocardiogram and the results of a trial without a stimulus were subtracted from the results of the preceeding trial with a stimulus.

The figure illustrates the outputs from a portion of the array from a measurement from somatosensory cortex stained with dye RH414 (similar to RH237, Grinvald et al., 1982). 128 trials were averaged. Each element received light from a 450x450 μm square area of cortex. The whisker A1 was moved by 20 degrees at the time of the square pulse in the lower left trace. A ball-electrode recording is shown in the lower right trace. The transient increases in fluorescence, which we presume to represent the average depolarization of the membranes imaged on the individual detectors, are localized in the middle of the field. The central responses are both larger and faster than responses on surrounding detectors. The diameter of the area that includes all signals that are greater than 50% of the largest signal was about 1800 μm in this experiment, larger than the diameter of the anatomical barrel measured at layer IV. When two whiskers, A1 and D4 were stimulated, the areas of cortical response were clearly distinguishable. It was also possible to measure signals of similar size from visual cortex in response to diffuse light flashes to the eye.



Optical monitoring may provide a useful tool for studying projections and organization in the mammalian central nervous system. Supported by NIH grants and a US-Israel BSF.

15.4 DIFFERENTIAL DISTRIBUTION OF NEURONS IMMUNOREACTIVE TO MONOCLONAL ANTIBODY CAT 301 IN THE MAMMALIAN CEREBRAL CORTEX. E.G. Jones, R.D.G. McKay, S.J. Hockfield and S.H.C. Hendry. James L. O'Leary Division of Neurology and Neurological Surgery and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO. 63110 and Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724.

The distribution of neurons immunoreactive to a monoclonal antibody, CAT 301, made by immunizing mice with aldehyde-fixed cat spinal cord was studied in the cerebral cortex of rats, guinea pigs, cats and macaque monkeys. Immunocytochemically labeled cells are present in all cortical areas, but the number of labeled cells varies markedly from species to species and area to area. In cat and monkeys the largest populations of CAT 301 positive cells are present in the primary motor area (area 4), the first somatic sensory area (SI) and the primary visual area (area 17). In most areas, CAT 301 positive cells are densely clumped in only 2 or 3 layers but the layers involved vary from one area to another. Because of the differences in the number and laminar distribution of CAT 301 positive cells, boundaries between many areas are readily apparent.

Several observations suggest that certain populations of cortical neurons are preferentially recognized by CAT 301: (1) laminar distribution of labeled versus unlabeled pyramidal cells suggests that neurons projecting to some targets are labeled but those projecting to other targets are not. Furthermore the vast majority of corticospinal neurons in monkey SI and area 4 labeled by the retrograde transport of Fast Blue are CAT 301 positive but commissural and associational neurons in these same areas labeled by retrograde transport are CAT 301 negative; (2) a patchy distribution of CAT 301 positive neurons is evident in monkey SI and areas 17 and 18, suggesting a relationship to particular afferent systems; (3) differences among areas in the numbers of CAT 301 positive neurons suggests that the relevant antigen or antigens may be present on subsets of pyramidal and non-pyramidal cells with particular functional characteristics.

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15.5 FEATURES OF THE ORGANIZATION OF MACAQUE MONKEY VISUAL CORTEX DEMONSTRATED WITH MONOCLONAL ANTIBODY CAT 301. S.H.C. Hendry, S.J. Hockfield, R.D.G. McKay and E.G. Jones. James L. O'Leary Division of Experimental Neurology and Neurological Surgery and McDonnell Center, Washington University School of Medicine, St. Louis, MO. 63110 and Cold Spring Harbor Laboratories, Cold Spring Harbor, NY 11724.

Using a monoclonal antibody, CAT 301, generated against a homogenate of aldehyde-fixed cat spinal cord, we have identified a patchy distribution of labeled neurons in areas 17 and 18 of cynomolgus monkeys (Macaca fascicularis) that can be correlated with certain aspects of cortical organization.

CAT 301 positive neurons in area 17 form densely stained bands in layers IVB and VI. Other layers contain fewer cells. Both pyramidal and non-pyramidal cells are labeled. Within layers IVB and VI, patches, 100-300 microns in diameter, of closely clustered, heavily labeled neurons are separated by gaps containing fewer, more lightly labeled cells. The gaps vary in width from 150-500 microns. Most patches in layer IVB line up radially with those of layer VI. Smaller patches of labeled cells are present in layers II and III and many of these, particularly in layer III, also line up with the patches in the deeper layers. In the dorsal lateral geniculate, cells only in the magnocellular layers are heavily labeled by CAT 301.

In three monkeys ocular dominance columns were labeled either by monocular enucleation followed by cytochrome oxidase (CO) histochemistry or by monocular injection of 2mCi of ^{3}H-proline followed by autoradiography. The CAT 301 positive patches in layers IVB and VI of the three animals are associated with the middle of both ipsilateral and contralateral eye dominance columns.

Patches or "blobs" of CO staining in layers II and III are also related to CAT 301 positive patches. The CO patches in layers II and III line up radially with CAT 301 positive patches of layers IVB and VI, and deep in layer III many CO patches and CAT 301 positive patches are coextensive. The relationship between CO patches and CAT 301 positive patches in the more superficial layers is being investigated.

In area 18 large (approximately 1mm) and small (200-300 microns) patches of CAT 301 positive neurons are present in layers III and V. The patches of labeled neurons overlap with most CO positive patches present in area 18 and at the 17/18 border.

These data suggest one or more as yet uncharacterized antigens recognized by a monoclonal antibody may be associated with neurons receiving certain afferent inputs and with certain classes of physiologically distinct neurons in areas 17 and 18 of macaque monkeys.

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15.6 **PERISTRIATE INPUT TO THE ENTORHINAL CORTEX FROM RETROCALCARINE REGIONS IN THE MONKEY.** C.L. Barnes, G.W. Van Hoesen and S.K. Itaya. Depts. of Anatomy and Neurology, Univ. of Iowa, Coll. of Med., Iowa City, IA 52242.

Physiological studies have shown that hippocampal neurons respond to visual stimulation. It is likely these are mediated largely via cortical pathways that relay in the entorhinal cortex (area 28), the major source of cortical afferents to the hippocampal formation. Several potential multisynaptic pathways have been demonstrated anatomically in the monkey, and involve such structures as the amygdala, perirhinal cortex (area 35) and the posterior parahippocampal region (area TF). All receive input from the visual association cortices, such as areas TE and OA, and send afferents to the entorhinal and subicular cortices. We report here a more direct visual input to the entorhinal cortex in the monkey that arises from the cortex in the mouth of the calcarine fissure and the retrocalcarine peristriate area along its upper bank. These observations were made after exposing the ventromedial temporal cortex via a lateral surgical entry into the middle cranial fossa, and injecting horseradish peroxidase (HRP) into the entorhinal cortex. The tissue was processed according to Mesulam's tetramethyl benzidine procedure. In these experiments, neuronal labeling in the peristriate cortex was confined largely to area OB, although neurons were observed in area OA, dorsal and ventral to the calcarine fissure. The labeling in area OB was located topographically in the anteriormost part of the calcarine fissure at levels coincident with the end of the hippocampus and the formation of the atrium of the lateral ventricle. It involved cortex in the depths of the fissure, along its upper bank and its dorsal continuation along the medial surface of the hemisphere where it adjoins cingulate area 23 and dorsal area OA. Cytoarchitectural study confirmed its characterization as area OB. In these locations labeled neurons occured at variable depths within layer III, with only a few labeled neurons in other laminae. Despite the location of labeled neurons within the calcarine fissure, several factors mitigate against considering this cortex area OC or 17. First, it fails to meet the relevent cytoarchitectural criteria, second, it does not contain the pattern of cytochrome oxidase staining characteristic of area OC, and, third, it does not receive direct lateral geniculate input as judged by the anterograde transneuronal transport of wheat germ agglutinin conjugated to HRP after intravitreal injections. Thus, the results represent a new and direct source of visual input to the entorhinal cortex from area OB. The anterior location of neurons in the calcarine region suggest a role in function related to peripheral vision. (Supported by Grant NS 14944 to G.W.V.H.)

15.7 PROJECTIONS TO THE FRONTAL CORTEX FROM THE POSTERIOR PARIETAL REGION IN THE RHESUS MONKEY. M. Petrides and D. N. Pandya. Dept. of Psychology and the Montreal Neurological Institute, McGill Univ., Montreal, Canada and V. A. Medical Center, Bedford and Boston Univ. Sch. of Med., Boston, Mass.

A detailed examination of the projections to the frontal cortex from various subdivisions of the posterior parietal cortex in the rhesus monkey has been carried out with the autoradiographic technique. The results showed that the superior parietal lobule (rostral and caudal area PE) projects to the dorsal part of area 6. This projection extends from the border of areas 4 and 6 to the caudal bank of the upper branch of the arcuate sulcus (AS). The projections from the medial parietal cortex (areas PEc and PGm) are similar to those of the superior parietal lobule but they tend to concentrate in the more rostral part of dorsal area 6. Both the superior parietal lobule and the medial parietal cortex are connected with a discrete part of the medial surface of the frontal lobe that appears to correspond to the supplementary motor area. The more ventral part of the medial parietal cortex also projects to area 8 in the concavity of the AS.

The projections to the frontal lobe from the inferior parietal lobule (IPL) are somewhat more complex. The anterior part of the IPL (area PF) projects to the ventral area 6 including the caudal bank of the lower branch of the AS, to the ventral area 46 below the sulcus principalis, and to a part of the cortex of the frontal operculum. The middle IPL (areas PFG and PG) projects to the ventral part of area 46 as well as to area 8. The projections directed to the ventral bank of the sulcus principalis appear to originate primarily from the more lateral parts of this region, whilst those directed to the part of area 46 lying below the sulcus principalis and to area 8 seem to originate from the more dorsal part of areas PFG and PG including the adjacant cortex lying in the lower bank of the intraparietal sulcus. The posteriormost IPL (caudal PG and area OPT) is connected with both dorsal and ventral area 46 as well as dorsal area 8.

The present investigation also showed that the frontal polar cortex (area 10) and the orbital frontal cortex do not receive projections from any of the subdivisions of the posterior parietal cortex.

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15.8 DOUBLE LABELING OF PARAHIPPOCAMPAL EFFERENTS TO THE CINGULATE GYRUS AND PARIETO-OCCIPITAL CORTICES IN THE MONKEY USING FLUORESCENT TRACERS. D.R. Brady, C.L. Barnes and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

The cortices of the posterior parahippocampal area (TF and TH) give rise to axons that terminate in all classes of cortex (allocortex, e.g., subiculum; periallocortex, e.g., entorhinal cortex; proisocortex, e.g., areas 23 and 24; isocortex, e.g., areas 7 and 19). Our aim has been to ascertain whether the neurons that underlie this connectional diversity are segregated into separate populations or whether this is sustained by axon collateralization from individual neurons. To initiate this, we have studied the posterior parahippocampal projections to the cingulate gyrus (proisocortical areas 23 and 24) and to the parieto-occipital area (isocortical areas 7 and 19) using both single and double labeling strategies. In five monkeys, tritiated amino acids (^{3}H-leucine, lysine and proline) were injected into areas TF and TH and the brains were processed for autoradiography. In four complementary experiments the retrograde tracers horseradish peroxidase (HRP) and fast blue were injected singly into either areas 23 and 24 or into areas 7 and 19. Lastly two monkeys received injections of two retrograde tracers, with fast blue injected into areas 23 and 24, and nuclear yellow into areas 7 and 19. Survival time for the autoradiographic and fast blue experiments was 7 days, while a 2 day survival was used for HRP and nuclear yellow. The single labeling experiments using tritiated amino acids, HRP and fast blue yielded complementary results and verified the fact that posterior parahippocampal areas TF and TH project to both the cingulate gyrus (areas 23 and 24) and to the parieto-occipital cortices (areas 7 and 19). The cells of origin for these projections were found in layers III, V and VI of areas TF and TH. An overall topographic difference was observed in double labeling experiments such that cingulate projections arose from more medial parahippocampal locations while the projections to the parieto-occipital area arose more laterally. However, overlap was observed between the two. Of interest was the fact that a population of double labeled neurons that project to both the cingulate gyrus and the parieto-occipital area was observed in the vicinity of the region of overlap. In view of the fact that the lateral parts of the posterior parahippocampal area are isocortical, whereas the more medial parts are more proisocortical or "limbic" in structure, it is tempting to speculate that via axon collateralization distinct populations of cortical neurons form an interface between both structurally and functionally diverse parts of the cortex. (Supported by Grant NS 14944 to G.W.V.H.)

15.9 PARAHIPPOCAMPAL PROJECTIONS TO POSTERIOR AUDITORY ASSOCIATION CORTEX (Tpt) IN THE MONKEY. D. Tranel*, D.R. Brady, G.W. Van Hoesen, and A.R. Damasio*. Depts. of Anatomy and Neurology, Univ. of Iowa Coll. of Med., Iowa City, IA 52242.

Area Tpt is a distinctive cytoarchitecturally-defined part of the auditory association cortex located at the confluence of the lateral and superior temporal sulci in the superior temporal gyrus. Lesions of this area in humans cause the distinctive and well-known language disturbance termed Wernicke's aphasia. In addition, area Tpt has been found to be significantly larger in the left hemisphere, a finding consistent with its role in language processing. A comparable area Tpt has been identified in the rhesus monkey. The acquisition of language depends, in all likelihood, on the appropriate locking of associations between auditory information and information from other modalities (e.g., visual, somatosensory), in what may be best described as a language-devoted memory process. Considering the central role of the hippocampal system in memory, we hypothesized that direct anatomical pathways between regions of the hippocampal system and the auditory association cortex must exist, and are probably demonstrable in even non-human primates. The brains of eight rhesus monkeys that had injections of tritiated amino acids (^{3}H-leucine, lysine and proline) in various parts of the parahippocampal gyrus (areas 28, 35, TF and TH) were studied. Using autoradiography, it was observed that both areas TF and TH project to the anterior auditory association cortices, but the more medial parts of the posterior parahippocampal cortices, including area TH, have extensive projections that course posteriorly to nearly the entire lateral extent of area Tpt. This observation was complemented by using the fluorescent retrograde tracer fast blue. Following the injection of this substance into area Tpt, labeled neurons were observed in the medial part of the posterior parahippocampal cortices, and particularly, in area TH. This result is of some interest, since it has been shown that area TH in the monkey receives a major hippocampal output from the subicular cortices. Thus, along with previous results demonstrating superior temporal projections to the hippocampal cortices, our findings complete a reciprocal pathway that mediates a direct structural coupling between higher-order auditory association cortices and temporal structures known to play a role in memory. (Supported by Grant NS 14944 to G.W.V.H. & A.R.D.)

15.10 PRIMATE PREFRONTAL CORTEX PROJECTS TO THE REGION OF THE LOCUS COERULEUS AND DORSAL RAPHE. Arnsten, A.F.T., Schwartz, M.L. and Goldman-Rakic, P.S.. Section of Neuroanatomy, Yale Medical School, 333 Cedar St., New Haven, CT 06510

The monoaminergic projections from brainstem to cortex have been firmly established in the rodent and primate. In contrast, the possibility of a reciprocal descending cortical projection to monoamine nuclei has been addressed less frequently. The substantia nigra appears to receive a widespread cortical innervation, (eg Br. Res. 117:423-435, 1976), whereas studies of cortical afferents to the locus coeruleus (LC) and midbrain raphe nuclei in the rat indicate that cortical projections to these monoamine nuclei may be restricted to the prefrontal cortex (PFC) (J. Comp. Neurol. 178:1-16, 1978; Br. Res. 122:229-242, 1977). We examined this issue further in the rhesus macaque by injecting anterograde tracers into PFC (principal sulcus and dorsomedial cortex) and nonPFC (parietal association cortex) areas. Injections of HRP or tritiated amino acids into the PFC areas resulted in anterograde labeling of the pontine central gray in the vicinity of the LC and the dorsal raphe. The labeling was densest at the anterior level of the ipsilateral LC, particularly surrounding the more ventrally-located cells. The projection extended medially to the dorsal raphe and ventromedially into the central superior raphe, but strikingly avoided the dorsal tegmental nucleus of Gudden. More posteriorly, the labeling became sparse and was present medial to the compact LC in a region where LC dendrites have been observed. Labeled fibers could be followed only as far as posterior levels of the LC, indicating that the PFC fibers terminated in the pontine central gray and were not in passage to more caudal areas of the brainstem. A contralateral projection to the LC-raphe region was also observed but was less dense than that to the ipsilateral central gray.

In contrast to the results with PFC areas, injections into the parietal association cortex did not result in anterograde labeling of the LC-raphe region. As parietal cortex shares most other PFC terminal fields, this negative finding is notable and supportive of a selective PFC innervation of the LC-raphe region.

Immunocytochemical analysis of the PFC terminal field revealed met-enkephalin- and Substance P-reactive fibers. These peptide neurons have been shown to synapse on LC cell bodies and dendrites in the rodent (Br. Res. 160: 387-400, 1979). Thus it is possible that the PFC influences LC neurons through indirect actions on peptide interneurons.

The results from these anterograde tracing and immunocytochemical experiments provide a morphological basis for PFC regulation of brain monoamine activity in the primate.

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15.11 DISTRIBUTION OF THE NEURONS OF ORIGIN OF THE CORPUS CALLOSUM AND ANTERIOR COMMISSURE IN THE MARMOSET MONKEY (Callithrix jacchus). Marc L. Jouandet, Laurence J. Garey, and Hans-Peter Lipp. Institute of Anatomy, Unversity of Lausanne, Switzerland.

The distribution of the neurons of origin of the great cerebral commissures of the marmoset monkey was investigated by means of Horseradish peroxidase (HRP) histochemistry. Large quantities of HRP were injected unilaterally throughout one cerebral hemisphere in order to label as many commissural neurons as possible in the contralateral hemisphere. The distribution of these labeled neurons was charted with the aid of a semi-automatic computer microscope. Six adult monkeys were used in this investigation: three were normal when subjected to the HRP injection operation; three others first underwent complete commissurotomy of the corpus callosum (CC), leaving the anterior commissure (AC) intact. Thus the three normals provided information on the origins of both the CC and the AC, whereas the three callosotomized monkeys allowed study of the origins of the AC alone.

All CC and AC neurons in the marmoset are pyramidal shaped. No columnar pattern is discernable for either the CC or the AC neurons; rather, these cells are densely and continuously distributed in the various lamina tangential to the cortical surface. Except for layer I, all cortical layers can possess commissural cells. The laminar distribution of these neurons varies according to cortical region. The cortical field of origin of the AC of the marmoset is similar to that in the rhesus monkey (layer III pyramidal neurons in an extensive temporal neocortical field; Jouandet and Gazzaniga, 1979). Significantly, there exists in the marmoset a progressive reversal in quantitative predominance between supra- and infragranular commissural neurons proceeding from the temporal pole, through the occipital cortex, through the parietal and into the frontal cortex. The predominance of infragranular neurons in portions of the parietal and frontal cortices of the marmoset differs greatly in organization from the prevailing laminar pattern seen in the cat. In the cat, commissural neurons present in supragranular layers almost invariably predominate in number over the commissural neurons in the infragranular layers (Jouandet, Lachat, and Garey, in preparation). The major acallosal zones are found in the primary visual cortex, and the fore and hindlimb representations of the somatosensory cortex. Correlations between commissural neuron distributions and previously described cyto-archetectonic areas are not obvious.

COCHLEA

16.1 POTASSIUM-INDUCED CHANGES IN THE LEVELS OF ENDOGENOUS AMINO ACIDS OF GUINEA PIG PERILYMPH. G. L. Jenison and R.P. Bobbin. Kresge Hearing Research Laboratory of the South, Dept. of Otorhinolaryngology, L.S.U. Medical Ctr., New Orleans, 70119

Among those studies intended to characterize the afferent neurotransmitter produced by cochlear hair cells, several have attempted to detect a stimulus-induced release of putative amino acid candidates in perilymph. Sound has proven effective only in elevating levels of β-alanine, and a γ-aminobutyrate-like substance (D.G. Drescher, ARO Midwinter Abstr. #116, 1982), however, neither β-alanine nor γ-aminobutyrate have any significant effect on cochlear potentials when delivered to perilymph. Therefore, we have chosen to examine the depolarizing influence of elevating potassium levels on the concentration of amino acids to perilymph.

Guinea pig cochleae were perfused with artificial perilymph (s. tympani → s. vestibuli, 2 μL/min.) through ports cut in their basal turn, after occlusion of the cochlear aqueduct. Trials began with a 40 minute perfusion period, during which natural perilymph was replaced with artificial perilymph. Samples were then collected during sequential perfusions of artificial perilymph containing 5 mM KCl (NORMAL), then 50 mM KCl (HIGH K^+) and finally 5 mM KCl (NORMAL). Amino acid levels for each sample were then determined by Dr. R. Thalmann of Washington U. School of Medicine using OPA derivatization followed by HPLC coupled with fluorescence detection.

Preliminary results (n=6) indicate significant ($P<0.01$) increases in four primary amine peaks which temporally correlated with the perfusion of HIGH K^+. In all four cases, baseline concentrations were reestablished with subsequent perfusion of NORMAL. Glutamate levels rose from a mean baseline concentration of 0.41 μM to a mean peak value of 0.68 μM within 20 minutes after exposure to HIGH K^+. Concentration increases were also noted for aspartate (0.23 → 0.38 μM) and tryptophan (0.74 → 1.27 μM) within 30 minutes after exposure to HIGH K^+. The fourth peak, consisting of the three co-eluting compounds: taurine, γ-aminobutyrate and α-aminobutyrate (R. Thalmann et al., Laryngoscope, 92: 321, 1982), increased by about 60% within the first 20 minutes of exposure to HIGH K^+. Unlike glutamate, however, this peak continued to grow over the following 10 minutes to approximately twice its original value. All other amino acids identified either decreased in concentration or remained unchanged. In view of the above mentioned co-elution problem, modified separation techniques capable of resolving relative taurine, α-and γ-aminobutyrate concentrations are currently being employed. [Supported by NIH grants NS-06575, NS-16080, NS-07058 and NSF grant BNS-8118772].

16.2 RESPONSE PROPERTIES OF SINGLE COCHLEAR AFFERENT NEURONS ALTERED BY RAISED PERILYMPHATIC MAGNESIUM CONCENTRATION. J.H. Siegel and E.M. Relkin*. Auditory Physiology Lab./ Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60201.

Divalent calcium antagonists of presynaptic transmitter release such as Mg^{++}, Co^{++} and Mn^{++} have been shown to inhibit spontaneous and sound-evoked activity of single cochlear afferent neurons (Robertson and Johnstone, Pflugers Archiv. 380:7-12, 1979). We have developed a technique for continuously perfusing scala tympani while simultaneously recording from single units in the eighth nerve of adult chinchillas (Siegel and Relkin, ARO Abstracts, 6:49-50, 1983). We are using this system to study mammalian synaptic transmission in vivo. Raising the magnesium concentration in the perfusate to 6 mM while holding calcium constant at 1.5 mM elevates single-unit threshold at all frequencies of the acoustic stimulus and reduces spontaneous activity to near zero. The maximum discharge rates are also reduced. Preliminary results from PST histograms for tone-burst stimuli show a greater reduction of the onset than the steady-state response, at a given stimulus level, with increased magnesium concentration. The change in shape of the PST response with elevated magnesium is thus quite similar to the effect of reducing the level of the acoustic stimulus when the perilymph contains normal calcium and magnesium concentrations. All these effects are fully reversible on return to control perfusate.

Our observations that cochlear microphonics and ear-canal acoustic distortion products are substantially unaltered by elevated magnesium suggest that the principal effect is on the presynaptic release of transmitter by the hair cell. Interspike interval histograms are evaluated to investigate possible effects on the excitability of the postsynaptic neuron. This system thus appears to be well suited to the study of synaptic mechanisms in a sensory organ where a controlled and graded stimulus can be delivered and extracellular fluid composition can be varied effectively.

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16.3 THE IMPORTANCE OF MEASUREMENT ERROR ON PARAMETER ESTIMATION FOR MODELS OF COCHLEA ELECTROANATOMY. B. N. Sabowitz*, A. E. Hubbard* and D. C. Mountain* (SPON: M. Feldman). Depts. Otolaryngology, Biomedical Engineering, Electrical,Systems and Computer Engineering. Boston University. Boston, MA, 02215.

Modeling of cochlear electroanatomy is of interest both as an aid to understanding cochlear electrophysiology and as a tool to predict current flows resulting from cochlear prosthetics. Parameters for these models are calculated from experimental measurements which are subject to a variety of potential errors. The present study examines the sensitivity of the model parameters to measurement error.

Johnstone et al. (1966) presented experimental data which allowed calculation of parameters for a purely resistive cross-sectional model of the cochlea. A review of this model and a sensitivity analysis of the determination of each resistance branch due to errors in voltage measurements will be presented. Results indicate that even for reasonable measurement errors (< 5%), derived values can vary by 100 % or more. In addition, simulations account for the occasional result of negative resistance branches as reported by Johnstone et al. The sensitivity of this model to voltage measurement errors sheds doubt on the ability to characterize parameters accurately unless measurements are performed in a judicious fashion. If one assumes that the measurements are highly accurate and that measurement errors are the result of damage caused by moving electrodes, the model allows one to predict the sequence in which electrodes may be placed in order to maximize the accuracy of derived model parameters.

16.4 HYSTERETIC EFFECTS IN THE COCHLEAR MICROPHONIC, D. C. Mountain*, A. E. Hubbard*, and T. A. McMullen* (SPON: R. Holub). Depts. of Otolaryngology, Biomedical Engineering, and Electrical, Systems, and Computer Engineering, Boston University, Boston, MA 02215.

The cochlear microphonic (CM) recorded from scala media of both gerbils and guinea pigs was studied for various sound pressure levels. The data was plotted as Lissajous figures with the x-axis corresponding to estimated basilar membrane displacement. At low and moderate sound pressure levels the resulting curves were similar to the transducer transfer characteristics reported by Hudspeth and Corey (Proc. Natl. Acad. Sci. USA, 74: 2407-2411, 1977) for frog saccular hair cells. A linear region about the origin and asymmetric saturation were noted. For higher input levels a pronounced hysteresis appeared. The major finding is that the negative-going slope of the CM becomes significantly steeper than the positive-going slope. The positive-going slope appears to be more sensitive to sound level than the negative-going slope. Qualitatively, the findings were identical in both species.

Computer simulations of several alternative models will be compared to the experimental data. These models include the effects of two populations of hair cells, effects of cochlear electro-anatomy, and transducer-channel opening and closing times.

16.5 THE RELATION BETWEEN THE ENDOCOCHLEAR POTENTIAL AND THE SPONTANEOUS DISCHARGE RATE OF AUDITORY NERVE FIBERS IN THE CAT FOLLOWING FUROSEMIDE ADMINISTRATION. William F. Sewell. Eaton-Peabody Laboratory of Auditory Physiology, Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA. 02114

The intravenous administration of furosemide produces concomitant decreases in the endocochlear potential and in the spontaneous discharge rate of auditory nerve fibers. By simultaneously measuring the endocochlear potential and auditory nerve spontaneous discharge rate in the same ear, following an intravenous injection of furosemide, the quantitative relation between the endocochlear potential and the spontaneous discharge rate was established.

Following furosemide administration the logarithm of the spontaneous discharge rate was proportional to the endocochlear potential. The slope of this logarithmic relation increased as a function of the characteristic frequency of the fiber. The slope of the logarithmic relation is steeper for fibers with "medium" spontaneous discharge rates (0.5 - 12 spikes per second) than for fibers with "high" spontaneous discharge rates (>18 spikes per second). Among "high" spontaneous rate fibers, there is no strong correlation between the slope and the spontaneous discharge rate of the fiber.

The decrease in the spontaneous discharge rate in auditory nerve fibers is interpreted as follows: (1) the injection of furosemide causes a decrease in the endocochlear potential; (2) a decrease in the endocochlear potential produces a linearly related decrease in the membrane potential of the hair cell; and (3) the rate of release of transmitter quanta decreases logarithmically with a change in the membrane potential of the hair cell, which decreases the rate of occurrence of action potentials in VIIIth nerve fibers.

16.6 ORGANIC ACIDS ATTENUATE COCHLEAR EFFECTS OF FUROSEMIDE. L.P. Rybak, C. Whitworth*, L. Wright* and T. Morizono*. Depts. of Surgery and Pharmacology, SIU School of Med., Springfield, IL 62708

A number of epithelial systems concerned with fluid transport possess organic acid pumps powered by aerobic metabolism. Such systems include kidney, eye, liver and choroid plexus. It is unknown whether the cochlea has an organic acid pump to control the local fluid balance.

Furosemide is an ototoxic loop diuretic which is actively taken up by an organic acid pump in the renal tubule (Odlind, B., J Pharmacol Exp Ther 208:515, 1979). Its mechanism of ototoxicity is unknown. The possibility exists that furosemide ototoxicity may be mediated in part by an organic acid uptake system in the cochlea. The purpose of this series of experiments was to investigate the effect of several inhibitors of organic acid transport on furosemide ototoxicity.

Chinchillas weighing 400-600 gm were divided into several groups. Each animal was anesthetized with ketamine 45 mg/kg IM followed by pentobarbital 30 mg/kg IM. A femoral vein was cannulated for drug administration. Cochlear function was monitored by placement of a microelectrode into the scala media through the basilar membrane to continuously monitor the endocochlear potential (EP) over several hours. Control animals received 0.5 ml of normal saline IV followed in thirty minutes by furosemide 25 mg/kg IV. Experimental animals were similarly treated as controls except that they received an organic acid transport inhibitor in the same volume as the controls followed by furosemide.

A series of organic acid transport inhibitors significantly reduced the EP decline produced by furosemide. Probenecid (50 mg/kg) was more effective than novobiocin (50 mg/kg). Diatrizoate (150 mg/kg) was the least effective in altering the furosemide related drop of the EP. An inhibitor of organic base transport, N-methylnicotinamide (50 mg/kg) had no effect on the furosemide-induced drop of the EP. These findings suggest that at least part of furosemide ototoxicity may be mediated by organic acid transport.

16.7 The inner-hair-cell (IHC) and outer-hair-cell (OHC) subsystems in the cochlea and the brainstem.
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Comparative neuroanatomy of IHC and OHC of the mammalian cochlea has been well documented: (1) They have prominent differences in their morphology; (2) They are selectively innervated in distinct ways by distinct neural populations for both afferent and efferent innervations; and (3) There are about four times as many OHCs as IHCs but the OHCs receive only about 5% of the total afferent neurons. Functional roles played by the IHC and OHC have not been well understood, and the marked underrepresentaion of the OHC in the afferent neural populations has been particularly puzzling. We propose and review evidence for the following set of hypotheses: (1) There are two distinct auditory subsystems in the cochlea and the brainstem, i.e., (a) the "IHC subsystem" consisting of IHCs and neural populations receiving input from IHCs; This subsystem includes the small olivocochlea-bundle (OCB) neurons of Warr (1978) with axons projecting to the IHC region, and (b) the "OHC subsystem" consisting of OHCs and neural populations receiving input from OHCs; This subsystem includes the large OCB neurons of Warr with axons synapsing on OHCs; (2) The OHC has active bidirectional transduction mechanism in the stereocilia and cuticular plate region which reduces the net mechanical damping of Corti's organ, even to negative damping under certain conditions; and (3) The function of the OHC subsystem is to actively enhance and control the sensitivity and tuning of the motion of the whole Corti's organ by regulating release of mechanical energy in the "motor function" of OHC, thereby confering high sensitivity, sharp tuning and wide dynamic range to the IHC subsystem through which the auditory information is channeled to the higher centers of the auditory system.
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16.8 PREFERENTIAL LABELING OF COCHLEAR HAIR CELLS FOLLOWING INCUBATION WITH H^3-GLUTAMINE. I.R. Schwartz and A.F. Ryan*, Div. of Head & Neck Surgery, UCLA Sch. of Med., Los Angeles, CA 90024, Div. of Otolaryngology, UCSD Sch. of Med., La Jolla, CA 92093 & Research Service, VA Med. Ctr., San Diego, CA 92161.

Identification of the afferent transmitter between cochlear hair cells (HC) and VIIIth nerve dendrites remains a major unresolved problem in cochlear physiology. Evidence that aspartic acid (ASP) or glutamic acid (GLU) is the transmitter is inconclusive, but evidence for other candidates is also equivocal. To further evaluate the role of ASP & GLU in afferent transmission, light and electron microscopic autoradiographic investigations of the amino acid uptake systems in the in vivo gerbil cochlear preparation have now been extended to seventeen H^3 or C^{14} labeled putative transmitters and related compounds with special emphasis on amino acids related to the metabolism of GLU. The methods used are described in Schwartz & Ryan (Hearing Research 9, 185-200, 1983).

Of the compounds tested, only glutamine (GLM) produced pronounced preferential labeling of cochlear HCs. Immediately after GLM incubations label was also found over efferent terminals beneath HCs, over satellite cells around spiral ganglion neurons and to a lesser extent over spiral ganglion neurons themselves. Vestibular HCs were unlabeled even though label was present over adjacent supporting cells. The accumulation of label in cochlear HCs following incubation with glycine, alanine and proline is only slightly greater than the labeling of surrounding cells in the organ of Corti. By contrast, labeling of HCs was less than that of surrounding tissues after taurine incubations. D- & L-GLU, D- & l-ASP, GABA, histidine, ornithine, arginine, α-keto glutaric acid, methionine, choline and muscimol produced no preferential labeling of HCs. At the light microscopic level afferent terminals did not appear labeled with any compound tested.

GLM uptake could play an important role in afferent synaptic transmission by providing transmitter precursor and/or by clearing the synaptic cleft of a transmitter breakdown product. It seems unlikely that GLM itself is a transmitter. However, in addition to possible transmitter related roles, it may also serve as a major substrate for HC metabolism.

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16.9 PREFERENTIAL GLUTAMINE UPTAKE BY COCHLEAR HAIR CELLS: CHANGES IN INTRACELLULAR DISTRIBUTION WITH INCREASING SURVIVAL TIME. A.F. Ryan* and I.R. Schwartz (SPON: B. Pfingst). Div. of Otolaryngology, UCSD Sch. of Med., La Jolla, CA 92093 and Div. of Head & Neck Surgery, UCLA Sch. of Med., Los Angeles, CA 90024.

Cochlear hair cells in the gerbil preferentially accumulate the amino acid glutamine, as described in the companion abstract. To determine the fate of incorporated label, both pulse duration and survival time were varied in a light microscopic, autoradiographic study.

Following pulse durations of 20 min, with immediate cold rinse and fixation, inner hair cells (IHCs) showed a marked preferential labeling when compared to supporting cells of the organ of Corti. The label was evenly distributed throughout the cell, penetrating even the stereocilia, and did not appear to be preferentially located in any cytoplasmic organelles at the light microscopic level. The outer hair cells (OHCs) were also uniformly labeled, although to a lesser extent than the IHCs. Preferential labeling of structures which we have previously shown to accumulate a variety of other amino acids (Schwartz and Ryan; Ryan and Schwartz; Hearing Res., 9:173&185, 1983), such as the cochlear efferents and spiral ganglion satellite cells, was also noted.

A similar, though more intense, pattern of labeling was noted after 40 min pulse duration. However, label in IHCs tended to be heavier at the apical pole of the cell, and in mitochondria. Label in OHCs tended to be in the subsurface cisternae (SSC) and in mitochondria. Spiral ganglion neurons showed a greater degree of labeling, relative to satellite cells, than after a 20 min pulse.

Following a 20 min pulse, cold perilymph rinse, and survival times of 30-120 min, label distribution changed markedly. With increasing survival time, IHC label shifted to the apical pole and, to a lesser extent, to the basal end of the cell. Label tended to be in mitochondria at 30-60 min survival times, but not at 120 min. In the OHCs, with increasing survival time, label was localized to the SSC. As with the IHCs, label was associated with mitochondria only with 30-60 min survivals. Disappearance of label from the cochlear efferents and the satellite cells surrounding the spiral ganglion neurons was more rapid than from the hair cells. Relative labeling of the spiral ganglion neurons increased, and preferential labeling of VIIIth nerve fibers in the modiolus was apparent at the longest survival times.

The kinetics of label distribution in hair cells are consistent with uptake of a transmitter precursor, with subsequent transformation into a different amino acid or peptide.
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16.10 IMMUNOCYTOCHEMICAL LOCALIZATION IN THE GUINEA PIG ORGAN OF CORTI AFTER PERIPHERAL EFFERENT LESIONS. J.A. Rubio*, R.A. Altschuler, B. Kachar, F. Eckenstein* and J. Fex. Lab. Neuro-otolaryngology & Lab. Neuropath. & Neuroanat., NINCDS, NIH, Bethesda, MD 20205 and Max Planck Institute, Martinsreid, Germany.

We have previously shown immunoreactive labelling in olivocochlear efferents in the organ of Corti using antisera against methionine enkephalin, choline acetyltransferase, aspartate aminotransferase and glutaminase. In this study peripheral lesions of the efferents were made and the organ of Corti was examined immunocytochemically both to confirm these findings and to determine if any non-efferent fibers or terminals are also labelled.

200-350 g. female NIH strain guinea pigs were pre-medicated with a mixture of atropine sulphate and chlorpromazine hydrochloride injected intramuscularly. Thirty minutes later ketamine hydrochloride was given intraperitoneally, with additional sodium pentobarbital often added to maintain the anesthesia level. A tracheostomy was then performed and ventilatory support given with a small animal respirator. Under aseptic conditions a posterior craneotomy was performed under a Zeiss operating microscope. Dura was incided and retracted medially. The parafloccular lobe of the cerebellum was then aspirated. Facial, vestibular and cochlear nerves were identified. Using a fine stapes hook, the vestibular nerves were avulsed at the internal auditory meatus, taking care not to lesion the auditory nerve or the internal auditory artery which lies between the nerves. Four to eight weeks after surgery, animals were fixed by vascular perfusion and immunocytochemical procedures carried out on the cochlea using antisera against methionine enkephalin, choline acetyltransferase and glutaminase.

In the cochlea ipsilateral to the lesion, no specific immunoreactive labelling was seen with antisera to methionine enkephalin or choline acetyltransferase. With antisera to glutaminase, in the cochlea ipsilateral to the lesion, immunoreactivity was observed by the bases of inner hair cells, though less was seen than in the contralateral, non-lesioned cochlea. This remaining immunoreactivity may be in the dendrites of spiral ganglion cells, whose cell bodies and terminals show glutaminase-like immunoreactivity.

16.11 TYPE 2 SPIRAL GANGLION CELLS IDENTIFIED BY GLUTAMINASE-LIKE IMMUNOREACTIVITY AND NEUROFILAMENT STAIN. A.M. Schwartz, R.A. Altschuler, R.J. Wenthold, and J. Fex. Lab of Neuro-otolaryngology, NIH, NINCDS, Bethesda, MD 20205, and Lab of Neurophysiology, Univ. Wisc., Madison, WI 53706.

The spiral ganglion of the guinea pig contains two morphologically distinct cell types. Type 2 spiral ganglion cells characteristically are smaller than type 1 cells, are unmyelinated, and have an abundance of neurofilaments within their cytoplasm. They are often but not exclusively located in the periphery of the ganglion in groups of two or three. In immunohistochemical studies, using antisera to glutaminase, which has been proposed as a marker for aspartergic/glutaminergic neurons, some spiral ganglion cells in the guinea pig were noted to lack immunoreactivity. These nonimmunoreactive cells had many characteristics of type 2 cells, but it was not possible to definitively identify them as such based solely on the immunohistochemistry. Therefore we took 10 μm cryostat sections of guinea pig cochlea and using indirect immunofluorescent techniques, reacted them first with antisera to glutaminase. After fluorescence photomicrography, the coverslip was removed and the same sections were restained with the Palmgren silver stain. With this silver stain, type 2 spiral ganglion cells appear darker than type 1 cells because of the abundance of neurofilaments in their cytoplasm. We see a close correspondence between cells with decreased glutaminase-like immunoreactivity and the darker staining type 2 cells in the Palmgren sections. We have thus identified a subpopulation of spiral ganglion cells based on their immunoreactivity to antisera to glutaminase, providing an immunohistochemical basis for future work on these two different cell types.

16.12 STAPEDIUS MUSCLE MODULATION OF AMPLITUDE AND PHASE OF COCHLEAR POTENTIALS IN AVES: PHYSIOLOGICAL AND HISTOLOGICAL EVIDENCE, S. Allen Counter. The Biological Labs., Harvard University, Cambridge, MA. 02138.

Aves, unlike mammals, possess only one middle ear muscle, the stapedius. This muscle, which is innervated by a branch of the facial nerve, is exceptional in the respect that it alone exerts its effects on an entire physiological system, viz. the middle ear as well as on intracranial pressure. Electromyograms from the m. stapedius, inner ear microphonic potentials (MPs) and 8th nerve responses were studied during activation of the m. stapedius in Aves in order to determine the muscle's role in phase and amplitude modulation of sensory input. The maximum MPs recorded in the control state were 200μV and were linear (on a log-log scale) over a range of 40-100 dB SPL. Tension levels of 50-400 mN in the m. stapedius caused a reduction of up to 20 dB in the MP amplitude, depending on the sound frequency. Both phase shifts and amplitude changes could be induced at the ipsilateral and contralateral cochleas during m. stapedius contraction. Intracranial MPs were out of phase at the contralateral ear by 180° at low frequencies (≤1 KHz) and approximately 2 μ at higher frequencies. Both histologic and physiologic studies show that the avian m. stapedius is a fast muscle with fast type (II_1, II_{12}, II_{123}) fibers of essentially uniform diameter (19μm). Teased fibers revealed en plaque endings, well developed sarcomeres, and a dense investment of both sarcoplasmic reticulum and transverse tubules. Preliminary evidence on embryo and early hatchlings suggest sensory pathology following m. stapedial deprivation.

16.14 AFFERENT INNERVATION OF OUTER HAIR CELLS IN THE ADULT CAT: AN HRP STUDY. D.D. Simmons* and M.C. Liberman* (SPON: D.K. Ryugo). Depts. of Anat. and Physiol., Harvard Med. School; Eaton-Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA 02114.

Recent morphological studies of the adult cat cochlea have concluded that afferent neurons of the spiral ganglion can be divided into at least two morphologically distinct types: type I neurons giving rise to radial fibers (RFs) which synapse exclusively with inner hair cells (IHCs) and type II neurons giving rise to outer spiral fibers (OSFs) which synapse with outer hair cells (OHCs) (Spoendlin, H., Arch. Klin. Exp. Ohren Nasen Kehlkopfheilkd. 200: 275, 1971; Kiang, et al., Science 217: 175, 1982). Data on RFs suggest that there may be significant differences in innervation patterns between the neonate and the adult, between the base and the apex of the cochlea, and between the fibers contacting opposite sides of IHCs (Liberman, M.C., Hearing Res. 3: 45, 1980). Compared to our current understanding of RFs we know little about the morphology of the OSFs. Most of the anatomical data concerning the innervation of the OHCs has been based on Golgi-stained material from neonatal animals.

The OSFs in the adult cochlea can be darkly labeled following extracellular injections of horseradish peroxidase (HRP) in the cochlear nerve trunk (Kiang et al., ibid.). This technique was used in the present study to produce the Golgi-like filling of OSFs necessary to study their innervation patterns. OSFs in the adult cochlea were found to pass unbranched through the IHC region, cross the tunnel of Corti along its floor, and spiral in a basal direction for at least 0.2 mm before sending branches to the OHCs. Many OSFs had branches which end in terminal swellings among the supporting cells well beneath the OHCs.

HRP injections were positioned in the nerve bundle so as to label OSFs innervating OHCs in different cochlear regions, exploiting the similarity of the tonotopic organization for the central axons of OSFs and RFs. Several morphological features of the OSFs were found to vary systematically with cochlear location. For example, toward the apex (the low-frequency region) of the cochlea, OSFs traverse longer distances, have more collaterals, innervate more OHC rows, have fewer branches terminating on structures other than OHCs, and have larger diameters and larger terminal boutons. Possible functional significance of these anatomical features will be discussed.

17.2 IN VITRO TISSUE CULTURE MODEL FOR STUDY OF AGENTS CAPABLE OF AFFECTING ADULT CNS REGENERATION. A. Leon, R. Dal Toso*, S. Mazzoni*° and G. Toffano. Fidia Research Laboratories, Department of Biochemistry, Abano Terme, Italy and °Divisione Neurologica, Ospedale per gli Infermi, Faenza, Italy.

Fetal neuronal tissue culture techniques are increasingly being utilized for the identification and study of growth-promoting environmental factors or pharmacological agents capable of positively influencing adult CNS neuronal regenerative capabilities. They also allow for analyses of the occurrence of toxic or specific growth-inhibiting environmental influences during chronic adult degenerative neuropathological diseases. Serumless dissociated fetal mesencephalic cells in culture were therefore utilized as a controlled model system for the study of factors capable of determining regeneration/degeneration of CNS dopaminergic (DA) neurons. In particular, facilitatory effect of monosialoganglioside GM_1 on dopaminergic regeneration in vivo (Toffano, G. et al., Brain Res., 261:163-166, 1983) and the possible occurrence of specific dopaminergic growth inhibiting agents in sera obtained from patients suffering from Parkinson's disease were investigated. The results obtained indicate that:

1. whereas the survival of serumless mesencephalic dopaminergic cells is highly dependent on the cellular density seeded, their dopaminergic maturation (measured as specific ^{3}H-DA uptake) is specifically affected by the presence of their target striatal cells. Such data are in accord with those already reported by Prochiantz A. et al. (Nature, 293:570-572, 1981) and Di Porzio U. et al. (Nature, 288:370-373, 1980).
2. The presence of GM_1 molecules on the neuronal cell surface, either endogenous or following stable insertion of exogenous molecules, affects neurite outgrowth and dopaminergic maturation of fetal mesencephalic cells. Addition of GM_1 at concentrations ranging from 10^{-8} to 10^{-7} M increases the proportion of neurite bearing cells and neurite length of co-cultured mesencephalic-striatal cells. GM_1 doubles ^{3}H-DA uptake in mesencephalic cells and the effect is additive to that produced by striatal target cells. In contrast GM_1 antiserum produces a marked decrease of both neurite extension and ^{3}H-DA uptake.
3. Addition of sera obtained from all male or female patients considered suffering from Parkinson's disease (15 patients, 40-60 years of age) impedes DA cell maturation regardless of the presence of optimal target support. The inhibitory effect is dependent on the concentration of sera (maximal concentration 1:50 dilution) and appears to be correlated with the severity of the disease. The sera are totally ineffective in modifying GABAergic maturation of striatal cells. Control sera obtained from normal subjects are totally without effect.

The relevance of such data will be discussed.

17.3 REGENERATION IN THE LARVAL LAMPREY: AN HRP STUDY. R. E. Coggeshall and B. N. Christensen. Depts. of Anatomy and Physiology & Biophysics Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

Recent experiments on lamprey suggest that regeneration of giant reticulospinal axons is often incomplete or inappropriate (Wood and Cohen, Science, 1979). This is in spite of the fact that the lamprey appears to regain its normal swimming behavior following spinal section. It has been suggested (Selzer, J. Physiol. 1978) that the return of coordinated swimming movements following spinal section are due to reinnervation of motor neurons via polysynaptic pathways. Both of these reports concluded that regenerating axons do not extend more than a few mm beyond the lesion. We investigated the regenerative capabilities of reticulospinal neurons, both "giant" and "non-giant" by examining the number of labelled cells found in the brain after application of HRP to the cut end of the cord from 0.5 - 1.5 cm caudal to spinal crushes made up to four months previously. Two other series of animals were done. In one, we determined the number of brainstem neurons projecting to the dorsal fin region of the spinal cord when HRP was applied to the cut end of the cord at the level of the first dorsal fin. In the second, we crushed the spinal cord 1 cm rostral to the first dorsal fin and then applied HRP to the cut end of the spinal cord 1 cm caudal to the lesion. The latter tested for the possibility that HRP could label cells within the brainstem by a route other than axonal transport along descending spinal axons.

By four months following spinal cord crush our animals regained normal swimming behavior. A large number of reticular neurons were labelled including some of the giant cells which give rise to the giant axons descending the length of the cord. In four animals without lesions, the number of non-giant cells labelled in the brainstem was 171 (+ 73.3 S.E.) and the number of giant cells was 8 (± 2.1 S.E.). In 9 animals transected 4 months previously, the number of labelled non-giant neurons was 57.6 (± 30.6 S.E.) and the number of giants was 3.8 (± 2.2 S.E.). Although there is more than a 50% reduction in the number of labelled cells in the experimental animals, it is clear that a large number of cells located in the brainstem send axons across the lesion for a distance of at least 1.5 cm. These results indicate that some axons regenerate for long distances past a transection and thus behavioral recovery may depend on these axons reaching their original synaptic sites. Second, since a large population of non-giant reticulospinal neurons regenerate axons past the transection, these cells, rather than the giant cells, may be responsible, at least in part, for the behavioral recovery. Supported by grants NS14429, 10161 and 17039 from the NIH.

17.4 DEGENERATION AND REGENERATION-ASSOCIATED POLYPEPTIDES IN RAT PERIPHERAL NERVE. H.W. Müller*, M.J. Ignatius and E.M. Shooter. Dept. of Neurobiol.,Stanford Univ. Sch. of Med.,Stanford, CA94305.

Non-neuronal sheath cells in rat sciatic nerve responded to denervation by the sequential induction of several new polypeptides which were secreted into the distal nerve stump. We were able to identify at least 7 specific denervation-induced proteins with apparent molecular weights from 37,000 (37k) to 45,000 (45k) and isoelectric points between 5.2-5.4. The appearance of these proteins correlated well with periods of axonal degeneration and regeneration. However, the time-course of induction as well as the rates of synthesis and disappearance of these proteins in nerve segments distal to injury were dependent on the particular type of nerve injury applied to initiate degeneration.

(i) When degeneration was initiated by a nerve crush leading to axotomy and subsequent axonal regeneration, the synthesis and secretion of a 45k polypeptide doublet was maximally induced within 3-6 days post-injury prior to the induction of any of the other proteins. Despite a decreasing rate of synthesis during the following weeks the 45k remained at a high concentration in the nerve for approx. 4 weeks until at 7-8 weeks after the crush it had slowly disappeared. A 37k protein (Skene and Shooter, PNAS, 1983, in press) was maximally induced at 1 week post-crush and appeared in the distal stump approx. 2 weeks after the 45k. The 37k remained at a high concentration for 2-3 weeks before it rapidly decreased to background levels.

(ii) In a transected sciatic nerve where axonal regeneration into the distal stump was prevented the same set of soluble proteins was induced and synthesized at similar rates as in (i), thus indicating that the induction of these proteins is not under regulation of regrowing axons.

(iii) When a segment of sciatic nerve was separated by excision from its neuronal input and from its target cells the time-course and rate of synthesis as well as the concentration of the denervation-induced proteins in the isolated piece of nerve was significantly altered. The initial high rate of synthesis of the 45k was maintained for several weeks and this polypeptide remained at high concentration in the nerve sheath for at least 6-7 weeks post-excision. However, the 37k did not accumulate in the isolated nerve segment in detectable amounts for 10 weeks suggesting a role for the target in the maintenance of 37k synthesis.

The other degeneration-induced proteins besides the 45k and 37k were less prominent and their rates of synthesis and accumulation generally resembled that of the 45k throughout the experiments.

17.5 EARLY CALCIUM DEPENDENT INCREASE OF TRANSGLUTAMINASE ACTIVITY IN THE RAT SUPERIOR CERVICAL GANGLION AFTER POSTGANGLIONIC NERVE INJURY. G. M. Gilad and V. H. Gilad*.Isotope Department, Weizmann Institute of Science, Rehovot, Israel.

As early as 2h after postganglionic nerve injury an increase in ornithine decarboxylase (ODC) activity occurrs within parent sympathetic neurons of the superior cervical ganglion (SCG). This increase in ODC activity is rapid and transient, and is most probably due to a transient accumulation of new enzyme molecules. The increased ODC activity reflects an enhanced polyamine biosynthesis which is required for the survival of the injured neurons(Gilad and Gilad, Brain Res., in press). Intracellular transglutaminase (TG) is an enzyme which catalyzes the covalent binding of diamines (e.g. putrescine), or polyamines to glutamine residues on peptide chains and thereby may lead to the formation of crosslinks between adjacent polypeptide chains. In the present study therefore we sought to determine: (a) whether TG activity is present in the SCG and if so, is it activated by calcium (Ca)? and (b) whether TG activity is altered in the SCG after axonal injury and if so, is it dependent on Ca fluxes? The activity of TG was found to be present in homogenates of rat SCG (65.5±6.5 pmol [^{3}H]putrescine bound/h/mg protein) and had an absolute requirement for Ca in the assay mixture. When the enzyme activity was measured at different times (15min to 7h) after unilateral postganglionic nerve crush, an increase of 175.5±8.5 percent ($p=0.001$) was detected by 30min postoperative in the ipsilateral SCG as compared to contralateral or unoperated controls. Such an increase was not observed before or after 30min postoperative. Application in vivo, of a saline (0.9% NaCl) solution containing verapamil (2mM) - an inhibitor of Ca fluxes across membranes of excitable tissues - completely prevented the observed increase in TG activity. The effect of verapamil could be reversed by addition of CaCl (10mM) to the applied solution. We conclude that: (a) within minutes after postganglionic nerve injury,putrescine dependent and Ca activated TG activity is briefly enhanced in the corresponding SCG, and (b) the enhanced TG activity is dependent upon Ca fluxes across membranes proximal to the injury site. The results imply that changes in Ca fluxes, which may be triggered by axonal injury, are essential for the observed increase in TG activity, the earliest biochemical change so far reported in the region of the parent nerve cell body.

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17.6 SELECTIVE REINNERVATION OF LIMB MUSCLES BY REGENERATING FROG MOTOR AXONS. Susan L. Powell* and Monte Westerfield (SPON: B. Gordon-Lickey). Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

We have examined the selectivity of reinnervation of limb muscles by the regenerating axons of frog spinal cord motoneurons following axotomy when the ventral root was crushed or cut. Two factors that might direct regeneration were investigated. 1) Do external factors in the nerve guide the regenerating axons? 2) Do the central connections of motoneurons from limb sensory neurons signal which muscles they should reinnervate?

The anatomical pattern of reinnervation was assayed by retrograde labeling after injection of HRP into individual muscles. The functional pattern of reinnervation by motoneurons was assayed by intracellularly recording antidromic activation of motoneurons upon stimulation of single muscle nerves, and the pattern of central connections between muscle sensory and motor neurons was determined by recording synaptic potentials elicited by stimulation of appropriate peripheral nerves. Our results demonstrate that:

1) Motoneurons randomly reinnervate limb muscles if the ventral root is completely severed by cutting.

2) On the other hand, if the ventral root is crushed, motoneurons selectively reinnervate their old targets. This difference may suggest that alignment of the nerve sheath is required for proper regeneration.

3) Motoneurons reform appropriate connections with muscles (after crushing the ventral root) even when their sensory innervation is absent due to removing the dorsal root.

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17.7 ^{3}H-URIDINE INCORPORATION IN NORMAL AND AXOTOMIZED IMMATURE HAMSTER FACIAL MOTOR NEURONS. Pamela Clark and Arthur LaVelle. Dept. of Anatomy, University of Illinois, Chicago, IL 60680.

A common feature of the axon reaction in mature peripheral motor neurons is increased RNA synthesis, reflecting the anabolic and regenerative nature of the response to injury. Immature hamster facial motor neurons do not survive axotomy as do mature hamster facial neurons. The capacity to survive axotomy is gradually acquired over the first two postnatal weeks. This temporally coincides with the early period of maturation of the neuron's protein biosynthetic apparatus, including the nucleoli which are sites of rRNA synthesis and processing. A study of ^{3}H-uridine incorporation was undertaken to determine how RNA synthesis is altered in facial neurons axotomized during the period when they cannot, or have a reduced capacity to, sustain a regenerative response.

Facial nerves of fetal and early postnatal hamsters were severed by electrocautery and by cutting, respectively, with the unoperated sides serving as control. Animals were sacrificed at times varying from 17 hours to 4 days after injury. One hour prior to killing by perfusion fixation, ^{3}H-uridine (3μCi/g body wt) was injected into each animal. Brain-stems were processed for light microscope autoradiography. Somal area, nuclear area and grain number per unit area cell body were averaged per facial nuclear group. Statistical analysis was by 2-way ANOVA.

Axotomy in fetal hamsters (14 days in utero) resulted in a significant decrease in grain density 17 hours after injury, even though uridine incorporation remained high. Significant changes in grain density were not observed after axotomy in the postnatal ages examined. Mean somal area decreased significantly in neurons axotomized at 4, 7, and 9 days and examined after 2, 2 and 4, and 4 days respectively. Nuclear area, however, did not change.

Thus, immature hamster facial neurons do not increase RNA synthesis to meet the demands of regeneration. Injured neurons from fetal hamsters show a large, rapid decrease in RNA synthesis. Since it appears that these neurons synthesize RNA at a high level normally, the pronounced effect of axotomy stems from interrupting a highly active process. In contrast, injured neurons from early postnatal animals continue to synthesize RNA at normal levels through the postoperative times examined. This correlates well with the gradually increasing capacity of these neurons to survive injury as they mature. The decrease in somal area after axotomy can be interpreted as an interruption of the rapid growth that accompanies neuronal maturation and suggests that synthesis of structural proteins necessary for growth may be significantly altered during this period.

17.8 A TIME COURSE STUDY OF AXOTOMY-INDUCED CHANGES IN RNA SYNTHESIS AND NUCLEOLAR MORPHOLOGY IN IMMATURE AND MATURE HAMSTER FACIAL NEURONS. Kathryn J. Jones and Arthur LaVelle. Department of Anatomy, University of Illinois, Chicago, Illinois, 60680.

We have previously observed that diffuse chromatolysis, cell swelling and increased protein synthesis characteristic of the adult response to axotomy do not occur in the 15-day postnatal neuron. This differential response to axotomy has been temporally correlated with the development, mainly after 15 days, of an intranucleolar body (INB) consisting of ribonucleoprotein (RNP) particles. Axotomy induces dispersion of this INB in the adult by 4 days postoperative (dpo). In this study we examined the initial effects of axotomy on RNA metabolism and on nucleolar morphology in neurons undergoing the two different modes of reaction.

Adult and 15-day old hamsters were subjected to axotomy of the right facial nerves, with the left sides serving as controls. At 1/2, 1, 2 and 4 dpo, some of the animals were injected subcutaneously with ^{3}H-uridine 1 h prior to sacrifice via perfusion-fixation and the brains processed for routine light microscope autoradiography. The remainder of the animals were sacrificed and processed for routine EM. The number of grains per unit area cell body, in 2 μm sections, were averaged per animal. The areas of nucleolar profile tracings from 1 μm Epon-embedded sections were calculated using a BioQuant Image Analyzer. All data were analyzed using 2-way ANOVA. Cytomorphic changes in nucleolar substructure were assessed by EM.

In the 15-day neurons, no changes in uridine uptake after axotomy were noted throughout the time course studied. Nucleolar morphology and size were also unaffected by axotomy at 15 days. In the adult neurons, axotomy induced a transient increase in uridine uptake at 2 dpo, with return to control levels by 4 dpo. This increase in RNA synthesis at 2 dpo coincided with the onset of nucleolar swelling, with an initial "loosening" of the fibrillar portion of the nucleolus and with an incipient dispersion of the INB granular material. Nucleolar area remained significantly higher at 4 dpo when the INB was totally dispersed.

Intranucleolar bodies are thought to be storage sites for ribosomal precursors. Their absence in normal 15-day and in axotomized adult neurons reflects the increased metabolic activity associated with both growing neurons and with regenerating adult neurons. Since it has been shown that the level of protein synthesis remains increased in these adult neurons for a prolonged time after injury, it is surprising that there is such a short spurt of elevated RNA synthesis after axotomy. Although a later increase in RNA synthesis cannot be excluded, we feel that the stored RNP particles in the INB contribute to and meet the initial metabolic demands placed on these cells by axotomy.

17.9 FLAT, WHOLE-MOUNT NERVE PREPARATIONS: A USEFUL TOOL FOR STUDYING PATTERNS OF REGENERATING AXON OUTGROWTH. Y. Geinisman and M.T. Shipley, Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60611 and Dept. of Anat. & Cell Biol., Univ. of Cincinnati Col. of Medicine, Cincinnati, OH 45267.

Present techniques for experimental analyses of regenerating axon outgrowth distal to the site of axotomizing injury have serious limitations. For example, the actual outgrowth distance cannot be measured for individual axons without their entire course reconstruction from numerous histological or ultrathin sections, which is difficult, if not impractical. Further, the direct differential examination of all axonal subpopulations that elongate at fast, intermediate or slow rates is impossible, since only one subpopulation of the fastest growing axons can be directly studied by axonal transport and pinch test techniques. To circumvent these limitations, we have developed a method which is based on the use of flat, whole-mount nerve preparations.

In young adult rats, the right sciatic nerve (SN) is crushed, and HRP (Sigma VI, 40% solution) is injected into the region of cell bodies of spinal motoneurons innervating the musculature of the right hind limb. Rats are perfused 16-24 h later, the SN is dissected out and the epineurium removed. The SN is flattened between two glass slides, removed, then reacted (floating) with TMB, mounted on a micro slide, counterstained with neutral red, dehydrated and coverslipped. At 16 h after the crush, all HRP labeled axons are found proximal to the crush zone. By 2 days, HRP labeled axons are observed crossing the crush zone and passing peripheral to it; the distance between the crush site and axon tips is progressively increased at 4 and 6 days. Many axon tips are enlarged and mark a distinct leading edge of several waves of outgrowing axons. In each wave, both single labeled axons and axonal bundles can be traced along their entire undulating course. The flat SN preparations have also been stained for acetylcholinesterase and reacted with antitubulin antibodies. With both techniques, outgrowing axons can be clearly visualized.

It appears, therefore, that flat, whole-mount preparations of regenerating nerves can be useful for obtaining: (1) accurate estimates of the rate of regenerating axon elongation by measuring the outgrowth distance of individual HRP labeled axons over time after axotomy; (2) values of the "initial delay" of outgrowth by assessing the time necessary for regenerating labeled axons to reach the nerve segment just distal to the lesion site; and (3) information concerning the nature of axonal subpopulations that elongate at different rates by employing histochemical, immunocytochemical and electron microscopic techniques. Moreover, virtually any staining method applicable to floating or mounted histological sections should work well in these preparations.

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17.10 NON-SELECTIVITY OF AXONAL REGENERATION IN THE OCULOMOTOR NERVE OF GOLDFISH. S.S. Scherer, Division of Biological Sciences, The University of Michigan, Ann Arbor, MI 48109

I have reinvestigated the question of whether the extraocular muscles of goldfish are selectively reinnervated by regenerating axons of the oculomotor nerve. Using the retrograde transport of horseradish peroxidase (HRP), I have mapped the motor pools of extraocular muscles that were innervated by normal or regenerated oculomotor nerves.

HRP was injected into either the left superior rectus (SR) (N=3), medial rectus (MR)(N=1), inferior rectus (IR)(N=1), or inferior oblique (IO)(N=2) muscle in 7 normal animals. After 4 days survival, the animals were perfused, and their brains sectioned transversely at 40-50 um. The sections were reacted with diaminobenzidine, and counterstained with neutral red. Labeled and unlabeled motoneurons were plotted with the aid of a camera lucida. As in other teleosts, the motor pools of the MR, IR, and IO were in the ipsilateral (left) tegmentum of the midbrain, whereas the motor pool of the SR was on the contralateral (right) side.

I similarly mapped the motor pool of the left SR in 4 animals whose left oculomotor nerve had been sectioned intracranially 17 (N=2) or 65 (N=2) days before. Labeled cells were found in 3 of the 4 brains; none were found in one brain (65 d). Most of the labeled cells were found in the ipsilateral tegmentum, in positions that corresponded to the motor pools of the MR, IR, and IO, but some labeled cells were found in the contralateral tegmentum, in the position that corresponded to the motor pool of the SR.

Two explanations could account for the wider distribution of labeled motoneurons in lesioned animals: (1) reinnervation of the SR was non-selective, or (2) regenerating axons selectively reinnervated the SR, but the intraorbital diffusion of HRP was more pronounced than in normal animals, and led to the labeling of the other motor pools. The second explanation seems unlikely since the orbital contents were not directly involved in the approach used to transect the oculomotor nerve. Furthermore, behavioral experiments in progress show that animals have abnormal eye movements after the transected oculomotor nerve has regenerated, which is consistent with the hypothesis that reinnervation is non-selective. (Supported by EY07022 to S.S.S. and EY00168 to S.S. Easter, Jr.)

17.11 ABNORMAL PHYSIOLOGICAL PROPERTIES OF REGENERATING SENSORY AXON TERMINALS IN THE CORNEA. Andrew J. Rozsa, Roger W. Beuerman, Brett Dupuy*, and Darrell L. Tanelian. LSU Sch. of Med., Dept. of Ophthalmology, LSU Eye Center, New Orleans, LA 70112.

Our previous report (Neurosci. Abst., 8:300, 1982) presented anatomical descriptions of the reorganizational process of epithelial axon terminals following various types of experimental wounds in the cornea. Collateral sprouting from intact terminals overlapped temporally the primary degeneration of the original terminals. In turn, the early collateral sprouts degenerated in 7-10 days and a second generation of regenerative neurites appeared. These latter neurites originated from the tips of the severed axons. The electrophysiological studies presented here sought to correlate the functional features of degenerating and regenerating corneal axon terminals to the morphological changes described previously.

Twenty-three albino rabbits (2-3 kg) were anesthetized with urethane (1.5 g/kg) and placed in a head holder. The periorbital skin was retracted and the conjunctiva was secured over a stainless-steel ring to ensure a stable preparation. Action potentials were recorded conventionally from dissected axon bundles of the long ciliary nerve. Circular (4 mm dia) wounds were produced by scraping the corneal epithelium with a scalpel. In post-operative preparations exceeding 24 hrs, distinct wound margins were preserved by trephine demarcation.

We examined two conditions: first, the immediate effects or response to acute injury; and second, the initial phase in the restoration of sensory competence to the corneal epithelium. The following neuronal activity and response characteristics were noted: 1) the generation of spontaneous abnormal patterns of neural impulses in unstimulated corneal nerves that are normally quiescent; 2) the replacement of the modality specificity of corneal nerves by a generalized responsiveness to all forms of stimulation; and 3) the prolonged excitation of sensory nerves denoted by after discharges that outlasted the duration of the stimulation. The area of tissue confined within the wound margins displayed a zone of absolute sensory loss; this area was surrounded by a narrow peripheral zone of partial sensory loss. The biophysical specificity of terminals was reestablished after 24 hrs, although the hyperexcitability to stimulation persisted for the duration of the post-operative period investigated (7 days). We hypothesize that the ectopic neural activity and responsiveness characteristics that follow corneal injury are neural substrates for acute pain. Collateral sprouts appear to possess sensory functions; however, their overreaction to stimulation is probably the neural substrate for hyperpathia. (Supported by USPHS grants EY04074 and EY07073, NEI).

17.12 NEUROMUSCULAR PROPERTIES DURING CLAW REGENERATION IN ALPHEID SHRIMP. P.J. Stephens and J.M. Leferovich*. Department of Biology, Villanova University, Villanova, PA. 19085.

The dimorphic claws of snapping shrimp consist of a smaller pincer claw and a larger snapper claw. In Alpheus californiensis the structural dimorphism is accompanied by a profound asymmetry in the properties of the closer muscle. The closer muscle in the snapper claw is composed of a near uniform population of slow fibers, while the pincer claw closer has two different types of muscle fibers. Analysis of sarcomere length and myofibrillar adenosinetriphosphatase (ATPase) activity revealed that a central band of fast fibers is located along the longitudinal axis of the muscle and intermediate-type fibers are situated on the dorsal and ventral margins.

The closer muscle in each claw is innervated by two excitatory motor axons. The fast closer exciter (FCE) supplies all closer muscle fibers whereas the slow closer exciter (SCE) makes functional connections only with fibers in the central portion of the muscle. In the pincer claw, injection of dye through the recording microelectrode demonstrated that muscle fibers supplied by only the FCE are fast, while those innervated by both excitatory axons are intermediate-type fibers. Thus there is a close matching between the innervation pattern of the SCE and the regional distribution of different fiber types in the pincer claw closer muscle. These data are consistent with the hypothesis that nerve-muscle interactions take place during development.

We have made observations on the closer neuromuscular system during regeneration of the pincer claw. Upon emergence of the claw from its limb bud, analysis of sarcomere length and ATPase activity revealed that the closer muscle is composed of a near homogeneous population of fast fibers. During the ensuing intermolt period the muscle fibers on the dorsal and ventral margin of the closer differentiate from fast to intermediate-type fibers. Examination of the innervation patterns of the two exciters revealed that at all stages of claw regeneration the peripheral fields are similar to those in fully developed claws. Thus the innervation patterns of the two excitatory motor axons are established before muscle differentiation, suggesting that any trophic interactions must proceed from nerve to muscle.

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17.13 LAMININ IN REACTIVE SCHWANN CELLS. LIGHT- AND EM IMMUNOLOCALIZATION IN TRANSECTED RAT SCIATIC NERVES. A. Bignami, N.H. Chi* and D. Dahl. Spinal Cord Injury Research, Brockton-West Roxbury VA Medical Center, and Department of Neuropathology, Harvard Medical School, Boston, MA.

Laminin is an extracellular noncollagenous matrix glycoprotein present in all basement membranes so far investigated. Laminin is produced by several cell lines and promotes the adhesions of cells to a substratum in vitro. The expression of laminin by early astrocytes in primary dissociated cultures of newborn murine brain has been reported recently (Liesi et al., J Cell Biol. 96:920, 1983). Immunofluorescence with laminin antisera revealed a striking change in the localization of this protein in rat sciatic nerve as a result of Wallerian degeneration. In normal sciatic nerve, the staining was confined to the endoneurium and the same was also found to be true during the first days of degeneration. On day 11 endoneurial tubes were not more identified in the distal stump of crushed nerves and of nerves that had been transected and tightly ligated to prevent regeneration. In both crushed and ligated nerves proliferating Schwann cells forming the cell-bands of Büngner were intensely laminin positive. As indicated by double-labeling experiments, laminin and neurofilament antisera revealed similar but not identical staining patterns in crushed nerves, thus suggesting a close relation between laminin and regenerating axons. Crushed nerves had recovered their normal appearance 3 weeks after operation while anti-laminin reactivity was markedly decreased in ligated nerves probably as a result of fibrosis. The localization of laminin in reactive Schwann cells was confirmed by immunoperoxidase labeling at the EM level. Schwann cell cytoplasm engulfing myelin breakdown products and surrounding regenerated axons was intensely stained. No reaction product was present in axons. Supported by NIH grant NS13034 and by the Veterans Administration.

17.14 RELEASE OF FAST-TRANSPORTED PROTEINS FROM REGENERATING NERVE. Bruce Tedeschi* and David L. Wilson. Dept. of Physiology & Biophysics, Univ. of Miami Sch. of Med., Miami, FL. 33101.

The secretion of axonally transported proteins from intact nerve was reported (Hines & Garwood, Brain Res., 125:141, 1977) but not confirmed (Tedeschi et al, Brain Res. 211: 175, 1981). In the present study, we have demonstrated the secretion of a subset of rapidly transported proteins from regrowing axons in previously-crushed frog sciatic nerve.

To study secretion from axons, proteins in DRG were labeled in vitro with ^{35}S-methionine. After axonal transport, any released proteins were collected in the bath of a small chamber surrounding the sciatic nerve at its crush site. The released material was subjected to 2-dimensional polyacrylamide gel electrophoresis and the pattern of labeled, released polypeptides detected with Kodak XAR film.

The results showed that: (1) A specific subset of rapidly transported proteins is released from axons at the crush site; (2) Most proteins that are rapidly transported and released from axons are different from those synthesized and released by the surrounding cells in the sciatic nerve; (3) One exception is a glycoprotein, designated B14 (Mr = 30,000 to 40,000 daltons, pI = 5 to 6), which also is released from a number of other tissues in a variety of organisms.

The release from axons appears to represent a physiological process because (1) the release is selective, (2) no release is seen during the first 24 hours after nerve crush, and (3) the release continues to occur for weeks after damage, while axon regrowth is occuring. The release may be associated with new membrane addition in the growing axons. The role of the released proteins is not known. (Supported by NSF grant BNS 81-17817, NIH grant NS18263 and NIH training grant NS07044.)

17.15 RELEASE OF NON-NEURONAL PROTEINS FROM REGENERATING NERVE. David L. Wilson and Bruce Tedeschi* (SPON: L. Dribin.) Dept. of Physiology & Biophysics, Univ. of Miami Sch. of Med., Miami, FL 33101

We have examined the proteins that are synthesized in and released from non-neuronal cells in the frog sciatic nerve after nerve damage.

Frog sciatic nerves were unilaterally crushed with forceps. The uncrushed contralateral nerves served as controls. At 1 to 28 days following damage the frogs were sacrificed, and the sciatic nerves were removed and desheathed. In small chambers, various parts of the previously-crushed nerve (proximal to crush, crush site, distal to crush) or control nerve were incubated in a bath containing frog Ringers and ^{35}S-methionine. Following labeling (3-7 hours), the material released into the baths was subjected to 2-dimensional polyacrylamide gel electrophoresis and the polypeptides detected with Kodak XAR film. For quantitative analysis, selected gel regions corresponding to polypeptide spots on the film were cut out and counted in a liquid scintillation counter.

One protein, designated B14 (Mr = 30,000 to 40,000 daltons, pI = 5 to 6), was found to be released in greater amounts from crush and distal nerve regions than from proximal regions of damaged nerve or control nerve. This increase was not seen initially (1 to 3 days post-crush) suggesting a possible association with the regeneration process. It is interesting that this protein, B14, is the one protein which we have found to be both a rapidly transported protein released from axons and a non-neuronal protein released from the cells surrounding axons in the sciatic nerve. The relevance of the present findings to reorganization of an injured nerve is not known. (Supported by NSF grant BNS 81-17817, NIH grant NS18263 and NIH training grant NS07044.)

17.16 FUNCTIONAL AND ANATOMICAL CONSEQUENCES OF NEONATAL INFRAORBITAL NERVE DAMAGE IN RAT. M. Math*, A. Brown*, M.F. Jacquin and R.W. Rhoades. UMDNJ-NJSOM & RMS, Piscataway, NJ 08854.

The infraorbital (IO) nerve's innervation of the mystacial vibrissae and its central representations at the various levels of the trigeminal (V) neuraxis have been used by numerous investigators to examine the central nervous system response to neonatal peripheral damage. Surprisingly, with only a few exceptions, the effects of neonatal IO damage upon organization in the V ganglion and brainstem have not been examined in detail. We have used a combination of Electronmicroscopic, HRP tracing and single unit recording techniques to assess the consequences of neonatal section of the IO nerve in rat.

In normal adult rats (N=4) the IO nerve contained from 17,480 - 22,259 myelinated and 12,088 - 15,851 unmyelinated axons. Electronmicroscopic examination of this V branch in a neonatally lesioned adult animal revealed only 5,260 myelinated and 2,747 unmyelinated axons.

Single unit recording from the ophthalmic-maxillary portion of the V ganglion also demonstrated substantial effects of this manipulation. In normals, 44% of the 96 units recorded were responsive only to deflection of the whiskers while in the lesioned rats only 13% of the 64 cells tested were activated by such stimulation. Moreover, 31% of the cells in the lesioned rats responded tonically to a noxious pinch or deep pressure while only 12% of the units recorded in the normal ganglia yielded such responses. In spite of these changes in single unit response characteristics, the normal topographic organization of the ganglion was maintained in the lesioned rats.

Transganglionic HRP transport was used to examine the innervation of the brainstem by the normal and regenerate IO nerve. In the normals, somal clusters resembling the barrels or barreloids were not visible. In the brainstem, however, barrelike aggregates of HRP labelled terminals could be seen in principalis, interpolaris, caudalis and the first cervical segment. In the lesioned animals, the number of V ganglion cells labelled by HRP application to the IO nerve was substantially reduced and the terminal distribution in the brainstem for this V branch was also markedly abnormal. Transganglionic transport from the regenerate IO nerve indicated an almost exclusive projection to the marginal layer of the medullary and rostral cervical dorsal horn.

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17.17 NERVE REGENERATION IN A SUPRAMAXIMAL GAP. L. J. Stensaas. Dept. of Physiology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Nerve regeneration in a supramaximal gap following severance of a nerve and removal of the distal stump depends on the growth and development of the regenerating unit. The regenerating unit (RU) consists of a small open-end perineurial sheath surrounding a cluster of axon sprouts and reactive Schwann cells. Axons and Schwann cells within the unit originate from single myelinated parent fiber in the proximal stump which grow out into the connective tissue matrix of the lesion accompanied by the new perineurial sheath. During the process of regeneration the RUs display a maturation gradient in which the more proximal axons are larger, more numerous and become myelinated early while those situated distally are smaller, unmyelinated and few in number. Thus, maturation of most cellular components of the RU occurs even in the absence of nerve union with the distal stump. Each of the many RUs emverging from the proximal stump maintains its individuality within the lesion by virtue of the rapid concomittant development of the perineurial sheath from mitotically active Schwann cells near the tip of the regenerating axons. Depending on the location and orientation of the units, they collectively give rise to three structures appended to the proximal stump: 1) the nerve regenerate, 2) stump regenerate (neuroma) and 3) epineurial regenerate. In addition to changes in the size and number of RUs along the maturation gradient of the nerve regenerate of a 21-day preparation, remarkable changes occurred in the connective tissue matrix of the regenerate. Such changes were most conspicuous near the "front" of regenerating axons where RUs were incompletely ensheathed by perineurial cells. The changes included a dense collagen network within which there were numerous hyperplastic fibroblasts and capillaries. The fact that such reactive tissue developed ahead of the nerve regenerate implies that factors elaborated within the RUs were liberated from the open, distal end of the regenerating units and served to stimulate cells in the connective tissue through which the regenerating axons will pass. With time a progressive deposition of dense (epineurial) collagen developed around RUs in the nerve stump and epineurial regenerate. The presence of a dense connective tissue matrix in the "capsule" of the long term terminal and epineurial regenerate may contribute to the development of a painful neuroma.

17.18 MORPHOLOGICAL ASSESSMENT OF MYELINATION OCCURRING IN ADULT PERIPHERAL NERVES REGENERATING THROUGH A SILICONE CHAMBER. J.M. LeBeau*, F.M. Longo* and M.H. Ellisman. (SPON: J.D. Hatton) Laboratory for Neurocytology, Department of Neurosciences, University of California, San Diego, School of Medicine, La Jolla, CA 92093.

A model system in which peripheral nerve regenerates across a 10mm gap between cut ends of the nerve has been described (Lundborg, Longo and Varon, Brain Res. 232:157-161, 1982). This system involves suturing 2 mm of the proximal and distal stumps of transected rat sciatic nerve into a 14 mm long silicone tube. Since regeneration and myelination occur somewhat separated in the intervening 10 mm space, this system provides a natural gradient of changes across the chamber representing the sequence of axon-Schwann cell interaction. This sequence has been examined with light and electron microscopic techniques, including freeze-fracture EM. Migration of vascular endothelial and Schwann cells from both ends of the cut nerve follows the development of an acellular matrix that spans the length of the chamber. Axonal outgrowths of the proximal nerve stump then invade the chamber. Axonal growth cones appear to associate with the Schwann cell surface. Several millimeters behind the advancing growth cone, multiple lamellar processes of the Schwann cell surround the axons. New nodes of Ranvier are defined before the lamellar processes of the encircling Schwann cell begin to compact into myelin. These are defined by the subaxolemmal densification, observed in thin sections, and clusters of axolemmal particles, observed by freeze-fracturing. The internodal spacing is short, similar to that observed for nodal spacing of early development and other regenerating systems. The newly formed nodes are normal in appearance, however, the annulus of heterogeneously sized particles, characteristic of the nodal zone of the axon, is prominent. (Supported by NIH grant NS14718, MDA and NMSS grants to M.H.E.)

17.19 REGENERATION OF STRETCH RECEPTORS IN RAT MUSCLE. D. C. Quick* and S. L. Rogers* (SPON: W. R. Kennedy). Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

In traumatized muscles, the muscle fibers undergo a process of degeneration and regeneration (B. M. Carlson, 1978, Physiol. Bohemoslov. 27:387). Histological studies have shown that muscle spindles are also capable of regeneration (S. L. Rogers, in press, Devel. Biol.). Nerve fibers are known to be present in regenerated spindles but their functional status has been uncertain. We undertook electrophysiological studies of regenerated rat muscles to determine whether functional stretch receptors could be found (see also D. C. Quick & S. L. Rogers, in press, Neurosci.).

One extensor digitorum longus muscle from each rat was traumatized by devascularization and injection of Marcaine; the contralateral muscle served as a control. After 2-4 months, the muscle nerve was tested for afferent electrophysiological activity as the regenerated muscle was stretched. Sensory units could be found in nearly every muscle tested, and could often be localized by probing the belly of the muscle. The responses in some units resembled those of normal muscle spindles, but the responses of most regenerated units were very rapidly adapting and many were only transiently sensitive to stretch stimuli. In addition, the inter-spike intervals were often highly irregular. We therefore conclude that regenerated muscle spindles can regain functional activity, but their response properties are abnormal for at least 4 months in rats. These results suggest that the criteria for evaluating the success of a muscle graft or transplant should include a consideration of whether the muscle can participate in sensory-dependent processes, including tone control, initiation of reflexes and kinesthesia.

This work will be extended to cats in order to study single regenerated muscle spindles in vitro and to make direct correlations between histological and electrophysiological data from individual spindles. (Sponsored by a grant from the National Science Foundation.)

17.20 THE SYNTHESIS AND AXONAL TRANSPORT OF GLYCOPROTEINS IN AXOTOMIZED FROG NEURONS FROM ANIMALS MAINTAINED AT 15°C AND 25°C. Marino De Leon and Richard C. Carlsen, Department of Human Physiology, University of California, Davis, CA 95616.

Previous studies in our laboratory have shown that axotomized spinal neurons in the frog do not undergo a cell body response when the animals are maintained at 15°C. In contrast, spinal neurons in frogs kept at 25°C show cell body changes beginning 5-6 days after injury. Injured neurons in 15°C animals initiate regenerative sprouting despite the absence of cell body changes, but regenerative growth is abortive and injured motoneurons do not reinnervate their former target muscles. The purpose of the present study was to compare injury-induced changes in the synthesis and axonal transport of glycoproteins in frogs kept at 15°C or 25°C. Glycoproteins are a major constituent of axon structure and have been shown to be increased during regeneration of peripheral nerves. Experiments were performed on Northern Rana pipiens maintained at either 15°C or 25°C. The synthesis and axonal transport of H^3-fucose labeled glycoproteins were determined in vitro at room temperature (21°C). Isolated ganglia were incubated with H^3-fucose for 4 hours. Axonal transport continued for 18 hours and transported glycoproteins collected at a ligature (normal nerve) or at the transection. At the end of this first transport period a second ligature was placed around the nerve 1.0 cm proximal to the first collection point and incubation continued for 6 hours. Glycoprotein accumulation distal to the second ligature was used as a measure of axonal "turnaround". Experiments were performed at 4,7,12 and 29 days after axotomy in 25°C frogs, and at 14,21 and 36 days after axotomy in 15°C animals. The general features of injury-induced changes in glycoprotein synthesis and transport were as follows: a) In 25°C Frogs; fast anterograde transport of glycoproteins in axotomized nerves increased 2 fold over control nerves within 12 days of injury. Synthesis of fucose labeled glycoproteins by axotomized cell bodies also increased by 40-50%. Late anterograde transport increased by 20% and "turnaround" of glycoproteins in injured nerves was approximately 250% greater than in ligated control nerves. b) In 15°C Frogs; the pattern of synthesis and transport in injured nerves was the opposite of that occurring in 25°C nerves. Axotomized 15°C nerves synthesized and transported about 50% less glycoprotein than control nerves. There was no apparent difference in synthesis and transport by control nerves from 15°C or 25°C animals. We conclude that one feature of the signal initiating the cell body response to axotomy is an induction of increased glycoprotein synthesis and transport, and that this induction is required to support continued growth and maturation of regenerating axons (Supported by NIH NS15065).

17.21 FORSKOLIN ACTIVATION OF ADENYLATE CYCLASE IN VIVO STIMULATES SENSORY NERVE REGENERATION. Suzanne L. Kilmer* and Richard C. Carlsen. Department of Human Physiology, University of California, School of Medicine, Davis, CA 95616.

The previous demonstration of an increase and redistribution of adenylate cyclase activity in injured nerve suggests that an increase in neuronal cyclic AMP concentration could play a role in peripheral nerve regeneration. We have determined that accumulating adenylate cyclase activity was translated into a two-fold increase in cyclic AMP concentration in the regenerating nerve stump, coincident with the initiation and elongation of regenerative sprouts. We sought to magnify the role of cyclic AMP in regeneration by using Forskolin, a robust activator of adenylate cyclase, to produce an additional increase in neuronal cyclic AMP in situ. Forskolin in vitro produced an approximately 40-fold greater elevation in neuronal cyclic AMP than an equimolar (10-5) concentration of isoproterenol. The increased cyclic AMP concentration was sustained for at least 60 minutes in the continued presence of Forskolin. Sensory nerve regeneration, measured by axonal transport of radioactive proteins to the most distal point of regenerative growth, was compared in Forskolin-treated and saline-treated frogs. Injection of Forskolin, in situ, using several patterns of application had a significant stimulatory influence on sensory nerve regeneration in freeze-lesioned sciatic nerves of Rana pipiens. Neurons in treated frogs regenerated at a rate 38% faster than their paired controls, 2.71 mm/day and 1.95 mm/day, respectively. The time to initiation of regenerative growth was not affected. In contrast, reinnervation of the gastrocnemius muscle by motor nerves was not enhanced. The degree of reinnervation was determined by comparing the tension produced by stimulating the nerve to that produced by direct stimulation of the whole muscle. Forskolin and saline-treated motoneurons reinnervated the muscle at the same post-injury day (21) and recovery of nerve evoked twitch and tetanic tensions occurred at the same rate. This contrast in the Forskolin effect on sensory and motor nerve regeneration does not support a role for Forskolin at the site of axonal growth, or the possibility that Forskolin may enhance regeneration by serving as a general metabolic stimulant. Instead, the positive effect on sensory regeneration suggests that Forskolin may act at injured cell bodies in dorsal root ganglia. The inability of Forskolin to cross the blood-brain barrier would exclude it from contact with axotomized motoneurons. These observations suggest that an increase in cyclic AMP concentration in axotomized cell bodies may stimulate processes involved in regenerative axonal growth.

(Supported by NIH Grant NS 15065)

17.22 AN IMPROVED METHOD UNMASKS HIDDEN POLYNEURONAL INNERVATION AT FROG NEUROMUSCULAR JUNCTIONS. A.A. Herrera. Dept. Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

Focal polyneuronal innervation (PI) is the convergence of 2 or more presynaptic axons to the same postsynaptic site. It is commonly observed at developing vertebrate skeletal neuromuscular junctions and, in frogs, persists throughout adulthood. In 16 normal adult Rana pipiens sartorius muscles, 102 of 639 endplates tested (16%) showed PI. The incidence was variable, ranging from 5 to 35% in muscles from different batches of frogs and in experiments done at different times of the year. The incidence of PI, however, was consistently enhanced in reinnervated muscles. In 10 frogs tested 67-107 d after the left sartorius nerve was crushed near its entrance to the muscle the incidence of PI was 40% (118/295), significantly higher than normal ($P<0.0002$). These measurements were made by careful application of the conventional method of estimating PI: Muscles stretched to 1.1x rest length were immersed in the minimum concentration of curare ($2\text{-}4 \times 10^{-6}$ M) necessary to block twitching and attempts were made to subdivide the intracellularly recorded endplate potential (EPP) by varying the amplitude, polarity, and duration of a stimulus applied to the nerve. Since spontaneous miniature EPPs, the smallest units of transmitter release, are never seen under these conditions, this method will obscure very weak inputs and therefore underestimates PI.

A new method of determining PI uses high concentrations of formamide to block excitation-contraction coupling (J. Cell Biol. 78,782). Muscles were exposed to 2M formamide in Ringer for 17-22 min at 7°C. This depolarizes muscle fibers to an average resting membrane potential of about -60 mV, a desirable feature that tends to block the generation of muscle fiber action potentials. Miniature EPPs were seen at all endplates with resting potentials more negative than -40 mV. In 6 reinnervated sartorius muscles so treated, 46 of 62 endplates examined (74%) showed PI, a level significantly higher than in curare (40%, $P<0.0002$). In two individual reinnervated muscles, the same groups of endplates were tested both in curare and after formamide treatment. Formamide revealed higher PI in both (curare 49 and 39%, after formamide 89 and 63%, respectively). Of the 28 endplates with PI seen after formamide treatment in these 2 muscles, 7 had inputs whose EPP averaged less than 3.5 mV in amplitude and showed quantal fluctuations. Three of these occasionally failed to release upon nerve stimulation. I hypothesize that the increased PI seen in reinnervated muscles after formamide treatment is due to the enhanced detectability of very weak polyneuronal inputs.

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17.23 IMPLANTED ELECTRODES FOR MONITORING ACTION POTENTIALS IN REGENERATING CAT NERVES. C. Krarup* and G.E. Loeb, Lab. of Neural Control, NINCDS, NIH, Bethesda, MD 20205.

The in situ study of conduction along regenerating and constricted nerve fibres requires the use of near-nerve electrodes because of the low amplitude responses and high threshold to electrical stimuli. To obtain a high signal-to-noise ratio and a fixed spatial relationship of the electrodes to the nerve, stranded stainless steel wires, 100 μm in diameter, were sewn along the inside of silastic tubes 2.6 and 3.3 mm in diameter and implanted around the tibial and sciatic nerves of cat. The leads were placed subcutaneously and connected to a socket secured to the back of the animal. Nerve action potentials were recorded by electronic averaging using a tripolar electrode configuration (Stein, R.B., et al., Brain Res. 128:21-38, 1977). The six independent leads spaced along each cuff allowed the local conduction velocity to be calculated from the responses recorded simultaneously at two independent tripolar sites. The amplitude of the normal compound nerve action potentials ranged from 3 to 7000 μV depending on stimulation and recording site. The evoked muscle action potential (10 - 30 mV) was recorded via a bipolar surface patch electrode implanted on the fascia of plantar muscles; a distal sensory branch of the plantar nerve could be activated via this electrode. The implanted electrodes remained stable over many months. Conduction along regenerating fibres in the tibial and plantar nerves after crush was examined weekly in lightly anesthetized animals. Four weeks after crush the action potential was 0.5 μV (0.25 percent of normal), conducted at 10 m/s (10 percent of normal), and had a 10-fold increase in threshold (2 - 3 ma). Within 100 days after crush, the conduction velocity along sensory and motor fibres increased to about 60 percent of normal. In other animals the tibial nerve was constricted by placing a silastic cuff 1 mm in diameter around the nerve. This caused 40-60 percent slowing in distal conduction velocity. In the most severely affected nerves the conduction velocity proximal to the constriction evoked by distal stimuli was 50 percent slowed as well, suggesting selective survival of small fibres. In less affected nerves the proximal velocity was normal, indicating survival of large fibres with slow conduction velocity and presumably smaller caliber distal to the lesion (Baba, M., et al., 54:197-208, 1982.)

Implanted electrodes are sensitive enough to record the action potential from regenerating nerve fibres and remain stable over many months. Distal to a constriction, conduction was slowed consistent with nerve fibre atrophy.

17.24 AXON-MYELIN ANOMALIES REVEALED BY DIAMINOBENZIDINE REACTION WITH INTRA-AXONAL HORSERADISH PEROXIDASE. R.J. Preston*, J.D. Kocsis and S.G. Waxman. (Spon: M. Weinrich) Dept. of Neurology, Stanford Univ. Medical Sch. and Veterans Admin. Med. Ctr., Palo Alto, CA 94304.

In studies of rat sciatic nerve, including samples from normal and demyelinated nerve and nerves with experimentally induced neuromas, the DAB reaction with HRP-filled axons differentiated myelinated vs nonmyelinated fibers and also seemed to provide a sensitive light-microscopic indication of aberrant axon-myelin structure. Central ends of 1-2 cm segments of desheathed nerves were soaked in HRP (Sigma VI; 3% in Kreb's soln.) in vitro for 1-2 hrs. at 5°C. Nerves were rinsed and stored in Kreb's for 12-45 hrs (5°C) prior to glutaraldehyde fixation (5%;5 hrs;5°C), PO_4 buffer rinse (pH 7.4; containing 0.2M sucrose; 12-18 hrs; 5°C) and processing with 3,3'-diaminobenzidine tetrahydrochloride (0.05%) and H_2O_2 (0.06%). Reacted nerves were examined in glycerol or after Vestopal embedding. In normal nerves, reaction product (RP) was continuously distributed in small diameter nonmyelinated fibers, but was mostly limited to nodal and paranodal axon of myelinated fibers where it formed a 6-12 μm long rod centered at the node and extending into adjacent paranodal areas. Internodal and continuous staining of myelinated fibers was less frequent and often in superficial fibers possibly damaged by dissection. However, when nerves were frozen whole or freeze-sectioned prior to DAB processing, continuous RP reliably occurred in myelinated fibers. In nerves with fibers demyelinated by loose ligature, RP appearance depended on location. Beneath and 0.6-1.5 mm central to the ligature, both normal and abnormal RP distribution occurred in myelinated fibers. The latter extended for 25-90 μm or more, sometimes asymmetrically about nodes and other times without apparent node or myelin profiles. More centrally, normal nodes prevailed and nodal sprouts were more frequent. In segments ending in neuromas, few or no normal nodes occurred within 2.5 mm of the terminal stump, and only scattered medium and large diameter fibers had continuous RP despite numerous stained axons in this zone after freezing. More centrally, normal nodal RP was less prevalent than distributions showing: densely stained nodes with extensive symmetric or asymmetric spread of RP to adjacent internodes; extensive stained axonal regions with one or both adjacent paranodes absent or thinned; occasional nodal sprouts and many fine, varicose nonmyelinated processes. We cannot presently specify the mechanism through which this histochemical procedure may differentiate normal and abnormal axon-myelin relationships, but we expect that the procedure will be useful for light microscopic study and for focusing ultrastructural study of pathological tissue. Supported by the National Multiple Sclerosis Society and the Veterans Administration.

17.25 MYO-INOSITOL UPTAKE IN REGENERATING SCIATIC NERVES OF MICE. D.E. Matsumoto and R.M. Gould. Dept. of Anatomy, Howard University Coll. of Med., Washington, D.C. 20059 and New York State Inst. for Basic Res. Ment. Retard., Staten Island, N.Y. 10314.

It has been shown that myo-inositol, when injected into sciatic nerves, is incorporated into phosphoinositides by normal axons and Schwann cells, and that CDP diacylglyceride:inositol transferase, the terminal enzyme involved in de novo synthesis of phosphatidyl inositol, is present in axons (M.H. Kumara-Siri and R.M. Gould, Brain Res. 186:315, 1980). In the present study, myo-inositol uptake was examined in regenerating nerves. To initiate regeneration, sciatic nerves of anesthetized adult mice were unilaterally crushed with fine-tipped forceps and, after survivals of 2,3 or 5 days, the nerves were injected (under halothane anesthesia) with 75 to 100 μCi of (^{3}H) myo-inositol. Glass micropipettes were used to make multiple injections at locations which were proximal and distal to the crush site. After 1 or 2 hr postinjection survival, the anesthetized animals were perfused with formaldehyde-glutaraldehyde fixative, the nerves removed, washed, osmicated and processed for embedment in epoxy resin. Semithin and thin sections of nerves were processed for autoradiography at the light and electron microscopic (EM) levels. In autoradiographs of nerves with 2 or 3 days postcrush survivals, the level of labeling was low except for an accumulation of grains in the region immediately proximal to the crush. In nerves with 5 days postcrush survival, the regenerating neurites have crossed the crush site and entered the distal portion of the nerve. The labeling pattern is correlated with the presence of the neurites so that the grain density is highest around the crush site and in the distal part of the nerve where regenerating axons occur, and the labeling is low in more proximal regions, and the general level of labeling is greater than that observed in specimens with 2 days survival. In EM autoradiographs, the neurites, including growth cones, had the highest labeling of all the structures in regenerating nerves. The processes of Schwann cells were also labeled to some extent, but grains rarely occurred over the nucleus and degenerating myelin. Examination by thin layer chromatography of lipid extracted from regenerating nerves established that injected (^{3}H) myo-inositol was incorporated into phosphoinositides. The evidence therefore indicates that in the early stage of regeneration axons are the predominent site where myo-inositol is incorporated into phospholipids. This study was supported by NIH Biomedical Research Support Grant 5807RR05361 and NIH Grant NS 16305.

17.26 REINNERVATION OF SKELETAL MUSCLES AFTER SCIATIC NERVE TRANSECTION AND REPAIR. S.S. Deshpande*[1], N.H. Goldberg*[2], C.K. Meshul[1], F.K. Toy*[2], K.S. May*[2], R.T. Smoot*[2] and E.X. Albuquerque[1] (SPON: T. Oh). Depts. of [1]Pharmacol. and Exp. Ther. and [2]Surgery University of Maryland School of Medicine, Baltimore, MD 21201.

Reinnervation of the fast extensor digitorum longus (extensor) and slow soleus muscles after nerve crush (J. Gen. Physiol. 61:1, 1973) or after blockade of axonal transport by batrachotoxin (Brain Res. 225:115, 1981) occurs at old endplates (EP). This study deals with the innervation of the extensor and soleus muscles after transection of sciatic nerve and after its immediate surgical repair. The sciatic nerve was cut 30 mm from its entry into the extensor muscle in Wistar female rats (200 g). Immediate microneurorrhaphy with 3-5 epineural sutures (10-0 nylon) was performed. At intervals up to 8 months, the regeneration in the nerve and reinnervation of muscles were studied using electrophysiologic and light and electron microscopic (EM) techniques. At 8 days after surgery, the extensor and soleus muscles showed 20% atrophy and were denervated as shown by membrane depolarization, absence of spontaneous miniature endplate potentials (mepps) or nerve-evoked muscle action potentials (AP). EM of EP disclosed absence of nerve terminals and presence of Schwann cell growth over junctional folds. At 2 weeks, extensor and soleus muscles were depolarized by 25-30 mV. The proximal nerve segment had grown through the lesion site but apparently had not made functional synapses in the muscle. By 22 days, the muscles were still depolarized by 25 mV, but mepps with low frequency (0.16 vs 3.2 Hz in control) and amplitude (0.4 vs 0.5 mV in control) were recorded indicating reinnervation. Nerve stimulation evoked APs with low rates of rise and amplitude. No muscle reinnervation was observed 22 days after nerve transection alone. At 50 days, the muscles showed complete repolarization, no signs of denervation and EM of EPs at 6 weeks showed normal EPs. At 8 months after nerve repair the soleus muscle still showed 45% atrophy. Although sensory function was established between 2-3 weeks after the operation, motor ability, particularly that of digital extension, had not been regained even at 8 months. Up to day 30, daily subcutaneous injection of GM 1 ganglioside (50 mg/kg) was ineffective in enhancing reinnervation after nerve repair. It is likely that, whereas regenerating axons after crush injury use old conduits (endoneurial tubes) for reinnervation in a precise fashion to bring complete functional recovery, the same axons after nerve transection and immediate repair could be misdirected to old or newly formed endoneurial tubes during the process of regeneration. (Supported by USPHS Grant NS-12063 (EXA) and Bressler Research Fund (NHG).)

17.27 FIBER ELONGATING FACTOR(s) ORIGINATED FROM THE GOLDFISH BRAIN: CHARACTERIZATION AND PURIFICATION. Y. Mizrachi*, Y. Kimhi*, M. Rubinstein*+ and M. Schwartz (Spon: I. Ginzburg). Depts, of Neurobiology and Virology*, The Weizmann Institute of Science, Rehovot 76100, Israel.

We have recently shown that goldfish brain contains factors that enhance neurite outgrowth from regenerating retinal explants. Two of these were purified and further characterized.

Crude preparation was obtained by collecting the 100,000g supernatant of homogenized goldfish brain. Several peaks of activity were separated by chromatography on a Sephadex G-100 column. Among them, two distinguishable low molecular weight substances (range of 5,000-10,000 daltons) could be detected by differences in their properties: One, designated SNEF (substrate neurite elongation factor) was found to exert its effect after preadsorption onto a poly-L-lysine treated substratum. A second, designated DNEF (diffusible neurite elongation factor) could not exert a significant effect when preincubated with the substratum. Whether this is caused by poor adsorption or "substratum-inactivation" is being investigated.

The ability of SNEF to exert an effect while being adhered, was employed to determine the nature of this peptide factor; we found that phytohemagglutinin, adsorbed onto plates, precoated with SNEF, inhibited the capability of SNEF to promote neurite outgrowth. This inhibition could be reversed by an addition of N-acetylglucose amine. The results suggest that this factor has an exposed sugar moeity.

With respect to activity *in situ*, we found that DNEF accelerated regeneration associated processes. Thus, a single injection of G-100 fraction (10 µg/eye) performed simultaneously with a crush of the optic nerve shortened the period required for expressing neurite outgrowth *in vivo* (extensive outgrowth was observed 3-6 days earlier than the regular schedule post injury). The DNEF fraction was further purified by HPLC, yielding a component of a very high specific activity (10^7 fold enriched in terms of specific activity relative to the crude preparation). The relation between the two factors is currently being examined.

17.29 THE EFFECTS OF MSH/ACTH PEPTIDES ON FAST AXONAL TRANSPORT IN RAT SCIATIC NERVE. L. Crescitelli*, °K.L. Keim and F.L. Strand, Dept. Biology, New York University, N.Y., N.Y., 10003 and °Dept. Pharmacology I, Hoffmann-La Roche Inc.,Nutley,N.J.,07110.

MSH/ACTH peptides are known to improve neuromuscular function [Strand, et al. Pharmacol Biochem Behav, 5: 179, 1976] and to promote the regeneration of peripheral nerve [Strand and Kung, Peptides 1: 135, 1980] in rats. To determine if peptide-induced changes in axonal transport (AT) were in part responsible for the previous findings, we studied the downflow rate of fast AT in intact rats treated with either MSH/ACTH 4-10 or MSH/ACTH 4-9 Met$(O_2)^4$D-Lys^8 [ie, ORG 2766] and in hypophysectomized (hypox) rats. Male Sprague-Dawley rats (225-325 gm; N=4 to 6) were used. Following treatment, a dorsal laminectomy was performed and 4 x 1 microliter injections of 3H-leucine (5 mCi/ml) were applied into the ventral horn of the 4th and 5th lumbar segments. Four hours later the animals were killed and perfused with 10% phosphate buffered formalin. The sciatic nerve was excised and divided into 3mm segments, and solubilized in Soluene 350 at 50°C for 18 hrs; the samples were counted for 3H at 35% efficiency for 10 min.

We determined the downflow AT rate in intact control rats to be 414 ± 16 mm/24 hr. This corroborated the findings of others [eg, 410 mm/24 hr; Ochs, Science, 176: 252, 1972]. In rats treated for 7 days with 10 µg/kg/day of MSH/ACTH 4-10 the fast AT rate was 416 ± 14 mm/24 hr. A fast AT rate of 412 ± 3 mm/24 hr was determined in rats treated for 7 days with 1 µg/kg/day of MSH/ACTH 4-9 Met $(O_2)^4$D-Lys^8. From 12-16 day hypox animals, a fast AT rate of 405 ± 12 mm/24 hr was found. Thus, neither an excess of MSH/ACTH peptides, those reported to be neurotropic in many other experiments, nor the absence of circulating endogenous MSH/ACTH peptides, as evidenced from experiments in hypox animals, affected fast AT.

However, more recent experiments indicate that MSH/ACTH 4-10 may alter AT in regenerating nerves. While the fast AT rate was not altered by nerve-crush in rats treated with peptide, the area under the AT curve (dpm x mm nerve) suggests an increase in the apparent amount of 3H-leucine incorporated protein present in sciatic samples from treated rats. That MSH/ACTH fragments might increase the amount of protein transported in regenerating nerves would support the concept that improvements in neuromuscular function and regeneration could be caused by such peptides.

17.30 TRANSIENT PROPERTIES OF MECHANORECEPTIVE AFFERENTS DURING REINNERVATION OF THE GLABROUS SKIN OF THE HUMAN HAND.
R Mackel*, EE Brink, G Wittkowsky* Dept. of Neurology, Technical University of Munich, Moehlstrasse 28, 8000 Munich 80, FRG.

The technique of percutaneous microneurography was used to record single unit activity from 64 mechanoreceptive afferents during the early stages of reinnervation (between 6 and 13 months postoperatively). The results were obtained from 13 patients who had suffered complete transsection, with subsequent repair, of the median or ulnar nerves. No functional connections were detected in another 4 patients examined at earlier postoperative (4-6 mos.) times.

Three types of mechanoreceptive (RA, SAI, SAII) and many unidentified units innervating deep tissues, but no Pacinian corpuscle units were found. Most receptors were in the palm and proximal parts of the fingers, consistent with the poor clinical recovery of the patients[1], and in contrast to distributions in normal subjects. The sampling distribution of afferents also resembled that seen in patients with poor recovery examined three or more years after surgery[1]. Additionally, response thresholds and discharge characteristics were comparable to those of afferents studied three or more years after nerve repair, and similar to normal[1]. However, two abnormalities not previously seen in humans were encountered. Firstly, multiple receptive fields innervated by single afferents (RA, SAI) and complex receptive fields (SAI) were found in plotting fields at 1oX von Frey hair threshold. Secondly, some SAI (3/14) and deep (5/22) units fatigued easily, showing a much decreased responsiveness to mechanical stimuli repeated with interstimulus intervals of 4-8 seconds. Normal afferents and those studied long after nerve repair showed no such fatigue to similar stimuli.

These abnormal properties are considered to reflect the active process of regeneration. It is suggested that the fatigue may be due to structural or metabolic alterations of the outgrowing axon. The disappearance of multiple receptive fields when regeneration is complete may be due to coalescing of separate fields or retraction of peripheral sprouts.

1) Mackel et al., Brain Res (1983), in press.

17.31 AGE-RELATED CHANGES IN THE STIMULATION OF THE REGENERATION OF THE HYPOGLOSSAL NERVE BY TESTOSTERONE. W.H.A. Yu. Department of Anatomy, Mount Sinai School of Medicine, New York, N.Y. 10029.

By quantitating the hypoglossal neurons labeled retrogradely by horseradish peroxidase (HRP) injected into the tongue, we have shown that administration of testosterone propionate (TP) accelerated the regeneration of the hypoglossal nerve in young adult rats (Exp. Neurol. 77:129, 1982; 80:in press, 1983). In the present study I have investigated whether regeneration of the hypoglossal nerve could be similarly influenced by TP in pre-pubertal rats. Three and 4 weeks old rats of both sexes were used. After unilateral hypoglossectomy under anesthesia, half of the rats in each experimental group were injected with 2 mg TP on every third day, and the other half with the oil vehicle alone. The rats were killed at the 11th, 13th, and 15th post-operative day, respectively Twenty-four hours prior to killing, 50 ul of 10% HRP solution was injected into the midline of the tongue. The total numbers of HRP labeled neurons in both hypoglossal nuclei were counted in each rat. The number of labeled cells in the lesion side was expressed as a percentage of that of the intact side and referred to as "the percentage regeneration". The percentage regeneration increased two folds between the 11th and 15th post-operative day. In 3-weeks old rats no difference in the percentage regeneration between the TP and control groups was observed at any post-operative period. In contrast, the percentage regeneration of 4-weeks old rats treated with TP was significantly greater than that in the control group at the first 2 post-operative periods. These data indicate that the responsiveness of the hypoglossal neurons to testosterone administration during regeneration is age dependent. The hypoglossal neurons of adult rats were shown to take up and retain radioactively labeled androgens into their nuclei (Sar and Stumpf, Science, 197:77, 1977). The ability of testosterone to accelerate nerve regeneration in mature but not in immature rats may be correlated with the level of androgen receptors in the neurons.

17.32 EFFECT OF EXTRACELLULAR MATRIX COMPONENTS ON THE REGENERATION OF ADULT SENSORY NEURONS IN CULTURE. Jean R. Wrathall, Department of Anatomy, Georgetown University, Washington, D.C. 20007.

The regeneration of peripheral neurons in vivo occurs in association with nonneuronal cells of the PNS and extracellular matrix (ECM) components produced by these cells. The importance of factors produced by PNS nonneuronal cells in neural regeneration has long been postulated but the mechanisms involved have not been determined. Studies on the regeneration of adult neurons in culture may be used to investigate these mechanisms.

The regeneration of adult sensory neurons in culture appears to be dependent upon the presence of PNS nonneuronal cells and is maximal upon co-culture of neurons with a mixture of Schwann-like and fibroblastic cells (Wrathall, J. Cell Biol. 95:92a, 1982). In the present experiments, neuron regeneration was studied in the absence of nonneuronal cells per se, but in the presence of types of ECM components produced by PNS nonneuronal cells.

Dorsal root ganglia from adult mice (9-12 months old) were dissociated with collagenase and the sensory neurons seeded at low densities (6,000 viable neurons/dish) onto 35mm tissue culture Petri dishes previously coated with type I collagen, type IV collagen, laminin or combinations of collagen and laminin. As controls, replicate cultures were prepared with neurons seeded onto uncoated dishes and onto monolayer "cell beds" of mixed adult PNS nonneuronal cells (cell line SNCT). The number of neurons that attached and the percentage that had regenerated neurites was determined 24 hours later. The percentage of neurons that regenerated neurites varied with the substrate according to the following progression: type I collagen < tissue culture Petri dish < type IV collagen < type I collagen + laminin < laminin < type IV collagen + laminin < mixed nonneuronal cell bed.

With the combination of ECM components tested that appeared optimal (type IV collagen + laminin) almost 60% of the attached neurons regenerated neurites as compared to about 5% on type I collagen and 70% on a living cell bed of mixed PNS nonneuronal cells. Thus ECM components appear to have a significant effect on the regeneration of adult neurons in culture. Those components associated with basal lamina (type IV collagen and laminin) appear to stimulate regeneration to a significant degree. Whether the stimulatory effect of living nonneuronal cell beds on neurite regeneration is due, at least in part, to their production in culture of these ECM components, is currently being investigated.

(The purified type IV collagen and laminin used in these experiments were a generous gift of Drs. Hynda Kleinman and George Martin.)

17.33 ANALYSIS OF PROTEINS RELEASED BY NERVE SHEATH CELLS DURING DEVELOPMENT. G. Jackson Snipes*, J. H. Pate Skene, and John A. Freeman (SPON: L. Aulsebrook). Dept. of Anatomy, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232, and Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA, 94305.

Molecules released by nerve sheath cells have been assigned many roles including tropic, trophic, growth inhibiting, maintenance and structural. We have undertaken a time course study to indentify interesting proteins released by nerve sheath cells from the time of birth to adulthood in rats. We have also compared proteins released by the central optic nerve sheath with the peripheral sciatic nerve glia. During the developmental period examined in this study, most myelin-forming cells will complete their final cell division. These experiments were performed essentially as described by Skene and Shooter (PNAS, in press). Briefly, the nerves were excised, washed in phosphate buffered saline, incubated in nutrient media containing ^{35}S-methionine. Labelled proteins in the supernatant were trichloroacetic acid precipitated and analysed on 2-dimensional O'Farrell gels visualized by flourography.

Among the interesting proteins released by optic or sciatic nerve sheath cells are proteins with molecular weights of 37 kilodaltons (kd), 51kd, 54kd, 47kd, and 75 kd. The 37kd protein is expressed at high levels in the neonate optic and sciatic nerves, but its synthesis is greatly reduced in adult nerves. These proteins co-migrate with a protein identified by Skene and Shooter as being induced in sheath cells following a nerve crush. The 51 and 54 kd proteins were found only in the sciatic nerve and could not be detected until about 2 weeks after birth. The synthesis of these proteins has been reported to be greatly reduced following nerve injury. The optic nerve releases a 47kd protein which, like the 51 and 54 kd proteins of the sciatic nerve, is expressed at maximal levels in the adult animal. The 75kd protein, which exhibits pronounced microheterogeneity, is found in both neonatal optic and sciatic nerves, but has not been detected in either the normal or regenerating adult nerves.

Thus, we conclude that nerve sheath cells in different functional states secrete different proteins. Neonatal sheath cells release proteins different from those of adult sheath cells. Sheath cells in the peripheral and central nervous system secrete proteins that are qualitativley similar (but not identical) to each other. The pattern of secreted proteins is also different between developing neonatal and regenerating adult sciatic nerves. We are presently preparing monoclonal antibodies against some of these proteins so that we may begin to answer questions on their function.

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17.34 REGENERATION OF A SENSORY AND A MOTOR NERVE. Chung Bii Jenq* and Richard E. Coggeshall. Marine Biomedical Institute and Neurosciences Graduate Program and Dept. of Anat. and Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas.

Previous work concerning axonal regeneration in peripheral nerves concentrated on the myelinated fibers. Since the majority of axons in peripheral nerves are unmyelinated, these fibers should also be considered.

Young adult (200-500 gm) male rats were anesthetized by nembutal (intraperitoneal injection 35mg/Kg). The right sciatic nerve was transected 2-3mm distal to the ischial tuberosity. Stumps of the severed nerve were reapproximated within a silicone tube. A stitch through the epineurium and the wall of the tube was made to secure the approximation.

The rats were sacrificed 8 weeks postsurgery by perfusion with aldehydes in cacodylate buffer. After perfusion, the nerve to the medial gastrocnemius (NMG), a typical somatic motor nerve, and the sural nerve (SN), a typical somatic sensory nerve, were removed and processed for EM embedding.

All myelinated and unmyelinated axons of the NMG or SN were counted. The data are presented in Table 1. Note that in NMG, numbers of both myelinated fibers and unmyelinated fibers from the operated side are always greater than those from the unoperated side whereas in SN, the unmyelinated fibers are less on the operated side. The differences are significant ($p<.05$).

The present study suggests that myelinated and unmyelinated fibers behave differently in our regeneration paradigm. We presume that a greater understanding of these differences may be useful in consideration of function and therapy.

	NMG				SN			
	Myelinated		Unmyelinated		Myelinated		Unmyelinated	
	Oper	Unop	Oper	Unop	Oper	Unop	Oper	Unop
#1	556	291	616	335	1552	1005	1580	3350
#2	656	436	814	395	1362	1050	1805	3483
#3	577	320	629	465	1931	957	2093	3143
#4	716	439	835	772	2273	1145	1670	3380
#5	310	314	636	348	2273	1042	1886	3842
#6	568	296	251	213				
	$p=0.005$		$p=0.018$		$p=0.026$		$p<0.001$	

This work is partially supported by Grants NS07377 and 17039 from NIH.

18.1 **THE EFFECT OF TRIFLUOPERAZINE ON ACETYLCHOLINE RECEPTOR SYNTHESIS IN CULTURED CHICK MYOTUBES.** M. Schneider*, B.H. Shieh*, L. Pezzementi*, and J. Schmidt. Department of Biochemistry, S.U.N.Y. Stony Brook, Stony Brook, N.Y. 11794.

Acetylcholine receptor appearance rate in the presence of the phenothiazines trifluoperazine and chlorpromazine was measured in cultured chick myotubes by means of ^{125}I-αbungarotoxin. At drug concentrations of 5 to 10 x 10^{-6} M, receptor appearance rate was significantly enhanced while receptor half-life, cellular protein, net protein synthesis rate, and acetylcholinesterase levels were not similarly affected. The phenothiazine sulfoxides were less potent by over an order of magnitude. Drug combination experiments revealed that receptor stimulation caused by phenothiazines, like that due to local anesthetics and blockers of voltage-gated sodium and calcium channels, is overcome by low concentrations of ryanodine, but, unlike that seen with lidocaine and tetrodotoxin, is insensitive to membrane depolarization with 20 mM KCl. The drug sensitivity of the trifluoperazine effect resembles that observed with D-600, suggesting that phenothiazines may block a voltage-gated calcium channel and thereby stimulate receptor synthesis. At concentrations of 3 x 10^{-5} M and above, both trifluoperazine and chlorpromazine inhibited receptor synthesis and caused myotube contracture and cell loss, presumably due to release of calcium from the sarcoplasmic reticulum. These results lend support to the role of calcium as an intracellular messenger in receptor synthesis regulation, but render unlikely the notion that calmodulin serves as the calcium receptor in this signaling pathway.

18.2 AGE-DEPENDENT CONVERSION OF SYNAPTIC PROPERTIES AT THE CRAYFISH NEUROMUSCULAR JUNCTION THROUGH ALTERED MOTONEURON ACTIVITY. G.A. Lnenicka and H.L. Atwood, Department of Physiology, University of Toronto, Toronto, Ontario, Canada.

Crustacean tonic and phasic motoneurons have synaptic properties which are in accordance with their functional requirements. Phasic axon synapses produce large EPSPs which depress rapidly during repetitive stimulation. Tonic axon synapses generally produce smaller EPSPs which are more resistant to fatigue. In order to test whether neural activity plays a role in the development of these properties, tonic activity was imposed upon a phasic motoneuron. The phasic "fast" closer excitor of the crayfish claw was chronically stimulated through an implanted electrode, and the population response of its neuromuscular synapses monitored via a recording electrode in the closer muscle. The fast excitor was stimulated for two weeks, two hours per day at 5 Hz, while the contralateral control claw was left unstimulated. After conditioning, the fast excitor initial EPSP amplitude (measured during 0.1 Hz stimulation) and the EPSP depression durin 5 Hz stimulation were measured intracellularly in a restricted subset of closer muscle fibers. A comparison of the conditioned and control fast excitor synapses demonstrated that in sexually immature juvenile crayfish, conditioning produced a 40% decrease in the initial EPSP amplitude, and a 9-fold increase in synaptic endurance (ratio of EPSP amplitude after 30 minutes of stimulation at 5 Hz to initial EPSP amplitude). In large adult crayfish this regime of stimulation produced no decrease in the initial EPSP amplitude and only a 2-fold increase in synaptic endurance. Measurements of muscle fiber input resistance, and membrane time constant indicate that changes in the EPSP produced by conditioning reflect changes in the amount and pattern of transmitter release. The effect of chronic stimulation upon the juvenile fast excitor synaptic properties has been found to persist for at least 7 days after the end of the conditioning regime.

Fast excitor synapses in recently hatched crayfish produce small EPSPs which facilitate during repetitive stimulation. During growth, these synapses gradually develop into the high output depressible synapses found in the adult. Preliminary measurements of fast excitor activity in free-moving crayfish indicate that the fast excitor is more active in small crayfish. It appears that the development of the phasic synaptic properties during growth could be a response to a decrease in the activity of the motoneuron, and imposed activity reverses or halts this change in synaptic function. This neuromuscular system should allow a cellular analysis of the influence of activity upon synaptic type, and the consolidation of synaptic properties during maturation.

18.3 HYPERTHERMIA-AND PENTYLENETETRAZOL-INDUCED NEONATAL SEIZURES FACILITATE KINDLING IN THE ADULT RAT. M.F. Gilbert and D.P. Cain. Dept. of Psychology, U. Western Ontario, London, Ont. N6A 5C2

Facilitation of electrically kindled seizures has been demonstrated in adult rats following a series of subconvulsive injections of Pentylenetetrazol (PTZ) (Cain, D., Pharm.Biochem.Behav., 17:1111,1982). A decreased threshold to PTZ seizures following neonatal treatment with hyperthermia (HYP) (McCaughran, J. & Schetcher, N., Epilepsia,23:173,1982), and an increased susceptibility to HYP-induced seizures as a result of neonatal PTZ convulsions has also been demonstrated (McCaughran, J. & Manetto, C.,Exp. Neurol.,79:287,1983). The present study investigated the effects of seizures induced by HYP and PTZ at various ages upon seizure susceptibility in the adult using the kindling model of epilepsy.

A single seizure was induced in young rats at 1 or 10 days of age with HYP or PTZ. A multiple seizure condition consisted of 3 seizures at 1, 3, and 5 days of age. Animals were left to mature and at 90 days of age or more were implanted with teflon-coated nichrome wire electrodes in the amygdala or pyriform cortex. One to two weeks postoperatively, afterdischarge (AD) threshold was determined, and thereafter a 1 sec train of square wave pulses at 200µA and 60 Hz was delivered daily in a standard kindling paradigm until 3 stage 5 generalized seizures were observed. Generalized seizure threshold was determined 2 weeks after the last seizure.

A significant facilitation in amygdaloid kindling was observed in the single HYP ($\bar{X}$=8.8 ADs) and PTZ ($\bar{X}$=8.1 ADs) conditions at 1 day of age relative to saline injected and handled controls ($\bar{X}$=13.8 ADs). No further facilitation was evidenced in the multiple seizure conditions ($\bar{X}$=8.8 and 10.1 ADs for HYP and PTZ, respectively). A similar facilitation was evidenced for pyriform cortex kindling following a single PTZ seizure at 1 day of age ($\bar{X}$=6.6 vs. 8.6 ADs for controls). No differences were found in AD or generalized seizure threshold between any of the groups.

This facilitation, however, appeared to be age limited. Animals receiving a single PTZ seizure at 10 days of age did not differ from controls in amygdaloid kindling rate ($\bar{X}$=12.8 vs. 13.8 ADs for controls). These results cannot be attributed to differences in the length or severity of the seizures between age groups as PTZ seizures in 10 day olds were longer and as severe as those observed in 1 day olds. In addition, similar kindling rates were observed in the HYP and PTZ conditions even though PTZ seizures were always of a much longer duration and frequently involved a tonic component never observed during HYP-induced convulsions. Similarily, multiple seizures did not result in any further facilitation in kindling rate. This age specific increase in seizure susceptibility therefore is believed to reflect a proclivity for neuroplasticity in the developing organism, rather than brain damage induced by neonatal convulsions. (Supported by a NSERC grant to D.P.C.)

18.4 NEUROMUSCULAR BLOCKADE WITH BOTULINUM TOXIN DECREASES THE INCORPORATION OF SODIUM CHANNELS INTO SKELETAL MUSCLE. L. Bambrick*, T. Gordon and J. Baumgold. Dept. of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The density of extrajunctional acetylcholine receptors (ejAChR) in skeletal muscle is regulated by the motor neuron during development, denervation and reinnervation. Studies using neuromuscular blocking agents to interfere with junctional transmission (Lavoie, P.-A., et al., Exp. Neur., 54, 148, 1977) or electrical stimulation of denervated muscle (Linden, D.C. and Fambrough, D.M., Neurosci., 4, 527, 1979) indicate that this effect is, at least in part, mediated by the nerve-evoked muscle activity rather than by some nontransmitter nerve-released substance.

The present study was undertaken to determine whether nerve-mediated activity may regulate the density of the voltage-sensitive channel (Na-channel), which is another integral membrane protein, and further whether incorporation of Na-channels and ejAChR are regulated reciprocally. Na-channel density increases during development (Sherman, S.J. and Catterall, W.A., J. Gen. Physiol., 80, 753, 1982) and declines following denervation (Barchi, R.L. and Weigle, J.B., J. Physiol., 295, 383, 1979) in contrast to the direction of change in ejAChR density. To discriminate between the effects of nerve-evoked muscle activity and of a possible nerve released factor on Na-channels, experiments were performed using botulinum toxin (BoTx). BoTx is a neuromuscular junction blocker which acts presynaptically to prevent the release of ACh while not disrupting the terminal, interfering with axonal transport or directly affecting the muscle.

Sprague Daley rats at different stages of development received subcutaneous injections of BoTx into one leg. The experimental and control contralateral leg muscles were removed at various days following treatment. The presence of ejAChR was assayed by using the contractile response to exogenously applied ACh as a measure of ACh sensitivity. The muscles were subsequently homogenized and Na-channels assayed using ^{3}H-saxitoxin (^{3}H-STX) a Na-channel specific neurotoxin. The number of ACh receptors was assayed using ^{125}I-labelled α-bungarotoxin (^{125}I-αBTX). The affinity constants (K_d) and maximum bindings (B_{max}) were determined by a Scatchard analysis as well as from a direct hyperbolic fit of the data. The effect of BoTx in all ages was to concurrently decrease Na-channel numbers and increase ACh sensitivity. The K_d of the bindings was unchanged. Our results indicate that nerve evoked activity mediates the incorporation of Na-channels into muscle membrane and that this incorporation may be reciprocally related to the decreased incorporation of ACh receptors into the extrajunctional membrane.

18.5 RAPID REMODELLING OF NEUROMUSCULAR JUNCTION DURING SHORT-TERM DISUSE. Fahim, M.A. and Robbins, N. Anatomy Dept., Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Disuse in adult neuromuscular junctions (NMJs) leads to rapid changes (within 3-5 days) in neuromuscular synaptic physiology (review; Robbins, Trends in Neurosci., p. 120, 1980). Therefore, it was of interest to investigate whether junctional structure was correspondingly modified. In an experimental disuse model, we employed a recently developed technique for obtaining large numbers of single fibre NMJs for topologic examination by scanning electron microscopy (SEM). Disuse was achieved by pinning the ankle and knees of male 150-180 g Sprague-Dawley rats, a procedure known to cause instant and long-lasting disuse of about 90% (Fischbach & Robbins, J. Physiol. 201, 305, 1969). After 3,5,7 and 10 days, muscles were removed and prepared for SEM (Fahim et al., J. Neurocyt. 12, 13, 1983) and light microscopy.

In soleus muscles, the motor endplate appeared as a smooth, slightly elevated, elliptical 'raised area' into which the synaptic clefts were etched. After only 3 days of disuse, the primary clefts became more frequently rounded in shape rather than linear channels, although the areas of the primary cleft and of the 'raised area' were unchanged. Corresponding to the rounded shapes, quantitative measurements of primary clefts showed a significant increase of the ratio of perimeter to center line length at 3,5 and 7 but not at 10 days of disuse. The changes may have receded either because of loosening of the pin, circumferential atrophy compressing the folds, or to some adaptation. Atrophy of about 20% was noted at 3-7 days and of 36% at 10 days.

In a pilot study of 5 day disused muscles, using the zinc iodide osmium method to visualize nerve terminals, there was enlargement of the nerve terminal area and most frequently, ultraterminal sprouting. Although nerve sprouting has been observed after total disuse obtained by various drugs (Brown, Ann. Rev. Neurosci. Abst. 4, 17, 1982), this is the first report of nerve terminal sprouting in disuse obtained without drug or nerve-muscle injury.

The SEM changes after 3-7 days of disuse differ from those seen after total TTX-induced disuse (Labovitz et al., Neurosci. Abst. 8, 755, 1982) where the 'raised area' is considerably reduced. Thus, "physiological" disuse, which is never complete, may differ in its consequences from that of artificial total disuse.

In conclusion, endplates undergo rapid structural remodelling in response to diminished activity. The presynaptic nerve terminals may be more plastic, exhibiting growth, while the postsynaptic structures undergo distortion. This work was supported by NIA grant AG 00795 and NS 18694.

18.6 SHORT AND LONG TERM SYNAPTIC STRUCTURAL CORRELATES OF ACTIVITY AND EFFICACY CHANGE IN RAT HIPPOCAMPAL SLICES. W. T. Greenough and F.-L. F. Chang. Depts. of Psychol. and Anat. Sci. and Neur. and Behav. Biol. Prog., Univ. Illinois, Champaign-Urbana, IL 61820.

Lee et al. (J. Neurophysiol., 1980, 44:247) reported increased density of shaft synapses and reduced variation in spine shape measures in hippocampal CA1 shortly after potentiating stimulation of Schaffer collateral afferents. However, their activity controls received less stimulation than the LTP preparations. We have further studied structural correlates of 1) long term potentiation (LTP), 2) equivalent low frequency synaptic activity, 3) continuous high frequency synaptic activation which did not produce LTP, and 4) synaptic inactivation by high Mg^{+2}/low Ca^{+2} incubation, in subfield CA1 in the in vitro hippocampal slice, and have examined the persistence of changes at 10-15 min, 2h, and 8h after stimulation. After potentiating stimulation (6 trains at 100 Hz for 1 s or 200 Hz for 0.5s), compared to an equivalent number of low frequency stimuli (1 Hz for 600s), there were increases in numbers of shaft and sessile synapses (synapses on stubby, headless spines). This suggested 1) an increase in the number of shaft synapses onto inhibitory interneurons and/or 2) an enhancement of synapse formation on pyramidal neurons possibly involving initial formation of shaft synapses and a transition from shaft, to sessile to spine synapses. Postsynaptic spine heads also assumed a rounder shape, as indicated by decreases in spine perimeter to area ratios, contact lengths, and the percentage of 'cup' shaped spines. There was no effect of potentiating stimulation on bouton or spine areas. After continuous high frequency synaptic activation (40 Hz or 100 Hz for 10 min), which produced no apparent LTP, there were no changes in synapse numbers or spine head shape parameters. However, in contrast to effects of LTP there was an increase in bouton mitochondrial area and a marginal increase in bouton area compared to the low frequency condition. Inactivation did not affect any of these measures. LTP associated increases in numbers of shaft and sessile synapses persisted over an 8h incubation period, while the effect on spine shape disappeared after 2h. Physiologically-demonstrable LTP persisted over the 8h period. Effects of continuous high level activation on mitochondrial and bouton areas were even more transient, disappearing 2h after stimulation.

These findings 1) confirm previously reported effects of potentiating stimulation on synapse numbers and spine shape, 2) indicate that spine shape changes are not necessary for the maintenance of LTP, and 3) indicate that continuous high frequency activation which does not produce LTP has different (and non-persisting) effects from potentiating stimulation. (Supported by NSF BNS-8216916 and NIH ST32 GM7143)

18.7 REGIONAL BRAIN 2-DEOXYGLUCOSE UPTAKE DURING PERFORMANCE OF A LEARNED REACHING TASK. Jannon L. Fuchs, Sandra M. Bajjalieh*, Cheryl A. Hoffman* and William T. Greenough. Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Regional brain activity in rats performing a learned reaching task was studied using [^{14}C] 2-deoxyglucose autoradiography. The reaching task has previously been used as a model learning system for investigating changes in brain RNA and protein chemistry (Hydén & Eghazi, 1964; Hydén & Lange, 1968). Recently, pyramidal cell apical dendritic branching was found to increase in the motor cortex forelimb area contralateral to the trained paw (Larson & Greenough, 1981). The present study was carried out to localize brain areas metabolically activated during the reaching task in order to provide a basis for further detailed anatomical and metabolic studies.

Fifteen male hooded rats were trained to reach through a hole in their plexiglass training cage for small pieces of food placed on a platform just outside the cage. A transparent cage partition was placed next to the hole so that the rat could only reach food using its initially non-preferred paw. As a consequence of the partition placement, the rat was positioned such that only one eye could view the food in the reaching situation. Animals were trained in 2 sessions per day (total 1 hr.) for 7 days. On the 8th day, subjects were injected i.p. with [^{14}C] 2-deoxyglucose (5 μCi/100g). The post-injection survival period lasted as long as the subjects kept reaching (usually for 35-55 minutes). Littermate controls (N=7) received the same treatments but were not trained, and were given either free access to food or no food during the post-injection period.

Increases in 2-deoxyglucose uptake were observed in the hemisphere contralateral to the reaching paw in areas including primary motor and sensory forelimb regions of cerebral cortex, and in prefrontal cortex, dorsal column nuclei, and caudate-putamen. Uptake in the ipsilateral hemisphere resembled that of controls in these regions. On the side contralateral to the eye which could view the food, increased label was observed in the superior colliculus and lateral geniculate nucleus. The asymmetry in superficial layers of superior colliculus was particularly marked, and was nearly abolished in 6 additional rats which performed the learned reaching task under very dim red light.

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18.8 A (^{14}C)-2-DEOXYGLUCOSE STUDY IN THE ADULT RAT SOMATOSENSORY CORTEX FOLLOWING ACUTE AND CHRONIC DEAFFERENTATION. W.D. Dietrich, R. Busto*, M.D. Ginsberg and D.W. Smith*. Cerebral Vascular Disease Research Center, Departments of Neurology and Anatomy, University of Miami School of Medicine, Miami, Florida 33101.

Previous quantitative histochemical studies demonstrated that chronic sensory deprivation produces changes in the activities of certain enzymes related to energy metabolism in individual barrels of the posteromedial barrel subfield (PMBSF) (Dietrich, et al., J. Neurosci. 2:1608-1613, 1982). Surprisingly, enzyme activities in barrels associated with intact whiskers were increased significantly over control values, apparently the result of an increased utilization of the intact sensory periphery by the animals. In the present investigation, we have examined the effects of deafferentation on the somatosensory cortex of adult rats to ascertain whether increased metabolic activity in this cortex could account for the previously reported enzymatic changes. Deafferentation was produced in experimental animals by first plucking all the large whiskers on one side of the face and next cauterizing the corresponding hair follicles. Following 1, 2, 5, 10 and 15 days post-whisker removal, 2-DG studies were performed according to the method of Sokoloff et al. for the determination of local cerebral metabolic rates for glucose (LCMRG).

Glucose utilization was reduced in the contralateral barrel subfield 1, 2, 5 and 10 days post-whisker removal, with the largest decrease occurring at 1 day (approximately 69% of sham-operated control values). Following this period, there was a progressive recovery of LCMRG at each subsequent time period. In contrast, an increase in LCMRG was demonstrated in the ipsilateral barrel subfield at selective time periods with the largest increase occurring at 5 days post-deafferentation. Isolated areas of high glucose utilization within the region of the PMBSF were apparent in groups of 3 to 5 overlaying the approximated region of lamina IV. Quantitative analysis of these individual regions demonstrated LCMRG to be approximately 130% of sham-operated controls. In contrast, increased glucose utilization in the region of the barrel subfield could not be demonstrated in 15-day animals.

The present findings demonstrate that following permanent removal of all the large whiskers on one side of the face, a transient increase in glucose utilization occurs in the PMBSF associated with the intact set of whiskers. The results are thought to be indicative of an increased utilization of the intact sensory periphery under these conditions. Finally, these results demonstrate that following a sustained period of decreased or increased stimulation, there is an apparent readjustment of the level of glucose utilization in the affected brain region.

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18.9 NUMERICAL DENSITY OF SYNAPSES IN NEUROPIL OF OCCIPITAL CORTEX OF RATS REARED IN COMPLEX, SOCIAL, OR ISOLATED ENVIRONMENTS. Anita M. Turner* and William T. Greenough (SPON: C. L. Prosser). Depts. Psychol. & Anat. Sci., and Neur. & Behav. Biol. Prog., Univ. Illinois, Urbana-Champaign, IL 61820.

Quantitative studies of Golgi-stained tissue have indicated that neuronal dendritic fields are more extensive in the visual cortex of rats reared in complex environments (EC) than in rats reared socially (SC) or individually (IC) in standard laboratory cages. While it has been widely assumed that this was associated with an increased number of synapses, reported synapse numbers per unit area, uncorrected for size, have been lower in EC than in IC rats (e.g., Diamond et al., J. Neurosci. Res. 1:109, 1975). Our results, using stereological calculations, conflict with this prior work. Members of 11 littermate triplet sets of male Long Evans hooded rats were reared in EC (grouped in a large cage with daily toy changes and daily exposure to a playbox), SC (paired in stainless steel hanging cages),or IC (individually in hanging cages) environments from 25 to 55 days of age. Micrographs representing a total area of 236 μm^2 (final mag., 41,786X) were randomly taken from each of layers I, III and IV of area 17, excluding cell bodies and blood vessels, from lead citrate-uranyl acetate stained sections from each of the 33 animals. All synapses exhibiting presynaptic vesicles and an asymmetric postsynaptic density were included. Synapse density was estimated by the method of DeHoff (DeHoff & Rhines, eds., Quantitative Microscopy, McGraw-Hill, 1968). Synapses were treated as discs of infinite thinness. Results, below, indicate slightly higher overall synaptic density in EC vs. SC rats, with IC values below these groups. Calculations using a number of other published formulae for volume density, including methods which correct the size distribution (Coupland, Nature 217:384, 1968), provide directionally equivalent results, although absolute values differ. Calculation of neuron densities, which will allow estimation of the number of synapses per neuron, is in progress. (Supported by NSF BNS 8216916 and U. Ill. Res. Bd.)

SYNAPSES/ μm^3

Layer	I	III	IV
EC	.4714	.4459	.4111
SC	.4124	.4832	.3794
IC	.3816	.4557	.4045

18.10 DEVELOPMENTAL CHANGES IN ACETYLCHOLINESTERASE IN NORMAL AND MED MOUSE MUSCLE. C.G. Reiness and J. Yeakley*. Dept. of Biology, Pomona College, Claremont, CA 91711.

We have investigated the effects of the hereditary motor endplate disease (med) on the development of the molecular forms of acetylcholinerterase (AChE) in mouse muscle. This mutation exists in two alleles, med and med^J which cause progressive muscle weakness and death of the mice by 3 (med) or 5 (med^J) weeks of age.

AChE forms from the biceps brachii muscle, which is severely affected by the disease, were separated by velocity sedimentation on linear sucrose gradients. Globular forms that sedimented at approximately 10S, 6S and 4S (the G_4, G_2, and G_1 forms; Bon et al. PNAS 76, 2546 (1979) were present. In normal littermates of affected mice, the fraction of total AChE in the 10S peak increased from 10% at day 10 to a plateau of 30% at day 20. A concomitant decrease in 4-6S forms from 50% to 30% of the total suggested that they were converted to 10S AChE. AChE profiles of med^J mice were indistinguishable from those of normal littermates until 14d of age (about 4d after symptoms of the disease are first detectable). Thereafter, med^J mice had relatively less 10S and more 4-6S AChE than unaffected mice. Med mice were similar except that they deviated from normals at an earlier age, and the 4S (but not 6S) form virtually disappeared by 19d of age.

Asymmetric 16S (A_{12}) and 13S (A_8) forms were also present in biceps brachii. The proportion of 16S AChE in normal muscle was constant at about 35±5%. Despite a 50% reduction in AChE specific activity in med^J mice, the mutation did not have a selective effect on 16S AChE as it did on the 10S and 4-6S forms.

All forms reappeared after inactivation of AChE in situ with DFP. Thus persistence of these forms in the muscle represents (at least partly) continued synthesis rather than maintenance of molecules existing prior to the onset of the disease.

The effects of denervation for 7d were similar to those of the med^J mutation. There was a decline in relative amounts of 10S AChE while 16S remained prominent.

We thus conclude that the effects of the med^J mutation on AChE in mouse biceps brachii muscle are similar to those of denervation. The muscle remains innervated throughout the course of the disease, indicating that some factor other than presence of the nerve must regulate AChE. Neither denervation nor onset of the disease results in the selective loss of the endplate specific 16S AChE. Therefore expression of this form does not require continuous innervation in mouse muscle.

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18.11 PAW PREFERENCE AND TRAINING IN CATS WITH ADULT OR NEONATAL REMOVAL OF ONE CEREBRAL HEMISPHERE. J.W. Burgess and J.R. Villablanca. Mental Retardation Research Center, Departments of Psychiatry and Anatomy, UCLA School of Medicine, Los Angeles, CA 90024.

In continuing studies on recovery of function and brain reorganization, we examined paw preferences after ablation of the left telencephalon (hemispherectomy) in neonatal (4-18 days old, N=10) or adult cats (> 180 days old, N=7); this lesion impairs posture and movement of the right (contralateral) limbs. Paw preference was evaluated with a 10-30 cm string manipulandum, passed alternately through L or R visual fields to elicit directed paw responses in 5-20 min testing sessions. Prior to two months of age, no limb preference was apparent, but after this age hemispherectomized kittens used the impaired paw less than similar-aged controls ($p<.02$). This bias persisted through adulthood, compared with intact adults (N=17; $p<.05$). Adult-lesioned cats also used the impaired paw less, and this bias was more marked than in neonatal-hemispherectomized cats ($p<.001$) or intact adults ($p<.001$). This abnormal paw preference showed plasticity to motor training in both age groups. By presenting the string stimulus repeatedly to the affected side, adult-lesioned animals were induced to respond with the impaired limb, and gradually reversed their marked preference for the non-impaired paw. In later unbiased testing, they reponded more with the affected limb than did untrained animals ($p<.01$). Directed paw use was also tested in a more complex food-retrieval paradigm: food-deprived cats were presented with a box containing food accessible only with the impaired paw. Neonatal-lesioned animals (N=4) mastered retrieval in 1 trial, either spontaneously or with brief restraint of the non-impaired paw. Adult-lesioned cats required significantly more trials (mean=7.57) to use the impaired limb ($p<.001$), and could do so only with continuous restraint of the non-impaired paw, extensive food deprivation, and at least 1 month of postsurgical recovery. However, after initial mastery, both groups retained proficiency in the task. Overall, these results show that abnormal paw preference resulting from hemispherectomy in cats shows increased recovery if: 1) lesions are sustained neonatally, or 2) appropriate motor training is administered. Concomitant studies of underlying anatomical mechanisms are presented in a companion abstract.
(supported by USPHS HD-05958 and HD-04612).

18.12 EARLY MONAURAL DEPRIVATION IN THE RAT INDUCES REORGANIZATION OF THE PATTERN OF METABOLIC ACTIVITY IN THE INFERIOR COLLICULUS. J.Maeder* and A.Matus. (SPON: J.G.Richards) Friedrich Miescher-Institut P.O.Box 2543, 4002 Basel, Switzerland.

Local metabolic activity in the central auditory system of the albino rat was assessed after free field auditory stimulation with 20 kHz sweeps, 5s/sweep for 45 minutes, using [I4-C]-2-deoxyglucose autoradiography. Autoradiograms were analysed using a Leitz-ASBA computerized image analysing system.

In the normal mature rat a high level of metabolic activity was observed bilaterally in all brainstem and mesencephalic auditory nuclei. The stimulus used did not markedly increase metabolic activity in the medial geniculate body (MGB) and cortical areas. Acute unilateral ear block induced a dramatic decrease of activity in the ipsilateral cochlear nuclei (CN), contralateral lateral lemniscus (LL) and inferior colliculus (IC). Metabolic activity in the contralateral superior olivary complex (SOC) was only slightly reduced.

Rats of 11-12 days old were raised with the left ear blocked until 25, 35 or 50 days old and were then presented with the auditory stimulus either with the left ear still blocked, with both ears ears open or with the left ear open and the right ear blocked. No modification of the pattern of metabolic activity was observed as compared to normal and acute controls in the CN,SOC,LL,MGB and cortex. However, in the IC contralateral to the ear blocked a complex pattern of changes was observed. In the central and caudal parts of this nucleus, a 30% decrease of activity in the medioventral region was seen after bilateral stimulation, while a 15% increase of activity was observed in the central and dorso-lateral part. In the frontal region of the nucleus an overall 10% decrease of activity was seen. These changes were already present in 25 day old rats and longer periods of deprivation did not induce any further alterations. No changes in mitochondrial enzymes (cytochrome C oxydase) or structural elements (tubulin, microtubule associated proteins MAP1 & MAP2, neurofilaments and synaptic membrane proteins) were apparent using enzymatic and immune staining techniques. Further studies will be required to evaluate the mechanisms underlining the observed metabolic plasticity.

19.1 DEVELOPMENT OF THE IPSILATERAL RETINO-COLLICULAR PROJECTION IN THE GOLDEN HAMSTER. R. Insausti , C. Blakemore, and W.M. Cowan. The Salk Institute, P.O. Box 85800, San Diego, CA 92138; University Laboratory of Physiology, Oxford, England; and Dept. of Anatomy, University of Navarra, Spain .

In most rodents that have been studied, there is, in the immediate postnatal period, an extensive projection from the retina to the ipsilateral superior colliculus, which later becomes restricted to the rostral part of the colliculus unless the contralateral eye is removed at, or shortly after, birth. We have analyzed the time course of the restriction of this projection in golden hamsters and, using a variety of retrograde labeling procedures, have determined the cells of origin of the early extensive retino-collicular projection, and their fate after unilateral enucleation on the day of birth.

From a series of brains of animals in which wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) was injected into one eye at different ages, it is evident that the ipsilateral retino-collicular projection is distributed across the entire rostro-caudal extent of the colliculus by the day of birth, and that it begins to withdraw from the caudal part of the colliculus towards the end of the first postnatal week. By day 12 or 16 it is reduced to its adult form, being restricted to the rostral pole of the colliculus. Retrograde labeling experiments with WGA-HRP or the fluorescent dyes Fast Blue, True Blue or Nuclear Yellow, indicate that the early-formed ipsilateral projection arises from ganglion cells that are scattered across most of the retina; however, as the projection within the colliculus becomes progressively restricted, its cells of origin come to be located predominantly in the inferior temporal quadrant of the retina. That this restricted distribution of the cells of origin of the projection is due to retinal ganglion cell death has been established by experiments in which True Blue was injected into the colliculus of one side on the day after birth in normal hamsters and in animals in which the contralateral eye was removed. Retinal whole mounts prepared when these animals were killed on day 16, showed a clear difference in the two groups: in the normal animals the labeled ganglion cells were essentially limited to the inferior temporal retina, whereas in the enucleated animals many were found widely scattered across the retina as in neonatal animals. Taken together these findings indicate: (i) that the initial ipsilateral retino-collicular projection has a diffuse origin within the retina; (ii) that its restriction during the first two weeks postnatally is due to the selective death of ganglion cells in the central and nasal parts of the retina; and (iii) that this cell death is dependent upon the integrity of the input from the contralateral eye.

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19.2 THE IPSILATERAL RETINAL PROJECTION TO THE SUPERIOR COLLICULUS IN THE RABBIT: RE-EXAMINATION OF DEVELOPMENT AND OF ENUCLEATION EFFECTS. L.H. Ostrach, K.L. Chow and J.W. Crabtree*. Dept. of Neurology, Stanford University, Stanford, California 94305.

A re-examination of the ipsilateral retinal projection to the superior colliculus (SC) in dutch-belted rabbits has revealed significant new information about the pattern and size of this projection during postnatal development and following unilateral enucleation. Rabbits aged 1, 6-7, 10-11 days, and adults received unilateral intraocular injections of 3H-proline. After appropriate survival times the animals were perfused with 10% formalin and the brains were frozen sectioned at 60 μ. Complete series of sections were identically prepared, exposed and developed to demonstrate the autoradiographically labelled retinal projection. Animals enucleated either at day 1 or as adults were identically prepared. Reconstruction of the entire surface area of the SC, dorsal view area of the ipsilateral projection to the SC and length (anterior-posterior extent) of both the entire SC and the ipsilateral projection were based on dark-field camera lucida drawings of every section.

In normal animals from day 1 to adult age increases in SC surface area (138%) and length of SC (41%) were observed. Significantly, the area occupied by the ipsilateral projection and its length also increased (110% and 62% respectively); thus, the proportional area of SC receiving ipsilateral retinal inputs was unchanged during postnatal development.

After 2-6 months survival, animals enucleated on day 1 showed expected decreases in SC surface area (37%) and length of SC (14%). However, the area occupied by the ipsilateral projection and its length was the same as in normal adults. Animals enucleated as adults showed smaller decreases in SC surface area (16%) and SC length (6%) and the area and length of the ipsilateral projection were similar to normal adult values.

In all cases the ipsilateral projection occupied approximately the rostral 1/3 of the SC and appeared wedge-shaped with a broad base aligned with the lateral margin of the SC.

The present results show that the ipsilateral retinal projection to the SC in rabbits is organized into its adult pattern at birth and develops to retain a constant proportional distribution within the SC. Furthermore, this pattern and distribution are not altered by enucleation. Additional data obtained from normal and enucleated fetal rabbits will be presented to support the generality of these conclusions. The present findings provide a more thorough and accurate representation of the ipsilateral retinal projection in the rabbit than has been previously reported (Ostrach et al., Invest Ophthal Vis Sci 20:(Suppl): 73, 1981).

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19.3 VISUALLY GUIDED AVOIDANCE BEHAVIOR BY RATS WITH EXPANDED IPSILATERAL PATHWAYS. G. C. Midgley (SPON: R. C. Tees) Dept. of Psychology, Mount Allison Univ., Sackville, N. B. E0A 3C0 Canada.

This study is part of an investigation of the plasticity of ipsilateral retinal connections to visual structures. Unilateral eye removal in infancy results in the development of new retinal connection and a different pattern of connections in the visual system of the rat. When small pieces of food or large objects approach the rat from the peripheral visual field they misdirect their initial head turn and behave as if the new visual pathways have aberrant connections (Midgley, 1981). Misdirected head turns could be the direct result of aberrant connections. However, it is also possible that the animals were attempting to avoid the visual stimuli. To test this notion we directly examined the visually guided avoidance behavior of rats with expanded ipsilateral visual pathways. If the pathways have aberrant connections then the rats should also misdirect their avoidance responses.

The visually guided behavior of Albino rats with expanded ipsilateral visual pathways was made entirely dependent upon the ipsilateral connections by severing the contralateral visual tract. They were trained to run between two goal boxes and visual stimuli that elicited avoidance responses from intact animals were presented in each of 4 quadrants of the visual field. The rats' avoidance responses were video-taped and an analysis of the relationship between the stimulus position, initial head turn and the direction that the animal ran was conducted. The success of the contralateral optic tract lesion and expansion of the ipsilateral retinal pathway was verified by the injection of horseradish peroxidose (5 ml, 40% solution) in the intact eye and microscopic analysis of the ipsilateral and contralateral superior colliculus.

The visually guided avoidance behavior of the rats with expanded ipsilateral pathways was appropriately directed and except for very small stimuli presented in the extreme peripheral visual field could not be distinguished from that of the normal rat. The misdirected approach response of these animals may in fact represent and attempt by the animal to avoid visual stimuli. The size and nature of the new subcortical pathways may result in a significant reduction of visual acuity. Reduced acuity would, in turn, result in avoidance of visual stimuli that the intact animal would recognize as food. These findings suggest that the new connections are not aberrant but, appropriate, and perhaps a plastic and adaptive response to neonatal visual damage.

19.4 AN AUTORADIOGRAPHIC ANALYSIS OF CELL GENERATION AND DEATH IN THE HAMSTER RETINAL GANGLION CELL LAYER. D.R. Sengelaub, R.P. Dolan*, and B.L. Finlay. Department of Psychology, Cornell University, Ithaca, N.Y. 14853

At birth, the distribution of cells in the hamster retinal ganglion cell layer is uniform across the retinal surface. Cell number increases through postnatal day 5, after which a substantial amount of cell loss occurs. Although this cell loss is apparent across the entire retinal surface, it is greatest in the peripheral retina. In order to determine if this greater peripheral cell loss is a product of retinal position or time of cell genesis, the initial number of cells, spatial distribution of cells, and relative loss of cells by generation day was studied using tritiated thymidine.

Following timed matings, pregnant females were injected with ^{3}H thymidine (5 uCi/gram body weight) on embryonic days 9-14 (E15 = day of birth). Pups were injected individually (20 uCi/gm) for postnatal injections on days 1-3. Animals were killed on either postnatal day 4 (prior to the period of maximal cell degeneration), on days 5 or 6 (the period of maximal observable degeneration), or at adulthood. After autoradiography processing, counts of labelled live and degenerating cells were made from horizontal sections through the retinal ganglion cell layer. The hamster retinal ganglion cell layer is generated from embryonic day 10 through postnatal day 3, with the number of cells labelled being small and decreasing with injections after postnatal day 1. Cell generation has two peaks on E12 and E14. The spatial distribution of labelled cells varies with injection day and shows evidence of both a temporal-to-nasal and a center-to-periphery progression. E10 injections label 32% more cells at the temporal margin of the retina than at the nasal margin, and 22% more centrally than peripherally. Injections on postnatal day 1 (day of birth) label 25% more cells nasally than temporally and 113% more cells peripherally than centrally. Migration into the ganglion cell layer of cells generated on E13 or later continues through postnatal day 6, increasing the number of labelled live cells from postnatal day 4 to day 6 for these cohorts. For cells labelled on E10 through E12, numbers of labelled live cells decrease over this same period.

The ratio of labelled degenerating cells to live cells is equal for all prenatal injections, indicating that no prenatal cohort is subject to more cell loss than any other. Injections after postnatal day 1 label few degenerating cells. Rates of cell loss are always relatively higher in the periphery than the center for each cohort, regardless of the spatial distribution of the cells in that cohort.

These results indicate that it is retinal position and not time of cell genesis that determines the probability of cell survival.

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19.5 TEMPORAL RETINA IS PREFERENTIALLY REPRESENTED IN THE EARLY INNERVATION OF THE SUPERIOR COLLICULUS IN HAMSTERS. Kenneth C. Wikler, Judy I. Raabe*, and Barbara L. Finlay. Department of Psychology, Cornell University, Ithaca, N.Y. 14853

The hamster retina shows distinct spatial patterning of neurogenesis (Sengelaub, Dolan, and Finlay, 1983). To determine if the spatial distribution of the first retinal cells to reach their targets is the same as the spatial distribution of the early generated cells in the retinal ganglion cell layer, the brachium of the superior colliculus was filled with horseradish peroxidase (HRP) during the early stages of tectal innervation.

The hamster retinal ganglion cell layer is generated from embryonic day 10 to postnatal day 3 (E-15/ day of birth = postnatal day 1). The tectum develops its mature retinal innervation pattern from embryonic day 14 to postnatal day 12. HRP gel was placed in the transected brachium of the superior colliculus in neonatal hamsters (n = 5) on the day of birth in order to label initial ingrowing axons. Retinal ganglion cells were examined under light microscopy for evidence of retrograde labelling (TMB reaction product). The retina contralateral to the HRP placement was densely and non-uniformly labelled on the day of birth. More cells were labelled in the temporal retina compared to nasal retina. A discrete lack of HRP backfill in the extreme temporal periphery of the contralateral eye and a corresponding label in the extreme temporal retina ipsilateral to the injection were observed.

In order to determine whether the pattern of innervation of the superficial gray layer of the tectum is similar to that of the brachium, unilateral HRP placements in the superficial gray layer of the hamster superior colliculus were made on the day of birth (n=3). Although labelling of the contralateral eye was less dense than that seen with brachial placements the same temporo-nasal spatial gradient of HRP label was evident.

Our results demonstrate that at birth a substantial number of retinal fibers have reached the brachium rostral to the tectum, with temporal retina having a larger representation than nasal retina. The pattern of innervation of the superficial gray layer is a sparser version of the pattern of representation in the brachium. Early representation of the retina in the brachium as revealed by HRP is similar to but more pronounced than the temporal to nasal gradient in retinal neurogenesis. Frost, So, and Schneider (1979) have demonstrated that the rostral portion of the tectum is the first area of the tectum innervated by the retina. This study indicates that the rostral part of the tectum is preferentially and appropriately innervated by temporal retina.

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19.6 CHANGES IN THE PROJECTION OF THE NUCLEUS ISTHMUS TO THE TECTUM WITH RETINOTECTAL COMPRESSION IN GOLDFISH. A.A. Dunn-Meynell* and S.C. Sharma. Department of Ophthalmology, New York Medical College, New York 10595.

It is well established that following removal of a portion of the optic tectum in teleosts, retinal fibers reorganize their tectal projection so that all ganglion cells project to the tectal remnant. The experiments described here were performed to examine whether tectal efferents from other areas of the brain could show a similar plasticity. The projection of the nucleus isthmus on to the optic tectum in goldfish was investigated.

In each of a group of goldfish the normal isthmo-tectal projection was examined by making a localized injection of horseradish perioxidase (HRP) into the dorsal surface of the optic tectum. Three days later each animal's brain was sectioned and reacted to reveal retrogradely transported HRP in the cells. Isthmal cells which contained HRP were thereby shown to send their fibers to the region of the tectum in which the injection was made. After an injection in the caudalmost tectum labelled cells were observed in the dorsal portion of the nucleus isthmus. Following a rostral tectal injection, labelled cells were seen in the ventral isthmus. An injection in the mid-dorsal tectum labelled cells midway between the dorsal and ventral poles of the isthmus. The dorso-ventral isthmal axis was thus shown to project caudo-rostrally along the optic tectum in goldfish.

Retinotectal compression was induced in a group of goldfish by removal of the caudal half of the optic tectum and crushing of the contralateral optic nerve, followed by 6 or more months of recovery. Compression of the visual field was verified in a sample of these fish by using electrophysiological mapping of the retinotectal projection. The fish were given HRP injections in the mid dorsal tectum (near the cut edge of the tectum). Following the injection, labelled cells were seen in the dorsal nucleus isthmus. This pattern of labelling was very similar to the pattern observed in controls which had received caudal tectal injections, and dissimilar to the pattern in controls given mid-tectal injections. It therefore appears that the isthmal cells previously projecting to the caudal tectum project after retinotectal compression to the caudal edge of the hemi-tectum, displacing the isthmal cells previously projecting there.

We conclude from these experiments that there is a topographically organized projection from the nucleus isthmus on to the tectum in goldfish and that this projection may reorganize to maintain a normal topographic projection after tectal surgery. This provides evidence of regeneration and plasticity of neurons in the central nervous system in adult fish.

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19.7 ISTHMOTECTAL CONNECTIONS IN LARVAL AXOLOTLS: DISCREPANCY BETWEEN ELECTROPHYSIOLOGICAL AND ANATOMICAL RESULTS. S.B. Udin and M.D. Fisher*, Div. Neurobiology; SUNYAB; Buffalo, NY 14214.

The nucleus isthmi (NI) is the primary relay for the frog's ipsilateral visuotectal projection; information is transmitted between the tecta via the crossed isthmotectal pathway (Gruberg, E.R. & S.B. Udin, J. Comp. Neurol., 178:487-500, 1978; Grobstein, P. et al, Brain Res., 156:117-123, 1978). In axolotls, electrophysiological methods have detected ipsilateral visuotectal activity only in animals which have been induced with thyroxine to metamorphose but not in animals in their normal larval state (Stirling, R.V. & K. Brandle, Dev. Brain Res., 5:343-345, 1982). In order to determine whether the crossed isthmotectal pathway develops during metamorphosis, as the electrophysiology suggests, or whether it is present but undetected in the larval state, we have investigated the structure and connections of the axolotl NI using horseradish peroxidase (HRP).

The anuran NI is characterized by (1) input from the tectum on the same side of the brain, (2) output to both tecta, (3) intense staining for acetylcholinesterase (AChE). Using injections of HRP into the tectum of axolotls, we have confirmed that there is a region in the axolotl's isthmus, caudoventral to the tectum, where HRP fills cell bodies bilaterally and also fills a restricted region of the nearby lateral neuropil unilaterally. The labelled cells send their dendrites into the tecto-recipient region of the lateral neuropil. AChE staining of HRP-labelled sections shows that the HRP-labelled region of the neuropil also stains heavily for the presence of AChE.

Thus, the larval axolotl does have a crossed isthmotectal projection which could relay ipsilateral visuotectal information. In order to investigate why such activity has not been detected electrophysiologically, we labelled crossed isthmotectal axons by injecting HRP into the NI of larval axolotls. The anterograde filling showed that isthmic axons do enter the opposite tectum but that they arborize only in the extreme rostrolateral margin. We offer three possible explanations for the apparent discrepancy between the electrophysiological and anatomical results: (1) the extreme lateral position and small extent of the crossed projection may have made it inaccessible for electrophysiological recording; (2) the synaptic connections in the NI may be immature and/or non-functional prior to metamorphosis; or (3) the terminal arborizations of the crossed projection may be too sparse to be detected by conventional extracellular recording methods.

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19.8 NORMAL DEVELOPMENT OF XENOPUS OPTIC TECTUM AND THE EFFECT OF OPTIC FIBER INNERVATION ON CELLULAR MIGRATION AND LAMINATION. S. M. Royer and P. Grant. Department of Biology, University of Oregon, Eugene, Oregon 97403.

While investigating the rules ordering retino-tectal connections during development, we studied normal development of lamination and synaptogenesis in the Xenopus laevis tectum and investigated the effect of unilateral enucleation on these processes.

To study the timing and pattern of lamination, we prepared a normal developmental series and one in which the left eye was removed at stages 26-31 (before any tectal innervation by retinal axons). For light microscopic analysis, toluidine blue-stained, serial semi-thin sections of plastic-embedded embryos and tadpole brains were used. For ultrastructural analysis, whole embryos or isolated brains were prepared using standard techniques.

The optic tectum of the frog is a laminated structure, consisting of 9 alternating fibrous and cellular strata. In Xenopus tadpoles lamination begins at about stage 47 with the appearance of a few cells in layer 8 in rostral tectum, long after the arrival of retinal fibers at stages 38-40. Lamination proceeds rostro-caudally, and the pattern of 9 layers is apparent in the rostral tectum by about stage 50 and appears later in more caudal regions.

In unilaterally enucleated tadpoles, the initial stages of lamination are similar in both tectal lobes, although the right tectum receives no optic fiber innervation. As reported for Rana pipiens (Rollros, J. Exp. Zool., 123:153, 1953), the thickness of the outer layers (7-9) of the right tectum, however, is later reduced, especially that of layer 9. Comparison of the ultrastructure of layer 9 confirms its reduced development on the non-innervated side. The neuropil is thinner and is characterized by fewer terminal processes and synaptic contacts and an abundance of small axons and extracellular space. At the ultrastructural level, differences between control and experimental tecta are much less apparent at early stages than during metamorphosis, when the reduced development of layer 9 is most pronounced. Even in the same embryo ultrastructural differences in neuropil organization are more apparent in the more mature rostral regions than in caudal regions.

These results suggest that early migration of tectal cells and the initiation of lamination are unaffected by the absence of optic fiber input. In later development, however, the absence of optic fiber innervation leads to a significant disorganization of the layer 9 neuropil.

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19.9 COMPETITION AND THE FORMATION OF EYE-SPECIFIC RETINOTECTAL BANDS IN GOLDFISH. A.S. Mednick and A.D. Springer. Dept. of Anatomy, New York Medical College, Valhalla, N.Y. 10595

When one tectal lobe is ablated, the severed optic nerve fibers regenerate to the remaining ipsilateral tectal lobe and displace some of the existing retinotectal fibers from the contralateral eye. The foreign fibers from the ipsilateral eye form rostrocaudally oriented tectal bands of innervation that interdigitate with bands that represent the native fibers from the contralateral eye (Springer, A.D. & Cohen, S.M., Brain Res., 225:23, 1981). Such fish (Group 1) had either the native or foreign fibers labeled with tritiated proline after bands had formed and the percent of the remaining tectal surface that was occupied by each set of fibers was determined. The foreign fibers covered 27% while the native fibers covered 73% of the remaining tectum. This difference existed over the entire tectum. In addition, the native fibers occupied 50% or more of the tectum in 95% of the transverse tectal sections examined, while the foreign fibers occupied 50% or more of the tectum in only 5% of the sections.

A second group of fish (Group 2) had one tectal lobe ablated and, in addition, the ipsilateral optic nerve was crushed at the same time. Thus, both optic nerves were regenerating and reached the remaining tectal lobe at about the same time. For this group, the foreign fibers increased their coverage to 42% and the native fibers decreased their coverage to 58% of the rostral 3/4 of the tectum. Thus, the foreign fibers in Group 2 increased their projection by 56% relative to Group 1 and covered an additional 15% of the tectum. The native fibers in Group 2 decreased their projection by 21% relative to Group 1 and covered 15% less of the tectum. There were no differences between Groups 1 and 2 for the caudal 1/4 of the tectum. Furthermore, the foreign fibers occupied 50% or more of the tectum in 26% of the sections, a five-fold increase over Group 1.

These results indicate that temporal factors are important in the formation of eye-specific bands. Foreign fibers are least successful in displacing the native fibers when the native fibers already occupy the tectum, but become more successful when they and the native fibers are both regenerating and simultaneously competing to occupy the tectum. Increased competitive success in the foreign fibers, however, is restricted to the rostral 3/4 of the tectum.

(Supported by EY-03552)

19.10 EVIDENCE FOR MOVEMENT OF TERMINAL ARBORS OF AXONS FROM RETINAL GANGLION CELLS IN THE TECTUM OF FISH. Anne C. Rusoff. Oklahoma State University, Stillwater, OK 74078.

The retina and the tectum of adult fish both add new neurons as the fish grows. New ganglion cells form a complete annulus at the peripheral margin of the retina (Johns, P.R. J. Comp. Neurol. 176: 343-358, 1977); however, new tectal cells--the synaptic targets of these ganglion cells--form only a horseshoe in the tectum; there are no new neurons added at the rostral pole of the tectum (Raymond, P.A. and Easter, S.S. Jr. J. Neurosci., in press). The terminal arbors of the ganglion cells must, therefore, continually shift caudally, if the map of the retina is to remain centered on the tectum.

I have used 3 species of perciform fish, the kissing gourami, the green sunfish and the rainbow cichlid, to demonstrate anatomically that the map of the retina does remain centered on the tectum. These perciform fish have a ribbon-shaped optic nerve in which axons are very clearly segregated by age (Scholes, J.H. Nature 278: 620-624, 1979). All the axons from a given annulus of retinal ganglion cells are together in a band across the ribbon. The youngest axons (from the most peripheral annulus) are at one edge of the nerve and the oldest axons (from the most central annulus) form the band at the opposite edge of the nerve. I inserted a pipette coated with horseradish peroxidase (HRP) into optic nerves in these fish, damaging just axons of a given age and filling only these axons with HRP from their cell bodies in the retina to their terminal arbors in the tectum. I prepared the retinas as whole mounts to demonstrate the positions of the cell bodies of the axons filled with HRP-reaction product; I also prepared the tecta as whole mounts. The tecta were used to demonstrate the positions of the axons as they crossed the tectum and the positions of their terminal arbors. In all the preparations made from adult fish the terminal arbors from an annulus of ganglion cells centered on the optic disc formed an annulus on the tectum, and this annulus was centered on the tectum, not skewed toward rostral tectum. This result was obtained in fish two months old to one year old even though the tecta from the young fish were only about half as long (measured from rostral to caudal) as the tecta from the older fish. These preparations provide visible evidence that terminal arbors of axons from ganglion cells shift caudally on the tectum as the fish grows. (Supported by NIH grant RO1-EY03666.)

19.11 TRAJECTORIES OF REGENERATING FIBERS IN THE OPTIC PATHWAY OF GOLDFISH. S.M. Fraley and S.C. Sharma. Department of Ophthalmology, New York Medical College, Valhalla, NY 10595.

The optic pathway of normal goldfish is highly organized such that fibers from different retinal sectors are discretely localized in the nerve, tract and bracium and thus follow an orderly course en route to the tectum. In the present study, horseradish peroxidase (HRP) labelled regenerating fibers from a selected retinal sector were traced in order to determine whether their course deviates from the normal trajectory.

The optic nerve of adult goldfish (7-10 cm, snout to tail base) was crushed until the fibers within their sheath were visibly separated. Fibers from the dorsal retinal sector were labelled with HRP 4-12 months after nerve crush. The retina was exposed, fibers located at the 12 o'clock retinal position were severed, and 30% HRP in 1% DMSO-phosphate buffer solution was applied to the cut area. All surgery was performed under anesthesia with MS-222. The optic pathway was reacted with benzidine dihydrochloride 4 days later to reveal HRP label. The fibers were followed through the intact wholemount optic pathway and in 40u sectioned material.

In normal animals, dorsal retinal fibers remained closely adjacent to each other throughout the nerve and thus were localized in only a small portion of the total nerve area. All these fibers entered the ventrolateral tectum via the ventral optic tact and ventral brachium. In contrast, regenerating dorsal retinal fibers were distributed throughout the nerve. A large proportion of these fibers coursed into the ventral aspect of the optic tract and projected to the ventral brachium. Fibers which entered into the dorsal portion of the optic tract maintained their course and thus entered the dorsal brachium.

The data indicated that regenerating fibers followed a relatively straight trajectory through inappropriate areas of the optic pathway and thereby entered the tectum via the incorrect brachia. The size of the deviant population may thus be randomly determined by the number of fibers which become deflected as they pass through the site of nerve crush during regrowth.

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19.12 CYTOLOGY AND FIBRE PATTERN IN THE FISH OPTIC NERVE AND TRACT A. Maggs* and J.H.Scholes (SPON: M. Brown). MRC Cell Biophysics Unit, Kings College London, 26-29 Drury Lane, London WC 2, England.

Newly extending retinal axons in growing lower vertebrates pass through various sharply defined cytological domains on the way to the optic tectum. Two of these are the optic nerve and the optic tract, and the boundary between them, in teleost fish at least, appears to coincide with an important change in the retinotopic layout of the visual afferents, related to the final disposition adopted by their terminals in the tectal map. In the fish optic nerve, growing axons are usually constrained to track in a single tightly packed fascicle, from which satellite cells are excluded. In the optic tract, however, they are free to spread out in a sparse array among glia and older myelinated fibres. A conspicuous cytological distinction between these two domains in the visual pathway is that, in the optic nerve, astrocytes are hypertrophied and their cytoplasmic intermediate filament protein is a dominant component of the polypeptide spectrum of the tissue, only sparsely represented in the tract. We have made and characterised an antiserum against this protein to study by immuno- and conventional anatomical methods the the relation between optic fibre patterns and the distribution of astrocytes and other satellite cells in the visual pathway.

19.13 SYNAPSE FORMATION IN DOUBLY INNERVATED TECTA OF GOLDFISH. Mark J. Airhart* and Jeanette J. Norden (SPON: G. C. Palmer). Department of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232.

It has been reported that removal of one tectal lobe in goldfish results in hyperinnervation of the remaining tectum (Levine and Jacobson, Brain Res., 1975; Springer and Cohen, Brain Res., 1981). It has also been shown, using light microscopic techniques, that initially the projections from the two eyes to the remaining single tectal lobe appear to be completely overlapping. Over time, however, the projections from the two eyes segregate into eye-specific termination bands, similar to those reported for 3-eyed frogs (Constantine-Paton and Law, Science, 1978), and following optic tract deflection in goldfish (Meyer, Develop. Brain Res., 1983). In the present study, we have examined the neuropil of doubly innervated tecta in goldfish using electron microscopy to determine if regenerating retinal fibers form synapses at a time when the projection from the two eyes overlaps.

Doubly innervated tecta were created by ablating one tectal lobe and positioning the distal end of the severed optic tract and brachia against the rostral pole of the remaining tectum. The induced ipsilaterally projecting fibers and terminals were labeled by injecting HRP (50%, aqueous) intravitreally (4 days before sacrifice) and by injecting the nerve (2 days before sacrifice). Animals were sacrificed at times ranging from 26-112 days, and their brains processed according to standard protocols. Sections (50 and 200 µm) were cut on a vibratome and alternate sections were reacted for HRP using tetramethylbenzidine for light microscopy and diaminobenzidine for electron microscopy.

Four animals in which a continuous ipsilateral projection extending throughout the rostro-caudal and medio-lateral axes of the tectum have been examined. In these cases, regenerating ipsilateral fibers and terminals overlapped in their distribution with the same tectal laminae as the normal, contralaterally projecting retinal fibers and terminals. In addition, labeled ipsilateral retinal synapses could be identified in the stratum opticum and through the stratum fibrosum et griseum superficiale across the entire tectum. These results indicate that induced ipsilateral retinal fibers do form synapses at a time when the projections from both eyes overlap. This implies that a dynamic reordering of fibers and synapses from both eyes must occur during the subsequent formation of eye-specific bands.

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19.14 SEGREGATION AND DENSITY OF SYNAPSES IN EYE-SPECIFIC TERMINATION BANDS IN THE TECTA OF 3-EYED FROGS. Jeanette J. Norden and Martha Constantine-Paton. Dept. of Anatomy, Vanderbilt University School of Medicine, Nashville, TN 37232, and Dept. of Biology, Princeton University, Princeton, NJ 08540.

Transplantation of an eye primordium to an embryo host in Rana produces frogs with three eyes, two of which innervate a single tectum in alternating bands (Constantine-Paton and Law, 1978). In these animals, each of the eyes has approximately the same number of retinal ganglion cells and in the doubly innervated tectum the supernumerary and normal eyes appear to equally divide the available neuropil. Our previous quantitative analysis of the number of synapses in the tecta of 3-eyed frogs, however, indicated that the total number of synapses in singly and doubly innervated tecta was approximately equal, indicating that supernumerary and normal eyes projecting to a single tectum make significantly fewer synapses (Norden and Constantine-Paton, 1982).

We are currently examining synapses within eye-specific termination bands of doubly innervated tecta after double labeling at different developmental stages to determine 1) if there is a strict segregation of synapses from the two eyes; 2) if supernumerary and normal eyes initially form the same number of synapses; and 3) if there is any time during development at which the supernumerary eye appears to be at a competitive disadvantage in terms of the number of synapses. To date, we have examined approximately 35,000 um^2 of the superficial tectal neuropil of one Stage XV tadpole who showed eye-specific bands following injection of HRP into the supernumerary eye and 3H-proline into both normal eyes. Using quantitative EM, some HRP-labeled "profiles" could be identified in all areas of the superficial neuropil in the doubly innervated tectum, but very high densities of HRP-labeled profiles could be seen only within the supernumerary eye's termination zone. Furthermore, while it was extremely rare to see a labeled synapse within the normal eye bands, approximately 25% of all synapses present in the supernumerary eye bands were labeled. While these results are preliminary, they indicate that in the tadpole there is a strict segregation of synapses from the two eyes within doubly innervated tecta. Furthermore, in this animal, there was no consistent difference between the total number of synapses in the supernumerary and normal eye bands. We are currently examining the tecta of 1- and 8-mo. post-metamorphic 3-eyed frogs to see if this strict segregation and equal density of synapses in eye-specific bands is a consistent finding in older animals.

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19.15 MORPHOLOGY OF RETINAL AXONAL TERMINAL ARBORS IN THE GOLDFISH TECTUM. C.A.O. Stuermer. Div. Biol. Sci., U. Mich., Ann Arbor, and Max Planck Institut, Tuebingen, W. Germany.

Crystals of HRP were inserted into the optic nerve of 7 cm fish. Three days later the fish were perfused with saline. The tectal lobes were removed, reacted with DAB for 1 hr, then fixed, dehydrated, cleared, whole mounted under Permount, and coverslipped for light microscopic examination. Labeled axons and terminal arbors (TAs) were drawn through a camera lucida.

TAs occur in three sizes: small (c. 80x100 um), large (c. 150x 200 um), and giant (c. 200x300 um). The larger dimension is always oriented rostrocaudally in central tectum, or parallel to the tectal edge in peripheral tectum. The diameter of the axons enlarges 4-6X at the origin of the TA and divides into numerous branchlets studded with varicosities and synaptic boutons. Small TAs, from axons no greater than 0.5 um diameter, are the most numerous. Large TAs originate from axons of c. 1-1.5 um diameter, and are less frequent. Giant TAs, from axons of c. 2.5-3 um, are the least numerous. This three-class distribution of axon diameters and TA dimensions correlates with the three size classes of retinal ganglion cells.

TAs contribute to two distinct layers in SFGS, superficial (50 um wide) and deep (20 um wide), which correspond with Grafstein's F3 and F4, respectively. F3 has mostly small TAs superficially and all three sizes more deeply. F4 consists almost exclusively of large TAs. In any column perpendicular to the tectal surface, the major branches of all TAs in both layers are oriented similarly.

The orientation of a TA's initial segment and the long dimension of the TA may be similar or different, depending on the tectal location. Centrally, TAs originate from long, caudally directed, extrafascicular axons derived from short fascicles in rostral tectum. Here, the long dimension of the TA is parallel to its initial segment. In peripheral tectum, TAs emerge from short, centrally directed extrafascicular axons derived from peripheral fascicles. Here, the TA's initial segment and its long dimension are perpendicular.

These observations are consistent with a detailed model of retinotectal growth, which we have described elsewhere, based on Gaze's hypothesis of sliding connections.

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19.16 BINDING OF EXOGENOUS LECTINS TO DEVELOPING CHICK OPTIC TECTUM. S. E. Raiguel (Spon: R. E. Marc) Univ. of Texas Grad. School of Biomedical Sci., Dept. of Visual Sciences. Houston, TX 77025

Embryonic chick optic tecta were examined for lectin-binding capacity during development. Chick embryos at 8, 12, and 18 days of incubation were perfused with ethanol-acetic acid (95:5, v:v) and the brains fixed overnight in this fixative, dehydrated and embedded in paraffin. Eight-micron sections were deparaffinized, rehydrated and incubated 20 min. with the following fluorescein-isothiocyanate-conjugated lectins: Concanavalin A (Con A), Dolichos biflorus agglutinin, peanut agglutinin, Ricinus communis agglutinin I, soybean agglutinin, Ulex europaeus agglutinin, and wheat germ agglutinin (WGA) (Vector Laboratories). Fluorescence was observed only in sections incubated with Con A and WGA, and could be eliminated by preincubation of the lectin with the appropriate hapten sugar (10 mM α-methyl-d-mannopyranoside or N-acetyl galactosamine, respectively). Fluorescence was most intense at day 8 and diminished in progressively older embryos. Although WGA staining was more pronounced, similar distributions were seen for WGA and Con A: labeling of the fibrous layers of the stratum grisium et fibrosum superficiale, of the ependymal cell layer, and of the large cell bodies of the stratum grisium centrale. In the earliest embryos, what appeared to be radial glial cells bound WGA in a broad cental lamina. Although different planes of section were used to examine different tectal axes, no regional variation in staining intensity could be seen which might indicate the presence of gradients of glycoconjugates such as have been proposed to serve as marker substances during development. These data suggest that such markers, if present, are not preserved by this fixation procedure, and hence may consist of glycolipid, or are simply present in such low concentrations as to be undetectable by these methods.

19.17 Intermediate Filament Proteins in the Goldfish Visual Pathway. Wolfgang Quitschke* and Nisson Schechter. Department of Psychiatry and Behavioral Sciences, SUNY at Stony Brook, New York 11794.

Biochemical events associated with axonal outgrowth and regeneration can be investigated in the goldfish visual pathway. An analysis of the goldfish optic nerve by two dimensional gel electrophoresis indicated that a predominant cluster of proteins (58K daltons) is composed of components of neuronal and noneuronal origin (Brain Res. 258 (1983) 69-78). The concentration of the proteins of neuronal (ON_1 and ON_2) is directly linked to optic nerve degeneration and regeneration while the concentration of the other proteins (ON_3 and ON_4) remains stable during the process. These proteins are distinct from myelin, actin and tubulin preparatioins. Since they can not be classified among the most common structural proteins the possibility was tested whether they are intermediate filament proteins.

The proteins were analyzed with respect to several known characteristics associated with intermediate filament proteins. The results are as follows: Proteins ON_1 and ON_2, which are synthesized in the retina, are transported via the slow phase of axonal transport. Differential phosphorylation is observed among the components of the cluster both in vivo and in vitro. Furthermore, phosphorylation of proteins ON_1 and ON_2 occurs as a posttranslation modification during axoplasmic transport. Proteins ON_1-ON_4 are exclusively present in cytoskeletal enriched fractions which are insoluble in nonionic detergents. Finally, the proteins from this fraction can be reconstituted into typical 10nm intermediate filament structures as shown by electron microscopy.

Cytoskeletal preparations of rat optic nerve and goldfish optic nerve were compared by one dimensional gel electrophoresis. The rat optic nerve contains glial fibrillary acidic protein and nonneuronal vimentin in addition to the neurofilament triplet. A typical mamalian neurofilament triplet is not detected in the goldfish optic nerve while the major cytoskeletal constituent is the 58K band which is indistinguishable from vimentin in the rat optic nerve (this work was supported in part by a BBSG grant RR05736).

DEVELOPMENT OF MOTOR SYSTEMS

20.1 CORTICAL PROJECTIONS TO REDUCED TARGETS. M. E. Goldberger and B. S. Bregman. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA and Univ. of Maryland, Sch. of Med., Baltimore, MD.

We showed previously (1) that spinal cord hemisection in newborn cats has diverse effects on motor function. Sparing of function is observed in some aspects of motor behavior but not in others. The present studies indicate that the anatomical sequellae are also diverse. In adult cats having had C_1-3 hemisection as newborns and unoperated controls, the sensorimotor cortex (contralateral to hemisection) was either injected with HRP-WGA or ablated for degeneration studies. HRP and degeneration methods gave the same results. We examined cortical projections to the damaged side of the spinal cord and to two brainstem nuclei whose development was altered by the neonatal lesion; the magnocellular red nucleus (MgRN) and the dorsal column nuclei (DCN). MgRN cells were axotomized, while the gracile, cuneate and external cuneate, (ECN) nuclei were deafferented. After neonatal hemisection the corticospinal tract takes an aberrant route around the lesion, enters the grey matter, forms a small fasciculated pathway that descends through the base of the dorsal horn to lumbar levels and subserves the sparing of tactile placing. In controls, cortical projections occupy all of the MgRN but are densest ventrolaterally. Dorsomedial to MgRN a dense termination is seen in nuclei in and around the periaquaductal grey (PAG; e.g. interstitial, Darkschewitch). After neonatal hemisection, the MgRN undergoes massive retrograde degeneration during development. The cortical projection to the area of the MgRN which has few remaining (usually small) cells is greatly decreased or absent. The projection to the adjacent PAG region appears normal. The control cortico-DCN projection is dense ventrally at the base of the gracile and cuneate, sparse in the cell nest regions dorsally but does not enter the adjacent ECN. Neonatal hemisection causes shrinkage and cell loss of the DCN due to deafferentation and the cortico-DCN projection is extremely dense both at the base and among the cell nests. In addition, an abnormal projection courses laterally, near the floor of the IVth ventricle to reach medial groups of ECN cells. In contrast to the corticorubral projection which was absent, the cortico DCN projection expanded. Thus the response of developing cortical afferents to the reduction of a target nucleus may depend on how the target was reduced. Axotomy-induced retrograde degeneration (MgRN) of target neurons may inhibit development of afferents; denervation (or the transneuronal reduction it induces) may stimulate that development.

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(1) Bregman and Goldberger, Science 217:553-555, 1982.

20.2 DEVELOPMENT OF PLACING REACTIONS IN SPINAL CATS. G. A. Robinson, C. T. Leonard and M. E. Goldberger. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Cats display hindlimb locomotion after spinal transection at 12-14 days of age, providing evidence for a spinal pattern generator for locomotion (SPGL). Cats transected as adults, however, show poor locomotion, suggesting an age related sparing of function, i.e. an 'infant lesion effect.' We examined the development of placing reactions, hopping and treadmill locomotion in normal kittens and those with spinal transections (T13) at 3 ages: 1 day postpartum (1 DPP), 11-17 DPP, and as adults. Placing reactions were examined using a force transducer to record the force of stimulation required to elicit placing. Placing was elicited by stimulating the dorsal, ventral, medial or lateral surfaces of the hindpaw. Responses were scored relative to those obtained in the normal adult. The 3 spinal preparations and the normal kittens differed quantitatively in the stimulus strength required to elicit, and in the execution of, placing. For example, backward placing in normals began in the first week as a low threshold, flexion-only response and developed to a low threshold weight-bearing response after approximately 1 month. The 1 DPP group showed low threshold backward responses but they never matured past flexion. Transection at or after 11 days abolished backward placing responses. Lateral placing developed differently from backward. Normals, after a brief period of low threshold, partially weight-bearing responses developed fragmented responses such as simple flexion which finally matured to low threshold weight-bearing placement by 1 month of age. The 1 DPP group developed low threshold weight-supporting lateral placement by 2 weeks of age. Lateral placement in the 11-17 DPP group and in the chronic adult preparation was elicited by strong proprioceptive stimuli only and did not mature to the point of providing weight support. These data suggest that the spinal networks underlying the SPGL also share responsibility for placing reactions and are consistent with the development of locomotion in spinal cats. In both cases, the earlier the lesion was made the more sparing of function occurred. One possible explanation for these age-related differences might be the failure of neonatally spinalized cats to develop an adult level of inhibition. Thus, the SPGL would be suppressed more in an adult-transected cat. We therefore hypothesized that pharmacological blockade of inhibitory influences would result in facilitation of locomotor performance. Preliminary data suggests that i.p. administration of bicuculline facilitates treadmill locomotor performance in the chronic adult spinal cat but not the chronic neonatal operates (tested as young adults). This suggests that transection in the adult may reveal the normally developed inhibitory influence which fails to develop completely in the 1 DPP group. Supported by NIH16629&NSF BNS241775.

20.3 DEVELOPMENT AND RECOVERY OF FUNCTION IN NEONATALLY BRAIN DAMAGED CATS. C. T. Leonard, G. A. Robinson, M. E. Goldberger. Dept. Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Interest in the 'infant lesion effect' (i.e. greater recovery from CNS damage in the neonate vs. the adult) led our laboratory to examine various behavioral and neurological parameters of motor function in kittens receiving sensorimotor (SM) cortical ablations on the day of birth (neonatal operates; NO's). The NO's were first compared with normal developing littermates and after reaching maturity, with adult cats receiving similar cortical ablations (AO's). Testing included: placing responses, with thresholds measured with a force transducer; monopedal hopping and locomotion, analyzed by kinematics. Our data indicate that neonatally brain damaged kittens differ in their development from normals and also differ in their response to damage when compared with AO's. The NO's exhibit a prolonged period of reflex maturation when compared with normal littermates but all reflexes follow a normal progression of emergence. NO's exhibit fewer deficits than AO's. AO's fail to recover tactile placing and exhibit obvious locomotor deficits. In contrast, NO's do exhibit tactile placing (an example of neurological sparing) and show only subtle locomotor deficits. For example, kinematics reveal that abnormal increases in hip extension occur at the end of stance phase (E_3) but this is compensated for by an increase in total limb flexion velocity. We refer to this type of behavioral compensation as masking of a deficit. Masking is seen in both AO's and NO's. However, the deficit (hip hyperextension) is greater in the AO's and the compensation less effective.

Preliminary neuroanatomical studies, using conjugated Horseradish Peroxidase (HRP/WGA) and Fast Blue fluorescence, indicate the presence, in normal one day old kittens of a widespread, diffuse uncrossed corticothalamic (CT) efferents. In addition, numerous fibers cross through the massa intermedia. With time, there is a gradual decrease in the CT efferent system along with a concomitant increase in thalamic localization. Removing left SM cortex, at birth, appears to preserve, in part, the widespread CT efferent system of the right unablated cortex. This increased projection is not seen in AO's. Results confirm the greater plasticity of the neonatal nervous system and suggest two possible mechanisms underlying the 'infant lesion effect': 1) Greater masking of deficits utilizing movements which differ from those of an intact animal; and 2) Sparing of neurological function in the developing animal. Difference in organization and sparing of the corticothalamic system is one example of the neuroanatomical differences between AO's and NO's which may account for the observed behavioral sparing but this remains to be investigated.

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20.4 THE DEVELOPMENT OF CORTICOSPINAL PROJECTIONS IN THE OPOSSUM, T. Cabana* and G.F. Martin. Dept. of Anatomy, School of Medicine, The Ohio State University, Columbus, Ohio 43210.

It has been suggested that the development of the corticospinal tract in the rat involves the production of excess projections and the subsequent elimination of inappropriate ones (Bates & Killackey, '80; Ivy & Killackey, '81; Stanfield et al, '82): in newborn rats, nearly all neocortical areas are retrogradely labelled by injections of fluorescent markers into the pyramids or the spinal cord. The adult pattern of cortical labeling is none the less achieved by the end of the second week. In the opossum, cortical fibers do not grow into the cord until about 30 days after birth (42 days after conception) (Martin et al, '80). We took advantage of that fact to investigate possible sequences and mechanisms in the development of corticospinal systems. In pouch young opossums, the origin of corticospinal fibers was determined by retrograde tracing (horseradish peroxidase, Nuclear Yellow) and their terminations were studied using the orthograde transport of HRP.

Cervical placements of markers in 30 day old opossums labelled cortical neurons only in presumptive parietal areas of the motor-sensory cortex. One week later, labeling was found in cortical areas known to innervate the cord in the adult animal ("trunk-limb" area), but also in other regions such as the "face" area of parietal cortex, the preorbital, cingulate and insulo-temporal cortices; labeling was rare in frontal and peristriate-striate areas. Such labeling could be demonstrated until at least postnatal day 65. During that period of development, injections of HRP into either the parietal or the preorbital cortex (assessed by the pattern of retrograde labeling in the thalamus) labelled axons as far as the fourth thoracic segment of the cord where they distribute to laminae III to VII. Occipital injections did not produce spinal labeling. In the adult opossum, corticospinal fibers supply generally comparable areas (Martin & Fisher, '68). By postnatal day 80, retrograde labeling had "disappeared" from all but motor-sensory cortex, but it was still present in the "face" area. This was still the case in a weaned, four month old opossum, but not in adults.

Our data indicate that: 1) corticospinal projections in the opossum arise initially from areas which provide them in the adult, 2) additional areas of cortex subsequently contribute to spinal projections which are eventually eliminated, 3) there exist species differences as regard the scope and duration of transient projections and 4) the development of corticospinal systems in the opossum is relatively protracted. Supported by BNS-8068675.

20.5 MOTONEURON PROPERTIES FOLLOWING MOTOR REINNERVATION IN CAT MEDIAL GASTROCNEMIUS. R.C. Foehring,* G.W. Sypert and J.B. Munson. Dept. of Neuroscience, Univ. of Fla. Coll. of Med., Gainesville, FL, 32610.

Electrical properties of motoneurons (MNs) innervating the cat medial gastrocnemius are closely related with the type of muscle unit innervated (Fleshman et al., J. Neurophys. 46). We used intracellular recording techniques to determine if the normal correspondence between MN and muscle unit properties is re-established 8-10 months following self-reinnervation. These data were obtained from 10 normal and 4 reinnervated cats (all values ± SEM):

Motor Unit Type:	FF	FI	FR	S
% of Population				
Reinnervated(n)	44% (33)	4% (3)	21% (16)	25% (19)
Normal(n)	54% (43)	8% (6)	23% (18)	15% (12)
Conduction Vel.(m/s)				
Reinnervated	96 ± 1	94 ± 5	96 ± 2	85 ± 2
Normal	99 ± 2	100 ± 2	97 ± 2	83 ± 2
Rheobase(nA)				
Reinnervated	24 ± 0	18 ± 3	12 ± 1	4 ± 1
Normal	21 ± 2	17 ± 1	10 ± 1	6 ± 1
Input Resistance(MΩ)				
Reinnervated	0.5 ± 0.2	0.7 ± 0.3	0.9 ± 0.1	1.2 ± 0.1
Normal	0.6 ± 0.1	0.8 ± 0.1	1.0 ± 0.6	1.8 ± 0.1
AHP Half Decay(ms)				
Reinnervated	20 ± 1	23 ± 1	22 ± 1	43 ± 3
Normal	18 ± 1	17 ± 2	22 ± 1	41 ± 4

MN properties were generally appropriate for each unit type. To determine whether there were individual mismatches between MNs and muscle units, MN type was determined by the methods of Zengel et al. (ms. submitted). Briefly, cells with AHP 1/2 decay time >30ms were designated Slow, while cells with AHP 1/2 decay time <30ms were designated Fast. MNs were further subdivided by calculating rheobase/input resistance: FF>18>FR>7. FI MNs are not identifiable by this technique. The normal population showed 5 mismatches by these criteria (6.8%, excluding FI units). The reinnervated population showed 6 mismatches (12.8%, excluding 3 FI MNs and 6 MNs not making functional connections). Conclusions: Following 8-10 months reinnervation, MNs exhibit normal electrical properties and usually innervate appropriate muscle units. We are uncertain of the extent to which this is due to the MN converting muscle fibers, the converse, or competition among MNs for individual muscle fiber connections. Supported by NIH grant NS 15913, the MRS and RERDS of the VA, and the W.L. Gore Co.

20.6 REINNERVATION OF MUSCLE RECEPTORS BY CUT AFFERENT FIBERS. J. B. Munson, W. F. Collins, III, and L. M. Mendell. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

We investigated the specificity with which afferent fibers of the cat's medial gastrocnemius (MG) muscle reinnervate muscle receptors during peripheral nerve regeneration. Using sterile procedures, MG muscle nerves of 6 cats were cut and resutured a few mm from the muscle. Three or 6 months later, the receptors supplied by individual MG muscle afferents (penetrated in dorsal root filaments) were determined from their responses during muscle stretch and contraction. Using criteria of Gregory, et al. (J. Physiol. 331), we categorized 42 MG receptors as follows: spindles (n = 20); tendon organs (GTO; n = 5); contraction receptors (CR; n = 3); high threshold (HT; n = 6); not connected (NC; n = 8). To classify afferent fibers, we electrically stimulated each one individually (Honig et al., J. Neurophysiol. 49) and noted the averaged field potential generated in the MG motoneuron pool. Spindle afferent fibers can generate a field potential in the motoneuron pool consisting of a terminal potential (TP) and focal synaptic potential (FSP; Munson & Sypert, J. Physiol. 296). In control cats fast spindle afferent fibers (>88m/s) reliably evoked an FSP whereas slow ones (<79m/s) did not when the recording microelectrode was placed in the MG pool close to the afferent fiber entry level. This difference parallels the weak projection of slow compared to fast spindle afferents to the motor pool (Sypert et al., J. Neurophysiol. 44; Luscher et al., ibid. 42). One control GTO (of 10) produced a tiny FSP in the MG pool; the other 9 produced none. After muscle reinnervation, central projections were studied for 32 of the 42 characterized muscle afferents. Ten of 17 fibers supplying spindles produced FSPs in the MG pool. Most of these (7/10) conducted >88m/s. Most spindle afferents failing to generate an FSP (5/7) conducted <79m/s. Fibers innervating GTOs or CRs ("in series" behavior) produced no FSP (7/8) or a very tiny FSP (1/8). Four (of 7) NC fibers generated a FSP and conducted more rapidly than the 3 failing to generate an FSP. No HT afferent generated an FSP. We conclude: (1) after 6 months some afferents had not reinnervated muscle receptors; (2) the number of afferents reinnervating muscle spindles was much higher than that reinnervating GTOs (20 vs. 5) in keeping with the normal numbers of spindles and GTOs; (3) fibers reinnervating spindles and GTOs had central projections similar to those in control cats. At present we cannot determine whether this apparent re-establishment of almost normal specificity results from selective reinnervation of muscle receptors by afferent fibers, or whether adjustments occur in the muscle or the spinal cord to bring center and periphery into register.
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20.7 DOWN REGULATION OF GLYCINE RECEPTORS IN RESPONSE TO MOTONEURON INJURY. A. Rotter, C. Schultz* and A. Frostholm*. Department of Pharmacology, College of Medicine, University of California, Irvine, Irvine, CA 92717

Several quantitative studies have shown that axotomy of the hypoglossal nerve results in a loss of synaptic contacts on hypoglossal motoneurons. Our own studies have established that mouse hypoglossal nuclei have high concentrations of glycine receptors. The receptors were labeled with [^{3}H]strychnine, a potent glycine antagonist, the binding of which to mouse brain sections can be demonstrated autoradiographically. We have used this method to study the effect of axotomy on the number of glycine receptors in the hypoglossal nucleus. The present results are based on a total of 6 adult male and female C57BL/6J mice in which the hypoglossal nerve was cut on only one side of the neck. After 7-9 days the animals were anesthetized with ether, the brains removed, mounted on cryostat chucks and frozen in dry ice. Coronal sections 20 μm thick, were prepared for autoradiography. The highest levels of binding were observed in the spinal cord and brain stem; binding levels progressively decreased rostrally along the brain axis. In coronal sections of the lower brain stem, several structures were densely labeled. These included the reticular formation, cuneate and gracile nuclei, and most prominently, the hypoglossal nuclei. The labeling over the hypoglossal nucleus was distributed throughout the neuropil. The cytoplasm and nuclei of the large hypoglossal neuronal somata were unlabeled. The density of the silver grains fell abruptly at all the boundaries of the hypoglossal neuropil. This clear demarcation was abolished if sections were incubated in the presence of 1 mM glycine. We examined the hypoglossal nucleus at 7 to 9 days after the unilateral sectioning of the hypoglossal nerve. A marked decrease in the intensity of [^{3}H]strychnine binding was observed. Since the nerve arises almost exclusively from the ipsilateral hypoglossal nucleus and the two nuclei lie immediately adjacent to each other on either side of the midline, the opposite nucleus may be used as a control in the same coronal section. A comparison of grain densities over the nuclei on the operated and unoperated side indicated that sectioning of the hypoglossal nerve resulted in a decrease of at least one half of the number of [^{3}H]strychnine binding sites in the axotomized of nucleus.

Several studies have shown that many of the effects of axotomy are reversible if the hypoglossal axons are allowed to regenerate and re-establish neuromuscular contacts. Our preliminary observations on mice showed that in cases where the hypoglossal nerve regenerated, binding of [^{3}H]strychnine in the hypoglossal neuropil had also returned to normal levels. This dependence of the receptor upon the establishment of peripheral neuromuscular contacts raises the possibility that the metabolic events required to produce receptors are influenced by factors which originate in the target tissue.

This project was supported by NIH grant NS18089.

20.8 PATTERNS OF FAST AND SLOW MUSCLE FIBERS IN EMBRYONIC CHICK MUSCLES INNERVATED BY FOREIGN MOTONEURONS, M. W. Vogel* and L. T. Landmesser. Biology Dept., Yale University, New Haven, Conn. 06511 and Physiology Section, Biological Sciences Group, Univ. of Connecticut, Storrs, CT 06268

In the chick hindlimb there is a characteristic pattern in the distribution of fast and slow muscle fibers within individual muscles, as revealed by myosin ATPase staining. For example, the anterior medial portion of the iliofibularis contains predominately slow myotubes, the posterior lateral portion, predominately fast.

To determine what role, if any, the innervation plays in the development of such characteristic myosin ATPase staining patterns, we caused a variety of hindlimb muscles to be innervated by foreign motoneurons. This was accomplished by rotating about the a-p axis either the lumbosacral neural tube at St 15-16, or the limb bud at St 17-18. We then determined the myosin ATPase staining characteristics of muscles in embryos ranging in age from embryonic stage 36-44. For some muscles, we also recorded EMG activity patterns and/or labeled their motoneurons with retrogradely transported HRP.

In the majority of cases (80/85 muscles examined in 15 limbs) the overall pattern of fast and slow myotubes was essentially normal. From this we conclude that either the pattern is intrinsic to the limb and unaffected by the source of innervation up to St 44, or that when fast and slow motoneurons are presented with a muscle containing both fiber types, they selectively innervate the appropriate region. The latter possibility is supported by several exceptions to our general finding. In these cases (5/85), changes in fiber type composition of individual muscles were observed, suggesting that the pattern of fast and slow myotubes can be altered at the stages studied. This could result from motoneurons either altering the staining property of individual myotubes, or favoring the development of one type of myotube. The low frequency with which such changes were observed may be explained by the fact that under the experimental conditions used, muscles would have been likely to be innervated by mixtures of both fast and slow motoneurons. We are therefore attempting to produce additional evidence for changes by experimental designs which force motoneurons to innervate myotubes of the wrong type. Supported by NIH Grant NS 19640.

CONTROL OF POSTURE

21.1 PARTITIONING OF CAT POSTERIOR THIGH MUSCLES INTO TWO GROUPS BASED ON EMG ACTIVITY DURING JUMPING. F. E. Zajac, W. S. Levine* and D. Dungan*. Univ. of Maryland, Palo Alto VA Med. Ctr. and Mech. Eng. Dept., Stanford Univ., Stanford, CA 94305.

In our study of maximum height jumps a theoretical result independent of the musculo-skeletal model used to represent hindlimb dynamics is that each muscle ought to be either maximally activated (on) or inactivated (off) during propulsion once the heels loose contact with the ground. Specifically, single-joint extensor and flexor muscles ought to be on and off, respectively, throughout this 2nd propulsion phase. While double-joint muscle activation should also be binary in this epoch, its on-off timing depends on model details. Intramuscular EMGs (Fig. 1) were recorded from 4 adult cats trained to jump from a force-plate to touch cotton balls suspended about 2.5m above the platform. Comparison of filtered EMGs occurring in the maximal jump with those seen in other jumps and movements suggests that on-off activity does exist in the 2nd phase. EMGs prior to heel-off (1st phase) can vary in both timing and amplitude from one maximal jump to another, consistent with the variability seen in ground reaction forces during this phase (Zajac, F.E. et al., J. Exp. Biol. 91:73, 1981). Based on their EMG pattern posterior thigh muscles can be grouped into those which only facilitate hip extension (semimembranosis anterior [SMa], biceps anterior [BA] and adductor femoris [AF]) and those which also act to flex the knee (semitendinosus [ST], biceps posterior[BP] and gracilis [GR]). Data from submaximal jumps acquired during training indicate that the double-joint group contributes to 1st phase propulsion in the maximal jump as evident by the occurrence of the first peak in vertical force (Fig.1, top), though quite high jumps are possible without this participation. Activity level in the single-joint group and in vastis lateralis (VL) is in accord with jump height. Calculated length trajectories show that activity from each posterior thigh muscle studied occurs just before and during its shortening and ceases with onset of lengthing. The partitioning of posterior thigh muscles into groups based on their activation patterns during jumping is thus in agreement with such partitioning based on origin-insertion geometry.

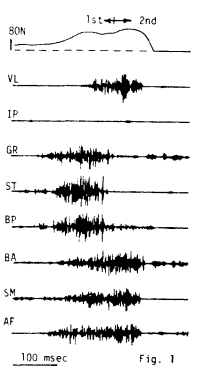

Fig. 1

(Supported by NIH NS 117662).

21.2 INTRALIMB COORDINATION DURING PAW SHAKE RESPONSES IN THE HINDLIMB OF CAT. D.M. Phillips* and J.L. Smith. Neuromotor Control Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024.

Paw shake responses (PSR) are the most rapid cyclic movements observed in cats involving simultaneous movements of the hip, knee and ankle. Intralimb coordination of PSR may be different from slower cyclic movements such as locomotion and scratching reflecting differences in the limb dynamics. The purpose of this study was to identify the PSR intralimb coordination in unrestrained and restrained hindlimb.

Data from 4 cats spinalized at T-12 as adults was collected 1-3 mos following spinalization. EMG sampled from selected flexors and extensors at the hip, knee and ankle included iliopsoas (IP), gluteus medius (GM), adductor magnus (AM), posterior biceps (PB), vastus lateralis (VL), lateral gastrocnemius (LG) and tibialis anterior (TA). Cycle durations (CD) defined as the interval between successive LG burst onsets were determined, and the onset time for each muscle was expressed as a percent of the average CD. PSR were elicited by wrapping tape around the hindpaw, and in some trials knee and ankle movements were restricted by a plaster cast at 115^0 and 100^0, respectively.

In the unrestrained limb the average CD was 97 ms during 12 cycles/PSR. Intralimb coordination consisted of an extensor synergy which included the coactivity of extensors at the hip and ankle (GM, AM, PB, LG), followed by a mixed synergy initiated at 57% of the CD which included coactivity of the ankle flexor (TA) and knee extensor (VL). The onset of the hip flexor (IP) was out-of-phase with the extensor and mixed synergies occurring at 33% of the average CD.

Mechanical perturbation restricting knee and ankle movements resulted in an increased CD of 120 ms and a reduction in the number of cycles/PSR to 8. The extensor synergy was unaltered; however, the coactivity of TA/VL was disrupted in 3 animals. In all cats, the TA remained reciprocolly active relative to the extensor synergy, although its onset was delayed to 73% of the CD. The VL exhibited two abnormal patterns: an absence of activity or a shift in onset resulting in coactivity with hip and ankle extensors. The IP activity was delayed in its onset to 42% of the CD.

PSR coordination is characterized by an extensor synergy at the hip and ankle followed by a mixed synergy at the knee and ankle. Perturbation by ankle-knee immobilization disrupts the mixed synergy especially the knee extensor suggesting that the dynamics of the moving limb may be necessary for consistent VL participation in the mixed synergy.

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21.3 INTERSEGMENTAL DYNAMICS OF THE PAW SHAKE RESPONSE.
M. G. Hoy*, R. F. Zernicke, and J. L. Smith. Dept. Kinesiology, UCLA, Los Angeles, CA 90024.

Angular motion of the cat hindlimb during the paw shake response (PSR) is determined by muscle activity and the dynamic mechanical interactions between the linked limb segments. The relative contribution of each force component to the resultant joint torque is important in understanding PSR control, because only active muscle force represents the direct participation of the CNS in movement dynamics. The purpose of this study was to quantify the role of the active musculature and passive mechanical dynamics in the PSR.

PSR were elicited in the chronic spinal cat by applying tape to the hindpaw; limb motion was filmed (200 f/s). Hip, knee, ankle, and metatarsophalangeal joints were digitized from projected film frames. Displacement-time data were smoothed and differentiated (Hatze, H., J. Biomech., 14:13, 1981) to provide linear and angular kinematics. The leg and paw were modeled as a planar, 2-segment rigid-body system. Body segment parameters were obtained, and kinetic equations of motion (Putnam, C., J. Biomech., In Press) were formulated to yield knee and ankle joint torques as functions of generalized muscle moments at the knee and ankle, angular acceleration and angular velocity of the leg, angular acceleration and angular velocity of the paw, linear acceleration of the knee, and gravity.

A PSR consists of a sequence of 8-13 flexion-extension cycles with an average cycle duration of 79 $\pm$ 6 ms. During the first half of a cycle, the knee flexes and the ankle extends; joint actions reverse in the second half of the cycle. These joint actions are consistent with the muscle synergy reported by Koshland and Smith (Neurosci. Abst., 1983). The range of motion of the ankle (56 ± 7^{0}) was greater than that at the knee (35 ± 5^{0}). Peak knee extension and flexion were 100 ± 3^{0} and 68 ± 2^{0}, and ankle values were 173 ± 1^{0} and 116 ± 6^{0}. Knee joint maxima preceded ankle joint maxima by 5-10 ms. The ankle angular velocities in flexion (-2584 ± 429^{0}/s) and in extension (3085 ± 306^{0}/s) were greater than the corresponding values at the knee (-1388^{0}/s and 1232^{0}/s).

Torque data demonstrate that passive mechanical interactions are especially important at the knee joint, while ankle joint torque is dominated by muscle moment. The segmental angular velocities and gravity contribute relatively little to the resultant joint torques during the PSR. The formulation of the kinetic equations of motion permits a detailed examination of the intersegmental dynamics of the PSR and highlights the capacity of the lumbosacral cord to generate and coordinate appropriate muscular moments to compensate for passive dynamic segmental interactions. Supported by NIH NS 16333.

21.4 *INTRALIMB COORDINATION OF THE PAW SHAKE RESPONSE: A MIXED FLEXOR-EXTENSOR SYNERGY.* *G.F. Koshland and J.L. Smith. Lab. of Neuromotor Control, Dept. Kinesiology, UCLA, Los Angeles, CA 90024.*

Two movements, locomotion and scratching, known to be generated within the lumbosacral cord follow a strict synergy of alternating flexor-extensor activity across all hindlimb joints. The paw shake response (PSR) also elicited in the chronic spinal cat, has a mixed synergy in which the knee extensors and ankle flexors are coactive. The purpose of this study was to determine whether this mixed synergy is typical of the PSR intralimb coordination of the normal, spinal and deafferented spinal cat.

PSR were evaluated in 4 cats, 1 mo prior to and 1-3 mos after cordotomy at T-12; and in one spinalized cat, PSR recovery was followed 1-4 mos after unilateral deafferentation (L_4-S_2). PSR were elicited in the usual manner by wrapping tape around the paw; however, in the deafferented limb, PSR were obtained from tactile stimuli applied to the lateral thigh. EMG from the vastus lateralis (VL), tibialis anterior (TA) and lateral gastrocnemius (LG) was analyzed for burst and cycle durations (CD, defined as the interval between LG burst onsets). Intralimb synergies were determined by the onset latencies of the VL and TA, normalized to percent of CD.

The mixed synergy of the VL and TA coactivity was common in all preparations. In the normal cats, the two muscles had onset latencies of 64% ($\pm$7) and the mean CD was 96 ms ($\pm$9) over 8 cycles/PSR. In the spinalized cats, VL and TA onsets were similar at 54% of the cycle and the mean CD was 85 ms ($\pm$8) over 11 cycles/PSR. In 21% of the 38 deafferented PSR recorded, the VL and TA were coactive at 86% of the cycle. This coactive pattern was not observed until later in the recovery period and was associated with CD of this cat's normal PSR (114 $\pm$24 ms). Earlier in the recovery, two other synergies were seen in which VL onset shifted as the onset of TA remained stationary at 86% of the PSR cycle. In one synergy, associated with PSR with longer cycles (158 $\pm$39 ms), the VL was coactive with the LG, while in the other synergy, the VL onset occurred at 93%, midway between the TA and LG and was associated with shorter cycles (123 +12 ms).

Our results suggest that PSR and its mixed synergy of ankle flexor and knee extensor coactivity is centrally generated. Thus, the lumbosacral cord may contain more than one, or a facultative network of unit generators to coordinate different intralimb synergies. *Supported by NIH grant NS 16333.*

21.5 RESPONSES OF LATERAL GASTROCNEMIUS INNERVATION SUBCOMPARTMENTS AND SOLEUS TO PAIRED PERTURBATIONS OF POSTURE. D.S. Rushmer, J.M. Macpherson, D.C. Dunbar*, and C.J. Russell. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209.

The stereotyped nature and the timing characteristics of EMG responses to controlled perturbations of stance suggest that they might be programmed within the central nervous system.

We have studied this possibilty by recording from the four innervation subcompartments of the lateral gastrocnemius and from the soleus muscles of awake behaving cats. Animals stood on an hydraulic posture platform and were subjected to two posture perturbations presented closely in time. The first perturbation was always a drop of support from beneath a single limb, evoking the "diagonal support response" described by Coulmance et al (Exp Brain Res 37:265,1979). The second perturbation, presented at latencies of 10-100 msec following the first, consisted of the drop of support from beneath the diagonal pair of limbs loaded as a result of the first perturbation. This second perturbation required the animal to change the strategy of postural response while it was still responding to the first perturbation.

Drop of a single limb evoked stereotyped EMG responses which were different depending upon the role in the postural response of the limb containing the recorded muscles. There were also differences between the response characteristics of the lateral, medial and intermediate innervation subcompartments on the one hand, and those of soleus on the other. Responses of the distal subcompartment, which contains a higher proportion of slow fibers than the other three, contained elements of both response types.

Responses to the second perturbation strongly suggested central programming of postural responses, since in many cases, EMG responses which were appropriate for the first perturbation but not for the second, continued to be observed even though the recorded limb was not subjected to the force changes evoked by the first perturbation. The timing characteristics of this programming are different for soleus and the four innervation subcompartments.

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21.6 POSTURAL CORRECTION IN FELINE AND HUMAN SUBJECTS DURING BOTH QUADRUPEDAL AND BIPEDAL STANCE. D.C. Dunbar*, F.B. Horak, J.M. Macpherson, D.S. Rushmer, and C.J. Russell. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

The use of the domestic cat as an experimental model for understanding human motor control evokes the issue of the degree to which a quadruped can legitimately serve as an archetype for a biped. This study addressed the problem as it pertains to investigating CNS mechanisms underlying postural maintenance by analyzing EMG activity, force changes, and segmental adjustments.

Cat and human subjects stood both quadrupedally and bipedally on hydraulic platforms that disturbed posture by suddenly translating the subjects either anteriorly (headwards) or posteriorly (tailwards).

The EMG activity and force changes of cats standing quadrupedally and human subjects standing bipedally during both anterior and posterior translations were comparable to those reported previously by Rushmer, et al. (Exp. Brain Res., 50:45, 1983) and Nashner (e.g., Exp. Brain Res., 26:59, 1976), respectively. Postural responses of feline subjects standing bipedally were, in general, in accord with the patterns seen in human bipeds and the responses in quadrupedal human subjects were similar to those in feline quadrupeds. Thus, during anterior translations, bipedal cats corrected for backward sway by activating muscles on the anterior aspect of the hindlimbs whereas quadrupedal human subjects activated muscles on the posterior aspect. The patterns were reversed during posterior translations. Bipedal cats rotated mostly at the ankles, less at the hips, and stiffened the knees; human quadrupeds used the hips and M-P joints while stiffening the knees and ankles. Furthermore, as in cats, muscle coactivity converted the human forelimbs into supportive struts while the hindlimb corrected posture. Variations appeared to be attributable principally to differences in limb morphology and to inexperience at adopting these postures.

These findings suggest that postural responses in feline and human subjects are determined primarily by the mechanical conditions of bipedalism and quadrupedalism combined with the particular linear dimensions and musculoskeletal designs of Felis and Homo. Thus, the CNS in feline and human subjects appear very similar physiologically, both systems dependent upon plasticity and adaptability to meet the specific requirements of a given posture. It is concluded, therefore, that cats provide an excellent experimental model for understanding CNS control of human posture.

21.7 POSTURAL BEHAVIOR OF INTACT CATS STATICALLY TILTED. E. Fava*, F. Lacquaniti*, C. Maioli*, G. Marini*, M.L.Sotgiu* and C. Terzuolo. IFCN-CNR, Milano,20131 ITALY.

Cats were trained to stand freely on a platform statically tilted at various angles (up to ± 30° from the horizontal) in the sagittal plane. Strain-gauges measured the normal components of the reaction forces at each paw. Geometrical parameters of the body posture (limb lengths, joint angles and head inclination) were obtained from photographs. The only requirements for trial acceptance were the maintenance of lateral simmetry in weight distribution and the fact that standard deviation of the forces recorded at each paw did not exceed a fixed value.

For all tilt angles of the platform, cats exhibited a remarkably consistent postural behavior, whose main features were: 1) the projection of the center of gravity on the support surface was approximately constant (about halfway the distance between fore- and hindpaws); 2) the distance between proximal joints and paws, and the orientation in space of both fore- and hindlimbs were also approximately constant; 3) head position instead varied widely from trial to trial, but it did not affect the postural parameters mentioned above.

On the basis of the described results, we argue that the process of postural regulation minimizes the changes in torques required for joint stabilization at different tilt angles. In fact, as long as limbs act as struts to support the animal's weight, no leverage is required in addition to that necessary for the maintenance of posture on a horizontal surface.

Notice that the observed behavior does not conform to that predicted on the sole basis of labyrinth and neck reflexes, as they are usually described (i.e. reciprocal flexion-extension at fore- and hindlimbs). Rather, it must be governed by the whole array of sensory information available to the animal.

21.8 ORIENTATION EFFECTS ON THE TENDON JERK REFLEX. B. Myklebust, G. Gottlieb, G. Agarwal. Dept. Physiology, Rush Medical College, Chicago, IL 60612.

The role of body tilt in soleus (SOL) motoneuron excitability has been studied in normal adults by correlating H-reflex amplitude with head position (1,2). The effects are attributed to vestibular influences, but results are contradictory, and inter- and intra-subject variability are significant.

We assessed the effects of body tilt on the stretch reflex by use of tendon jerks. Subjects were restrained on a Circolectric bed with a parachute harness; head and limb positions were controlled. Tendon taps were applied at a range of tilts from vertical (0 deg), horizontal supine (-90 deg), horizontal prone (+90 deg) and head down 30 deg from horizontal (+120 deg). The force of hammer tap was measured with a strain gage mounted on the hammer head. SOL EMGs were recorded with surface electrodes.

The ratio of mean peak-to-peak SOL amplitude to mean hammer force was computed for 10 to 20 taps at each body tilt. This ratio was greatest from 0 to 90 deg, and it was depressed in the supine positions (up to -90 deg) and in tilts greater than +90 deg. Thus it appears that outside the range of normal functional body attitudes, the gain of the tendon jerk reflex is depressed.

This supports an argument that tonic vestibular input maintains the excitability of the stretch reflex arc in body tilts characteristic of normal posture. However, uncontrolled exteroceptive input, poor correlations between hammer force and EMG amplitudes, and inter- and intra-subject variability suggest the need for further study. 1) C. Chan, R. Kearney: Neuroscience Letters 33: 333-338, 1982. 2) I. Aiello, G. Rosat, G. Serra, V. Tugnoli, M. Manca: Exper. Neurol. 79: 18-26, 1983.

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21.9 POSTURAL ALTERATIONS FOLLOWING VIBRATION IN THE TENDO-ACHILLES: A STUDY IN THE CALF MUSCLES OF ELDERLY SUBJECTS. M. E. Melnick, D. Biggs*, S. Zilber* and J.B. Redford*. Department of Physical Therapy Education, Univ. of Kansas College of Health Sci, Kansas City, KS 66103

Falls in the elderly are a major medical problem. The purpose of this study was to quantitate posture in elderly as compared with young subjects and to determine the response to vibration applied over the triceps surae. The H-reflex was also studied to see if it was correlated with stance and the reaction to vibration. The differential response in these parameters would give clues of the nature of equilibrium changes with aging and direction for future studies and treatment. Volunteers over the age of 68 with no overt neurologic symptoms or complaints of gait disorders were given a brief evaluation. Using standard recording techniques, latency of response and peak-to-peak amplitude of the H-reflex were measured in each leg. For the postural assessment, the subjects were asked to stand comfortably on a stabilograph connected to an X-Y plotter which traced any shifts in center of gravity (COG). Stability was tested with and without vibration and with eyes open and closed during repeated 30 sec epochs. Vibration (150 cycle/sec) was applied simultaneously to both triceps surae muscles. The X-Y plotter was adjusted so that tracings were obtained before, during and after vibration epochs. The results were compared to those from young volunteers 20-40 years old. COG measurements showed the following significant differences between the two groups: increase in lateral sway at rest in the elderly (20mm in elderly; 13mm in young, $p=0.05$); a decrease in response to vibration in the elderly (10mm vs 24mm, $p < 0.01$); a decrease in variability in the elderly. There was no change in response in either group between eyes open or eyes closed conditions. The H-reflex showed a tendency toward an increase in latency and decrease in amplitude in the elderly subjects but was not correlated with postural adjustments. These data suggest that there may be a decreased response to peripheral sensory input in the elderly. The elderly subject may then increase postural rigidity for support, making the elderly less able to respond flexibly to changes in walking surface.

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21.10 MODULATION OF H REFLEX EXCITABILITY BY LATERAL TILT. C.W.Y. Chan and M.A. Dalzell*. School of Physical & Occupational Therapy, McGill University, Montreal, Quebec, Canada, H3G 1Y5.

In a previous report (Chan, C.W.Y. & Kearney, R.E., Neurosci. Lett., 33:333, 1982), we demonstrated that the H reflex amplitude reached a minimum when the head was near vertical, and often increased as a sinusoidal function of whole head-body rotations in the sagittal plane. The present study extends this work to map changes in soleus motoneuronal pool (MNP) excitability due to lateral tilt.

Subjects were blind-folded and fixed to a modified circo-electric bed by means of partial casts for the neck and legs, and straps around the body and arms. A sling and saddle system held the body firmly in place. Static lateral head-body tilt was achieved by rotating the bed in random steps of 30°, to a maximum of 90° on either side of the vertical. Variability in soleus MNP excitability was measured with the H reflex technique. At each test position, 25 responses were recorded and analyzed to determine the mean and standard deviation of the peak-to-peak amplitude of H and m waves. These values were then compared with those obtained in the control position (30° ipsilateral) immediately before, and 1 min after, each change in head position. If there were significant changes in (1) the pre- and post-test control H values, and (2) the test and control m responses, the trial was rejected. The mean H reflex amplitude per test position was then normalized with respect to pre- and post-test control values and plotted as a funciton of head position.

1 major and 2 minor patterns of response emerged: (1) In 10 of 17 subjects studied, the H reflex was progressively facilitated with increasing amount of ipsi- and contra-lateral tilt. (2) In 3 subjects, it was facilitated by ipsilateral tilt but inhibited by contralateral tilt. (3) In the remaining 4 subjects, it was inhibited by ipsi- and contra-lateral tilt. Furthermore, the observed H reflex data could be described by a sinusoidal function in 15 of 17 subjects, with 81-99% of the variance accounted for.

The 3 patterns of H reflex modulation by lateral tilt represented real inter-subject differences, as the data from the same subjects were reproducible. Since the effect of rapidly adapting cutaneous receptors were minimized by waiting 1 min before each recording, our results could be attributed mainly to otolithic afferents whose unit recording showed similar behavior (Fernendez, C. & Goldberg, J.M., J. Neurophysiol., 39:996, 1976).

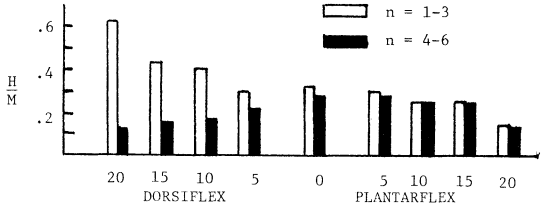
In conclusion, we have demonstrated that the soleus MNP excitability in man is often facilitated by ipsilateral tilt, a behavior that is consistent with the asymmetric tonic labyrinthine reflex described in decerebrate cats.

Supported by a grant from the Canadian Medical Research Council.

21.11 ADAPTATION OF THE SOLEUS H-REFLEX IN MAN TO STANDING ON INCLINED SURFACES. J.S. Frank* (SPON: A. Baylor). Dept. of Kinesiology, Univ. of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Passive movement of the ankle joint in man has been shown to modify soleus motoneuron excitability (Mark et al., Exp. Brain Res., 6:130, 1968; Robinson et al., J. Neurol., Neurosurg. Psychiat., 45:699, 1982). Since this may be important to the control of upright posture, this investigation examined the influence of ankle joint position on soleus H-reflex excitability in man when standing on inclined surfaces. Eleven subjects were required to stand for 2 min. on 4 upward-inclined (5, 10, 15 and 20° dorsiflexion) and 4 downward-inclined (5, 10, 15 and 20° plantarflexion) surfaces. Surface emg recorded over the right soleus and tibialis anterior muscles and H reflexes were elicited to the soleus muscle by stimulation of the posterior tibial nerve. The stimulus intensity was adjusted to evoke an H reflex of approximately half the maximum amplitude, as well as a small M wave. H reflexes were elicited at 15 sec. intervals while the subject stood alternately on a flat surface and then on inclined surface. A total of 8 reflex measures were averaged for each inclined surface.

Of the 11 subjects examined only 6 subjects showed a stable M wave which was used to verify constant stimulating conditions across the testing conditions. The results of these subjects are presented in the graph below. H-reflex amplitude is expressed as a percent of the maximum M response for each subject. All subjects revealed no change in reflex excitability during dorsiflexion of the ankle of up to 15°. There was a depression of reflex excitability at 20° which was accompanied by tibialis anterior muscle activity. Plantarflexion of the ankle when standing on downward inclined surfaces revealed a progressive increase in reflex excitability for 3 subjects and a progressive decrease in reflex excitability for 3 other subjects. These results differ from those reported for passive rotations of the ankle and suggest a different control of soleus motoneuron excitability with changes in ankle joint angle in standing man than non-standing man.



21.12 TWO DISTINCT STRATEGIES FOR STANCE POSTURE CONTROL: ADAPTATION TO ALTERED SUPPORT SURFACE CONFIGURATIONS. F.B. Horak and L.M. Nashner. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

Biomechanical models of the standing human predict two strategies for adjustment of the center of body mass with respect to the base of support: 1) "Ankle strategy", a pattern of muscular contractions with which the body moves as a flexible inverted pendulum about the ankles by exerting torque against the support surface and 2) "Hip strategy", rapid flexion and extension of the hips moves the center of body mass by exerting a shear force rather than torque against the surface. This study examines how the width of the support surface influences the choice of postural strategy and how these strategies interact.

Equilibrium was perturbed by platform translations in the anterior-posterior direction in 9 subjects standing alternatively on a normal surface and on a narrow beam which limited the ability of their ankles to exert torque. The width of the support surface was varied with respect to the amplitude of the perturbations such that the center of body mass disruptions were within and beyond the boundary of the "ankle strategy".

EMG activity from six trunk and leg muscles revealed two distinct synergic patterns available for stance control, each with a consistent temporal sequence and strength ratio of proximal and distal synergist activity. On the normal support surface, a distal-to-proximal muscle activation sequence was associated with a rapid increase in ankle torque and approximate in-phase coupling of the ankle and hip joints. In situations in which torque production by the ankles was limited, a hip strategy was used involving a directionally opposite muscle synergy in a proximal-to-distal sequence leading to rapid rotation of the trunk about the hips. Latencies of initial muscle activation for both the ankle and hip strategies were 75-100 ms suggesting automatic control. In trials immediately following a change in the width of the support surface the changes in strategy were often made gradually with a progressive shift toward proportionately less of the inappropriate strategy and more of the appropriate one. In some individuals, the shift was complete after 5-20 trials, while in others a small mixture of the two strategies remained under all circumstances. During adaptation to novel surfaces, normal subjects exhibited a specific interaction between the ankle and hip strategies such that the muscle synergies associated with each were activated sequentially rather than simultaneously.

21.13 VISUALLY MEDIATED FEEDBACK CORRECTIONS DURING ISOMETRIC FORCE TRACKING IN HUMANS. P.J. Cordo. Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

It is generally accepted that voluntary movements may be modified during the course of their execution provided that the total duration exceeds sensory feedback processing times. Although the role of somesthetic feedback in the production of adjustments to ongoing movements is presently undefined, visually-mediated modifications to movement trajectories have been demonstrated. The present study was undertaken to provide a more complete characterization of the properties of visually-mediated adjustments to a simple voluntary motor activity.

Eleven standing subjects supported at the chest and under the right elbow produced isometric force tracking responses to step stimuli. The right wrist was placed in a cuff attached to a vertical shaft such that force exerted on this manipulandum was produced exclusively by the elbow musculature. Two horizontal lines were displayed on an oscilloscope screen in front of the subject: 1) a stationary reference line and 2) a "tracking" line representing the mathematical difference between the stimulus voltage and the force produced on the manipulandum. The subject's task was to respond to a sudden upward displacement of the tracking line by exerting force on the manipulandum in the flexor direction in order to return the tracking line to the reference line position as quickly and accurately as possible. During sequences of 80 trials, the stimulus amplitude was randomly varied between limits requiring force responses of 2-12 kg. In 40 trials, the tracking line was unexpectedly extinguished within 10 ms of the onset of the subject's response. The stimulus amplitude, the force response of the subject and the electromyographic activity of the biceps brachii, brachioradialis, triceps brachii and posterior deltoid muscles was recorded.

By comparing average responses in the 40 trials with continuous visual feedback and the 40 trials where visual feedback was interrupted, the appearance of visually-mediated adjustments was determined to occur between 200-300 ms after response onset. Examination of single, selected trials revealed the occurrence of individual adjustments, beginning as early as 180 ms following response onset. Analysis of electromyographic recordings demonstrated the participation of flexor muscles in these visually-mediated corrections for both overshoots (decreased activity) and undershoots (increased activity). The extensor muscle (triceps) co-contracted with the flexor musculature and, therefore, was not directly involved in the production of force adjustments.

21.14 FEEDFORWARD CONTROL OF POSTURE IN NORMAL ADULTS S. D. Lucy*, C. L. Riach*, and K. C. Hayes*. (SPON: J. P. Girvin) Dept. Physiology, Fac. Med. Univ. Western Ont. Ontario N6G 1H1

It is well documented that activity in postural muscles, such as erector spinae, precedes upper limb movements in man and that this feedforward control of posture aids in the maintenance of truncal stability. Little is known, however, of the mechanisms by which this is accomplished, or the exact functional interpretation. The present study was designed to determine whether or not this muscle activity induced reliable shifts in the center of pressure (CP) of ground reaction forces in the 600 msec immediately preceding voluntary arm movements. Consideration was also given to the relationships between preparatory excursions in CP and the altered location of CP introduced by the torques transmitted through the musculo-skeletal system during arm motion. Healthy adults (n=32) stood quietly on a strain-gauged force-platform (AMTI OR-6) and generated a series of self initiated rapid straight arm raises (right arm). CP excursions were derived from the orthogonal force and moment of force signals and were sampled (A/D), at 512 Hz. Onset of arm movement was determined from an Endevco 7264-200 accelerometer secured to the wrist. 21 of the subjects exhibited clear evidence of preparatory shifts in CP in the lateral direction. 22 showed evidence of preparatory CP excursions in the fore-aft direction although these were less distinct and more variable. For the lateral CP shifts two distinct phases were evident, an early change, commencing 400-500 msec prior to arm acceleration (38%) and a later change <150 msec prior to arm motion (61%). These preparatory CP deviations were always to the left of midline; the CP excursions concurrent with arm acceleration were always to the right. These results provide evidence that the preparatory muscle activity leads to definite shifts in CP prior to arm movement (as distinct from an increase in stiffness without CP excursions) and that these are appropriate for offsetting the imbalancing torques resulting from arm motion that are transmitted through the kinematic linkage.

21.15 POSTURAL SWAY IN SUBJECTS WITH CEREBELLAR ATAXIA, K. C. Hayes*, J. D. Spencer*, S. D. Lucy* and C. L. Riach*. (SPON: W. F. Brown) Dept. Physiology, Fac. Medicine, Univ. Western Ont., London, Ontario N6G 1H1

The periodic fluctuations in location of the center of pressure of ground reaction forces during quiet standing ie postural sway, have been investigated in ten subjects with cerebellar ataxia. Of these subjects, 2 were diagnosed with Freidriech's Ataxia, 2 with Multiple Sclerosis, 1 chronic alcohol abuse and the remainder with brainstem or cerebellar infarcts, stroke or degenerative conditions. Subjects stood with feet parallel, and positioned 6 cm apart on a stable strain-gauged force-platform (AMTI OR-6) for a period of 20 seconds. They were examined with eyes open (EO) and eyes closed (EC). The coordinates of the center of pressure were derived from orthogonal force and moment of force signals sampled (A/D) at 51 Hz. In the EO condition cerebellar subjects demonstrated increased sway ($p<.05$) in both the fore-aft (RMS_y = 7.37mm ± 1.23 [S.E.]) and lateral direction (RMS_x = 8.1mm ± 1.43) when compared to age matched normal subjects (n=66). With EC the sway values in the fore-aft direction increased (RMS_y = 9.26mm ± 1.36 and RMS_x = 7.57mm ±2.0) yielding Romberg quotients ranging from R_y = 92% to 350% and R_x = 35% to 154%. Two subjects were unable to maintain equilibrium with EC. Power spectral analysis of the CP excursions of normal subjects, using a Fast Fourier Transform, indicated the principal energy to be <1Hz, with peaks generally occurring <.2Hz. Three of the cerebellar patients revealed prominent peaks in the spectrum between 1-3Hz in the fore-aft direction; others exhibited spectra that were essentially normal apart from the general increase in power.

21.16 BIOMECHANICAL CORRELATIONS OF POSTURAL MUSCLE ACTIVITY. W. G. Friedli*, M. Hallett and S. R. Simon*. Gait Laboratory, Children's Hospital Medical Center and Neurology Section, Brigham and Women's Hospital; Harvard Medical School, Boston, MA 02115.

When a standing human subject makes an arm movement muscles throughout the entire body participate. Muscle activity in the trunk and legs precedes the arm movement and is highly specific for different tasks. Presumably this muscular activity has significance for posture, defined as maintainance of the body segments in appropriate relationship to each other and stabilization of balance. The purpose of the present study was to understand the precise functional consequences of this postural muscular activity.

Normal human subjects standing on a force plate made bilaterally symmetric elbow flexion and extension movements (focal movements). For some movements subjects were free-standing and for others they were strapped to a rigid wall behind them. Vertical and fore-aft translational forces as well as center of foot pressure were measured from the force plate. Body motions were derived from high speed cinematography.

For movements made when free-standing, analysis of the postural instabilities revealed that the principal problems are those of fore-aft translational force and rotational force in the saggital plane. Sources of postural perturbation are (1) muscular forces arising from the muscles moving the limb which also act on the body and (2) the momentum of the moving limb. Our data show the former to be more significant. Hence for flexion movements the dynamic perturbation is a forward translational force and clockwise (viewing the body from the right) rotational force; for extension movements the perturbations are oppositely directed. The fore-aft translational forces seen by the platform during the movement are backward for flexion and forward for extension. Postural agonist muscles for a flexion movement are the erector spinae, hamstrings and triceps surae which produce counterclockwise force; postural agonist muscles for an extension movement are rectus abdominus, quadriceps and tibialis anterior which produce clockwise force. Actual movement of body segments is small and reflects interplay of forces from the focal and postural movement. Movements made with the subject strapped to a rigid wall have less need for compensation and reduced postural force was seen. Vertical forces seen by the platform appear to reflect changes in center of foot pressure and do not seem directly compensatory.

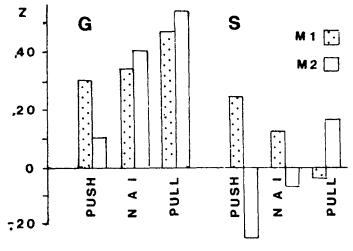
We conclude that a principal function of postural muscle activity seen in association with a focal movement is to compensate for the most destabilizing dynamic forces produced by the movement.

21.17 MODULATION OF POSTURAL RESPONSES DURING PREPARATION FOR VOLUNTARY MOVEMENT. M.H. Woollacott, M. Bonnet*, K. Yabe*. Département de Psychobiologie Expérimentale, CNRS-INP 3, 31 chemin J. Aiguier 13009 Marseille, France.

During preparation for voluntary movement, processes occur at a variety of levels within the nervous system, which facilitate the speed and/or accuracy of the task to be performed. The following experiments were designed to determine if the postural regulatory system, which is activated in advance of a voluntary movement as a function of the direction of movement, is modulated by preparatory processes, either in a global fashion, or more specifically, in relation to the direction of the prepared movement.

Electromyograms (EMG's) were recorded from the gastrocnemius medialis (GM) and soleus (S) muscles of the right leg while standing subjects performed a reaction time (RT) task involving pushing or pulling on a handle with the right hand. A warning signal gave advance information about the direction of the arm movement to be performed. Modulation of postural muscle excitability by preparatory processes was tested by rotating the right ankle joint to elicit a GM and S stretch reflex, randomly, at different times during the 1.5 sec. preparatory period: 100, 300 and 500ms before the response signal. The amplitude of EMG activity recorded after the stretch was measured in terms of 2 successive components: M1 (40-60ms) and M2 (60-80ms). Each series of trials consisted of randomly distributed control trials, advance information push, advance information pull, and no advance information (NAI) trials. 6 subjects were tested.

1) Statistical analysis of EMG amplitude (ANOVA) showed that the preparatory effects were different for the 2 muscles: GM showed a generalized facilitation, M1 and M2 being more strongly facilitated for pull than for push trials; 2) The time course of stretch response modulation during the second half of the preparatory period was different for the 2 muscles, an increasing facilitation being observed in GM only; 3) Significant interaction between advance information conditions and response components were shown for GM and S muscles; 4) These EMG response modulations were emphasized in faster RT performers (N = 3, see figure). Thus, when standing subjects know in advance the direction of rapid arm movement, direction specific preparatory changes of the stretch reflex response appear in both ipsilateral GM and S muscles.



21.18 DEVELOPMENTAL ASPECTS OF POSTURAL CONTROL IN CHILDREN. A. Shumway-Cook and M.H. Woollacott, Dept. of P.E. and Inst. of Neuroscience, University of Oregon, Eugene, Oregon 97403.

The development of neural mechanisms underlying postural control is critical to the acquisition of complex motor skills and requires the maturation of two potentially separate, though interactive processes: 1) those responsible for organizing muscles and joints into appropriately coordinated response patterns, and 2) those responsible for ensuring that responses remain consistently context dependent, by integrating and acting upon redundant and sometimes conflicting sensory inputs to the postural control system.

This study compared central nervous system organizational processes underlying balance in children of 3 age groups: 15-31 mos., 4-6 yrs., and 7-10 yrs., using a moveable platform capable of antero-posterior (A-P) displacements or dorsi-plantarflexing rotations of the ankle joint. A servo system capable of linking platform rotations to A-P sway angle allowed disruption of ankle joint inputs, to test the effects of incongruent sensory inputs on response patterns. Surface electromyography was used to quantify latency and amplitude of the gastrocnemius, hamstrings, tibialis anterior, and quadriceps muscle responses. Cinematography provided biomechanical analysis of sway motion.

While directionally specific postural response synergies to A-P perturbations were present in the youngest children tested, response durations were longer than in adults and did not adapt to changing contexts. Transitions from immature to mature response patterns did not occur in a linear fashion, but showed stage-like properties. Variability in latency and amplitude relations between synergic muscles was low in the 15-31 mo. group, large in 4-6 yr. group, and subsequently decreased in the 7-10 yr. old, as responses acquired adult characteristics. Motion analysis in conjunction with EMG data suggests that in the 4-6 yr. old, the lack of tight temporal coupling between distal and proximal muscles was less efficient in minimizing inertial lag associated with mass of trunk and thigh and resulted in a motion of the knee joint. Results from balance tests under altered sensory conditions (eyes closed and/or ankle servoed) suggested that 1) with development a shift in controlling inputs to posture from visual dependence to more adult-like dependence on a combination of ankle joint and visual inputs occured in the 4-6 yr. age group; 2) the ability to resolve multi-modal sensory conflict in balance control was not present in the 15-31 mo. old, emerged in the 4-6 yr. old, and reached adult form in the 7-10 yr. old. It is proposed that the age 4-6 is a transition period in the development of posture control. At this time the nervous system 1) uses visual-vestibular inputs to fine tune ankle-joint proprioception in preparation for its increased importance in posture control; and 2) fine tunes the structural organization of the postural synergies themselves.

21.19 VISUAL, VESTIBULAR AND VOLUNTARY CONTRIBUTIONS TO HUMAN HEAD STABILIZATION. N. Wereley*, D. Guitton, R.E. Kearney, B.W. Peterson. Montreal Neurological Institute and Biomedical Engineering Unit, McGill University, Montreal, Canada, and Northwestern University, Chicago.

We have investigated the ability of humans to stablize their heads in space and assessed the influence of mental set and the relative importance of visual and vestibular cues.

Subjects were fixed firmly to the chair of a turntable facing a screen on which was projected a target spot. A 'gunsight' spot generated by a small projector fixed to the head provided feedback of head position. Four conditions were studied. 1) Gunsight (GU), subjects were instructed to stabilize the head in space by superimposing the 'gunsight' spot on the fixed target spot while the chair position was displaced according to a random pattern with a bandwith from 0 - 1 Hz. 2) Imagined gunsight (IGU), identical to (1) except that the subject was blindfolded and so had to imagine the target position. 3) Mental arithmetic (MA), subjects did mental arithmetic while the chair was displaced. 4) Visual tracking (VT), subjects were instructed to track the target spot with the 'gunsight' spot while the chair was fixed and the target spot driven to follow the chair displacement trajectory used in conditions 1,2 and 3.

In GU normal subjects stabilized their head position extremely well (mean HEAD/CHAIR gain = .95). Significant stabilization was present in IGU although the gain (mean = .59) was significantly reduced compared to GU. There was very little stabilization in MA (mean gain= .08). In VT, subjects tracked the target well but with a lower gain (mean =.68) than for GU .

The impulse response functions (IRF) for GU had two distinct components: one near the origin and the other at about 250 ms. The initial peak reflects short latency mechanisms (inertia, prediction, vestibulo-collic, cervico-collic). The limited bandwidth of our turntable precluded a definitive statement regarding this peak. Nevertheless it tended to have a similar amplitude in GU and IGU and to be greatly reduced (40%) in MA. This result suggests voluntary control of neck mechanics.

The timing of the second peak was appropriate for visual tracking and indeed was similar to that of the single peak observed in VT . However, the second peak was not entirely dependent upon visual input since it was present, although reduced (80%) in amplitude, under IGU. It was clearly under voluntary control since it was further reduced (40%)in MA.

We have also studied GU, IGU, and MA in a patient with no vestibular function. Head stabilization in GU was good (gain=.75) although lower than normal. However, in contrast to the normal case, head stabilization of the patient in IGU was almost as poor (gain=.07) as for the MA condition (gain=.01): furthermore, in both of these conditions the second peak of the IRF was absent.

These results suggest that vestibular information, under voluntary control and acting at both short and long latencies, plays an important role in head stabilization.

Supported by the Canadian MRC.

OCULOMOTOR SYSTEM: MECHANICS AND PSYCHOPHYSICS

22.1 EFFECTS OF LIGHT AND DARK ENVIRONMENTS ON PRIMATE FIXATIONAL EYE MOVEMENTS. D. Max Snodderly. Eye Research Institute, 20 Staniford Street, Boston, MA 02114.

Many studies of the primate visual system compare behavioral performance during light and darkness. This is often done to eliminate visual influences on oculomotor performance or to compare the properties of photopic and scotopic systems. I have compared the fixational eye movements of two Macaca fascicularis monkeys when performing the fixation task devised by Wurtz under two illumination conditions: The fixation target was presented either with the room lights on, creating a background adjacent to the spot of 1 cd/m^2 or the fixation target was presented in total darkness.

For each trial, mean eye position was calculated for the last half second as a measure of eye position during the trial. Eye position at the end of dark trials was higher than at the end of light trials for both monkeys. Between-trial scatter of eye position was also greater on the vertical meridian in the dark than in the light. A human subject tested under the same conditions did not show this deterioration of performance in the dark.

When one examines the movements of the eye within the trials, the performance of the monkeys and the human subject is more comparable. In the dark, all subjects made fewer but larger fixational saccades than in the light. I interpret this to mean that stimulation of the peripheral retina during fixation increases the frequency of fixational saccades.

22.2 VISUAL SUPPRESSION DURING VERGENT EYE MOVEMENTS. K.A. Manning* and L.A. Riggs (SPON: F. Volkmann). Hunter Laboratory of Psychology, Brown University, Providence, RI 02912.

Vision is known to be impaired near onset of saccadic eye movements. This suppression of vision, which is usually called saccadic suppression, appears to arise through central, neural processes when tested under appropriate conditions. We now provide evidence that suppression is associated with non-saccadic eye movements in human subjects, vergent movements.

Visual suppression was measured psychophysically in 3 observers during convergence and divergence. Subjects executed 2 degree vergent movements by fixating alternately a near or far point under bright, Ganzfeld viewing conditions. A two-alternative, temporal, forced-choice procedure was used to measure subjects' sensitivity to brief, full-field decrements in illumination that occurred 1) at onset of vergent movements, and 2) during steady fixation. All subjects showed roughly 0.5 log unit suppression during convergent and divergent movements.

We conclude that saccades are not the only eye movements accompanied by visual suppression. Since visual suppression is present during convergence and divergence, suppression appears to arise as a corollary to initiation of an eye movement.

Supported by EY-03169

22.3 HUMAN SACCADES ARE VERY CONJUGATE. J.L. McFarland*, A.F. Fuchs, C.R.S. Kaneko and J. Wallman. Depts of Physiology & Biophysics and Psychology, and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195; Dept. of Biology, City Univ. of New York, New York, NY 10031.

For saccades to be conjugate, agonist motoneurons must be activated nearly simultaneously and with similar discharge patterns. Although the motoneuron pools innervating vertical extraocular muscles are all located in the mesencephalon, the motoneurons for horizontal gaze lie in both the pons and the mesencephalon. Recent electrophysiological studies suggest that for vertical saccades, information is probably delivered simultaneously over parallel pathways, while for horizontal saccades, first signals impinge on abducens motoneurons and then internuclear neurons relay these same signals to medial rectus motoneurons in the mesencephalon.

To investigate whether these differences in pre-motoneuronal circuitry are reflected as differences in the trajectories of conjugate horizontal and vertical saccades, we measured the movements of both eyes simultaneously and independently using the scleral search coil technique. Two human subjects made 5^{o}, 10^{o}, and 20^{o} horizontal, vertical and oblique saccades between stationary targets. Saccade amplitude, duration, peak velocity, onset time, and time-to-peak velocity were determined.

Both horizontal and vertical saccades had virtually identical timing and trajectories in the two eyes. On the average, the two eyes started together (within our 1-msec measurement error), moved the same distance (average difference of less than 0.2^{o}), ended together, and attained the same peak velocity. Both eyes reached maximum velocity simultaneously during all vertical and small horizontal saccades. During the larger (20^{o}) horizontal saccades, however, the adducting eye reached peak velocity 2.5 to 4.0 msec, on average, before the abducting eye.

Since the saccades of each eye are essentially indistiguishable, we suggest that the extraocular muscles of each eye must receive virtually identical and simultaneous neural inputs during horizontal and vertical saccades.

Our data are in contrast with those of previous binocular electro-oculographic studies (White et al., J. Opt. Soc. Am. 52:210, 1962; Miyoshi et al., Ann. N.Y. Acad. Sci. 374:731, 1981) in which significant differences between the timing and trajectories of horizontal saccades in the abducting and adducting eyes were observed.

(Supported by NIH Grants RR00166 and EY00745.)

22.4 HORIZONTAL AND VERTICAL COMPONENTS OF OBLIQUE SACCADES ARE TEMPORALLY COUPLED IN HUMANS AND MONKEYS. W.M. King, Dept. of Physiology, Univ. Rochester Med. Ctr., Rochester, NY 14642, S.G. Lisberger, Dept. Physiology, UCSF, San Francisco, CA 94143, and A.F. Fuchs, Dept. of Physiology & Biophysics and Regional Primate Research Center, Univ. of Washington, Seattle, WA 98195.

The trajectories of purely horizontal or vertical saccades are stereotyped, and are characterized by approximately linear relationships between amplitude and duration, and between amplitude and velocity (ADV relationships). For example, in humans, a 5 deg saccade typically has a duration of 35 msec and a peak velocity of 200 deg/sec whereas a 20 deg saccade has a duration of 70 msec and a peak velocity of 400 deg/sec. Most saccades, however, are oblique with differently sized horizontal and vertical components. Previous studies in humans (T. Bahill and L. Stark, Arch. Ophthal. 1977) suggested that oblique saccade components were temporally independent, each obeying separate ADV relationships. In contrast, studies in cat (C. Evinger et al., Exp. Br. Res. 1981; D. Guitton and G. Mandl, Vis. Res. 1980) suggested temporal coupling of oblique saccade components with failure to obey separate ADV relationships.

We have examined this issue in humans and in monkeys using an electromagnetic search coil to obtain accurate measurements of horizontal and vertical eye movements. In some animals, single unit recordings from short lead burst neurons and abducens motoneurons were obtained to corroborate the behavioral evidence.

Consistent with the cat studies, our data demonstrate strong temporal coupling between the horizontal and vertical components of oblique saccades. The duration of the smaller component is stretched to nearly equal the duration of the larger component. Consider, for example, an oblique saccade with a 5 deg horizontal and a 20 deg vertical component. Using the typical values given above, the duration of the 5 deg component would be nearly 70 msec instead of 35 msec. Quantitatively, our results may be summarized by two relationships: First, component duration is linearly related to the polar or vector amplitude of the saccade. This relationship is a generalized amplitude-duration function valid for the components of a saccade in any direction. Second, component peak velocity is linearly related to component average velocity (defined as component amplitude divided by component duration). This relationship implies that a single peak velocity - vector amplitude function does not exist for oblique saccades.

Our results suggest that the premotor circuits producing the horizontal and vertical components of saccades are centrally coupled.

22.5 ALEXANDER'S LAW: DOES IT REALLY INTERFERE WITH THE VESTIBULO-OCULAR REFLEX (VOR)? D. A. Robinson, D. S. Zee, A. M. Holmes*, T. C. Hain* and L. F. Rosenberg*. Depts. Ophthalmology and Neurology, The Johns Hopkins Univ., Baltimore, MD 21205.

In 1912, G. Alexander observed that the intensity of spontaneous, vestibular nystagmus decreased with gaze in the direction of the slow phases and increased in the other direction. In 1982, Doslak et al. (Ann. Otol. Rhinol. Laryngol., 91: 316) showed this occurred with caloric nystagmus in normals and intensity can be defined as slow-phase eye velocity. Does this mean that centripetal slow phases are more compensatory than centrifugal?

To test this possibility, we rotated four subjects at 0.5 Hz, 30 deg/sec peak velocity. With a visible target straight ahead, gains of the VOR (eye velocity/head velocity) were 0.914 ± 0.024. At 25 deg left and right, gains decreased to about 0.787. This decrease on eccentric gaze is a new finding but there was no difference between left and right peak eye velocity; no Alexander's law. When subjects imagined a target in the dark, all gains dropped by about 4% but again there was no Alexander's law. During rotation at a constant velocity (30 deg/sec) in the dark, subjects could suppress quick phases by imagining a target on the chair or on the wall. In the latter case it was necessary to imagine a target about 30 deg ahead and track it well into the other direction creating a giant, 60 deg, slow phase 2 sec long. In either case there was no consistent evidence for Alexander's law.

We did find Alexander's law during per- and post-rotatory nystagmus. Gains measured using initial eye velocity were low (0.437) because subjects were concentrating on maintaining gaze left or right periodically. Thus, mean initial eye velocity was 13.1 deg/sec. It rose or fell by 14.5% on 25 deg eccentric gaze in the quick-phase or slow-phase direction respectively.

In all the cases without Alexander's law, subjects suppressed normal nystagmus by attending to a real or imagined target. We felt that when quick phases are allowed to rapidly and repeatedly change gaze position it is impossible to form the percept of such a target. We suggest that the normal VOR is opposed by a parallel pathway normally used to cancel the VOR. The percept of an earth-fixed target drops the gain of this path to zero so that VOR gain is 1.0. The percept of a chair-fixed target raises it to 1.0 so that VOR gain is 0.0. Without a percept (e.g. mental arithmetic), the parallel gain goes to a default value near 0.5 so that VOR gain is also near 0.5. Under these conditions, nystagmus occurs, gains are low, and Alexander's law appears. We still do not know why it occurs but we can state that it only appears in situations where the VOR is not or cannot be used to assist in attending to the percept of a target.

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22.6 POST-SACCADIC EYE STABILITY REQUIRES A COMPLEX, ACTIVE POST-SACCADIC INNERVATION. H. P. Goldstein* and D. A. Robinson (SPON: E. Young). Wilmer Institute, The Johns Hopkins University, Baltimore, MD.

We recently reported that after averaging data from nearly identical horizontal saccades in the monkey, the abducens motoneuron firing rate consistently showed a slow decay to the steady state level for saccades where the unit burst ("on saccades") as well as for saccades where the unit paused ("off saccades"). This decay, with a major time constant of about 90 msec, lasted hundreds of msec after the end of the saccade even though the eye remained stationary in the orbit. From analysis of the dynamic orbital properties one can deduce that this "pulse-slide-step" (and "pause-slide-step") innervation is required to hold the eyes steady after a saccade.

To have consistency of neural and mechanical data in a single species we measured the mechanical dynamics of the passive orbital tissues in the monkey thereby extending previously determined data from humans and cats. Monkeys were anesthetized and the horizontal recti detached in one eye. That eye was displaced and then quickly released; the returning time course measured with the magnetic eye coil technique. The time course showed an initial fast, 20 msec time constant decay followed by a slower decay with a major time constant of about 170 msec. Mathematical analysis shows that the force needed to maintain the passive orbital tissues at a fixed position after an abrupt change in position is an exponentially decaying force with a time constant of 90 msec. While dynamic relationships exist between discharge rate and muscle force, we still feel that the long, post-saccadic drifts in firing rate are part of a programmed command (vision being too late to assist) preventing stress-relaxation of orbital tissues from creating eye drift. We have carried this further by simulating a more realistic nonlinear model on a digital computer. Both theory and simulation show that if the brain fails to compensate for these slower decays the eye would drift at velocities in excess of 10 deg/sec, a rate which would preclude clear vision after a saccade. We further suggest that patients with post-saccadic drift anomalies may correctly match the pulse and step, but not correctly match the slide to that pulse and step. (This work supported by NIH grants EY07047, EY01765, and EY00598.)

22.7 A RESTRICTED MAIN SEQUENCE FOR PURKINJE IMAGE RECORDINGS OF VERTICAL SACCADES. J.R. Hotson, K. Dehnad*, E.B. Langston*, A. Owen*, B.W. Brown*. Dept. Neurology and Div. Biostatistics, Stanford University and Santa Clara Valley Med. Ctr., Stanford 94305 and San Jose 95128, Ca.

The main sequence parameters of saccades provide quantified measurements of the saccade systems. This method has mainly examined horizontal saccades because studies of vertical saccades are limited by techniques that are insensitive, inaccurate, or invasive. These technical limitations may explain the paucity of studies of the vertical saccade system.

Purkinje image recordings using the SRI eyetracker can measure vertical saccades, however the recording range is limited to +/- 9 deg arc, and there is a lens overshoot artifact that interferes with amplitude and duration measurements. This recording approach however is particularly sensitive and records eye movement in the same range as most naturally occurring saccades. For these reasons, we used Purkinje image recordings to develop a restricted main sequence and provide a method for accurate comparative studies of the human vertical saccade system.

The peak velocity and amplitude was plotted for 1-15 deg arc vertical saccades excluding glissades and overlapping saccades. Saccade displacement rather than amplitude was measured to omit the lens overshoot artifact. Dynamic overshoot is not prominent in vertical saccades and thus position displacement gives an approximation of saccade amplitude. A power law (Log Vel=α + β Log Ampl) and an exponential law (Log Vel= α + β Ampl) with and without quadratic terms were tested for best fit of the plots. These models were compared in 8 control subjects by plots of residuals, the standard deviation around the linear regression, and a quadratic regression coefficient. A power law gave a better fit than an exponential law for this restricted region of the main sequence that lacks an asymptote. Adding a quadratic term did not significantly improve the model.

Next tested was the ability of Log Vel= α + β Log A to discriminate between control subjects and patients with Huntington's Disease and Joseph Disease. These diseases may selectively slow vertical saccades. A comparison of the estimated peak velocity at 10 deg arc amplitude (V_{p10}), the scattergram of the slope, and intercept of the linear regressions, and the plot of the standard deviation about the regression (SD_{reg}) were used as tests of discrimination. Both V_{p10} and plots of the regression line discriminated equally between 8 control subjects and 9 patients with 83% of the observations in the patients and none of the control subjects falling below a discrimination boundary (V_{p10}=5.9 or 365 deg arc/sec). SD_{reg} separated only 44% of patient measurements but when combined with V_{p10} further enhanced its discrimination to 89%.

22.8 STRETCH RECEPTOR AFFERENTS FROM EXTRAOCULAR MUSCLES IN THE SEMILUNAR GANGLION OF THE RAT. W. J. Daunicht* (SPON: W. Lichtensteiger). Div. Biocybernetics, Univ. Duesseldorf, D-4000 Duesseldorf, F.R.G.

In order to investigate the functional role of proprioception from extraocular muscles (EOM) in eye movements of mammals, the rat was chosen as a model. In a first basic study the presence of EOM stretch receptors was demonstrated in this animal, and sensitivity and dynamic properties of receptor impulse rate in relation to passive eye movements were determined.

Single unit activity was recorded in anesthetized albino rats, while EOM were passively stretched using an electromagnetic puller with position feedback. The impulse rate modulation was studied in response to sinusoidal stretching with frequencies f between 0.01 and 10 Hz and amplitudes between 140 and 355 µm corresponding to 2.7 to 6.8 deg of eye rotation.

Units responding specifically to passive stretch of EOM were found in the ganglion semilunare, but not in the mesencephalic trigeminal nucleus (mes V). The activity was not correlated to touch of surrounding tissue or movement of the jaw. The majority of afferents had a very high sensitivity S, e.g. at 1 Hz an average of 200 imp/s/mm corresponding to 10.5 imp/s/deg of eye rotation; others had a lower sensitivity, e.g. at 1 Hz an average of 30 imp/s/mm corresponding to 1.6 imp/s/deg. In the range between 0.1 and 10 Hz the sensitivity increased by 6.8 dB/decade. Such a behavior could be described by the relation $S \sim f^k$ with k = 0.34, predicting a constant phase lead of 31 deg in case of linearity. Experimentally a constant 34 deg phase lead was found. At frequencies below 0.1 Hz the sensitivity plots flattened out and the phase lead tended to decrease. The static sensitivity S_0 ranged from 0 to 130 imp/s/mm (6.8 imp/s/deg).

In a second series of experiments the localization of first order neurons was confirmed by horseradish peroxidase injections into EOM. Only the ophthalmic subdivision of the ipsilateral ganglion semilunare (besides the corresponding oculomotor nucleus) contained labelled neurons, but never the mes V. Up to 18 first order neurons per muscle were found.

It is concluded that rats are suitable to study the function of EOM proprioception and that the high sensitivity of their stretch receptors enables them to monitor eye movements with high precision. Dynamic properties and distribution of sensitivities are consistent with findings at primary and secondary endings at cat hind limb muscle spindles. In a next step it is planned to study the afferent activity in the alert animal.

22.9 ELECTRICAL ACTIVITY OF MUSCLE FIBERS OF RAT EXTRAOCULAR MUSCLES. D. J. Chiarandini, E. Stefani and J. Davidowitz*, Depts. of Ophthal. and Physiol. and Biophys., New York Univ. Med. Ctr., New York, NY 10016 and Dept. Physiol., Center for Advanced Studies, I.P.N., Mexico D.F. 14

Extraocular muscles (EOMs) of mammals have two types of muscle fibers, singly and multiply innervated (SIFs and MIFs). These are intermingled in two muscle layers, the global and orbital. Global MIFs (diameter:15-25 µm) have poorly delineated myofibrils, generate tonic tension, are polyneuronally innervated and lack action potentials. They show, instead, Na-dependent graded responses or slow peak potentials (SPPs) (1-5). Orbital MIFs (diameter:5-15 µm) have a morphology that varies from large ill-defined myofibrils toward the ends of the fibers to small well defined myofibrils in their middle region (6). Their contractile properties are unknown. In inferior rectus muscles of rat, we have found in vitro that orbital MIFs are polyneuronally innervated by 2-5 axons. These axons usually have a threshold of 0.3-2.0 V which is comparable to that of axons to SIFs but lower than that of axons innervating global MIFs (3-10 V). Measurements of the membrane time constant, using a bridge circuit, show that it is 7 msec in orbital MIFs, larger than in orbital SIFs (3 msec) but much smaller than in global MIFs (150 msec). The input resistance of orbital MIFs is about 14 MΩ, higher than in orbital SIFs (5 MΩ) and comparable to that of global MIFs (20 MΩ). Nerve stimulation of orbital MIFs evokes compound end-plate potentials which in most cases do not trigger action potentials, even if the fibers have a normal resting potential (RP). However, action potentials were recorded in some orbital MIFs. In global MIFs with a normal RP, nerve stimulation evokes compound end-plate potentials which do not trigger action potentials but which, if large enough, can elicit SPPs. These results demonstrate that in EOMs the contraction of most MIFs is due to graded, non-conducted depolarizations. Moreover, the differences in the threshold of the motor axons, passive electrical properties and ability to fire action potentials, indicate that orbital and global MIFs are functionally different. (Supported by grants from NIH (EY01297 and EY00309), NSF (INT 7920212) and CONACyT (790022)).

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22.10 AN INEXPENSIVE EYE-MOVEMENT MONITOR USING THE SCLERAL SEARCH COIL TECHNIQUE. R.S. Remmel, Department of Physiology and Biophysics, University of Arkansas Medical Sciences, Little Rock, Ar 72205.

The magnetic search coil method for measuring eye movements was developed by D.A. Robinson (IEEE Trans. Bio-Med. Eng., BME-10 (1963) 137-145) and offers very low error, drift and noise. In an animal a coil of wire of 1-3 turns can be surgically implanted either under or adjacent to the four recti muscles. A contact lens containing the coil can be used with humans. The subject is placed inside horizontal and vertical oscillating magnetic fields, which induce voltages in the scleral coil which are proportional to the sine of the eye angle.

Our two pairs of Helmholtz coils are 51 cm in mean diameter and spaced 51 cm apart. Each coil has 5 turns of #16 wire wound on a plywood frame. The pairs of coils are in series and have an inductance of 80 µH. The field is uniform to 1% out to 2.5 cm from the center, a distance appropriate for a cat's eye.

The horizontal field coils are driven by square waves at 50 KHz and the vertical ones at 75 KHz. These frequencies are derived from one master oscillator and are phase-locked. This 2:3 ratio of frequencies means that the 50 KHz and the 75 KHz square waves have no ODD harmonics in common (a square wave has only odd harmonics). The field-coil amplifiers use common switching transistors and supply 30 V p-p to the coils. No tuning nor other adjustments are needed.

The output of the scleral coil is (0.00035 AN $\sin\theta$) volts, where A is the area in sq. cm., N is the number of turns and θ is the eye angle.

The scleral-coil amplifier is made from three fast operational amplifiers. This signal and its inverse are connected to the two contacts of a single pole-double throw switch (made from MOS-FET transistors); this switch is operated by the 50 KHz signal to accomplish phase-sensitive demodulation for the horizontal channel. Next come a 3-pole filter, a D.C. amplifier and a 1-pole filter, which eliminate the carrier frequencies. The filter network has a rise time (10% to 90%) of 1.2 msec. An identical circuit is provided for the vertical channel. The amplifier has a noise level of 0.32 µV at the input (after demodulation). The gain is 7000 (D.C. output/p-p input). Gain and offset controls are provided.

For a typical one-turn coil of 2 cm diameter, the sensitivity is 0.13 V/deg. The amplifier noise is then equivalent to 1.0 min. of angle. The circuit consumes 0.68 A from each of two 15 V regulated supplies and costs less than $300 to build.

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22.11 SPECIFICITY OF SMOOTH PURSUIT EYE MOVEMENT DYSFUNCTIONS IN SCHIZOPHRENIA. P.S. Holzman*, C. Solomon*, S. Levin, C. Waternaux*. Mailman Res. Cr., McLean Hosp. and Harvard U., Belmont, MA 02178.

Smooth pursuit eye movements (SPEM) are disrupted in 50-80% of schizophrenics (SZ's) (review in Lipton et al., 1983). The eye tracking disruption (ETD) consists of saccadic intrusions and of saccadic smooth pursuit tracking (Levin et al., 1983). The previously reported prevalence of ETD in 50% of SZ's relatives, and data from twin studies showing a high concordance of ETD (r=.77) in MZ twins, but not in DZ twins (r=.39), discordant for schizophrenia (Holzman et al., 1977, 1978, 1980) suggest that ETD may represent a biological marker for vulnerability to schizophrenia. ETD are also present, however, in 30-50% of patients with major affective disorders, primarily manic depressive psychosis (MDP) (Shagass et al., 1974; Lipton et al., 1980). The appearance of ETD in many organic disorders also indicates that in themselves they are not pathognomonic to SZ. To establish the trait status of ETD in schizophrenia would therefore require evidence that they are prevalent in families of SZ's but not in families of MDP's. We present here such evidence.

80 SZ and 46 MDP subjects fulfilling DSMIII and St. Louis diagnostic criteria were tested. All were receiving medications at the time of testing. 59 parents from 34 families were also tested. Parents from the two groups did not differ in age. Eye-movements were recorded binocularly in the horizontal plane with the electrooculographic technique. Target characteristics and scoring methods were as described in Levin et al. (1981).

As in previous studies, 59% (47/80) of the SZ's and 41% (19/46) of the MDP's had ETD. A chi-square test was not significant. In contrast, 34% (13/38) of the parents of SZ's, as compared to 10% (2/21) of the parents of MDP's showed ETD. When counting the number of families with ETD, 55% (12/22) of SZ families had ETD but only 17% (2/12) MDP families had it. The probability that a parent or a family of a SZ offspring has ETD is significantly higher than the corresponding probability for a MDP parent or family. The significant predictive variable for the parent's eye tracking quality is the offspring's diagnosis rather than eye tracking status. There were no differences between ETD patterns in the records of parents or of patients. The data replicate a previous report of ETD in 34% of SZ relatives and 11% of non-SZ relatives.

The results suggest that ETD represent a trait-related disorder in SZ but not in MDP. Although they characterize only a subgroup of SZ's, ETD now appear to be a biological marker that transcends surface symptoms of this psychiatric condition.

22.12 SMOOTH PURSUIT AND FIXATION IN THE PARENTS OF SCHIZOPHRENICS L. Whicker, Jr.*, L.A. Abel and L.F. Dell'Osso. Ocular Motor Lab, V.A. Med. Cntr. and Dep't of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

In the past decade a large body of work has grown up concerning ocular motor function in schizophrenics. Abnormalities of smooth pursuit were described in patients and in their relatives. Early work postulated an actual defect in the pursuit pathways of schizophrenics and their relatives, but the methodologies used were crude and qualitative. More recent work on patients using quantitative recording techniques has shown poor fixation and tracking, but questions of interpretation have left it unclear as to whether an actual defect in pursuit exists or if poor tracking might be attributed to impaired attention. Of particular interest would be documentation of an abnormality in the clinically normal relatives of schizophrenics, using quantitative oculographic techniques. We therefore studied the fixation and smooth pursuit of 8 parents (6 F, 2 M) of schizophrenic patients admitted to the Psychiatry Service of the VA Medical Center. The subjects' mean age was 61.9 (s.d.=7.64); all subjects denied hospitalization for psychiatric reasons or use of neuroleptic drugs. None showed evidence of loose associations or illogical thought during interviews.

Subjects were instructed to "hold their eyes still" or "stare at a spot" for two minutes. One subject's square wave jerk (SWJ) frequency lay outside the published normal range;he also had some other saccadic intrusions. Instruction showed no effect on SWJ incidence. Pursuit of ramp targets at 5, 10, 20 and 40 deg/s showed that all patients could produce normal gains; pursuit per se was not impaired. SWJ persisted during pursuit, but at a lower frequency. In 3 subjects at 5 deg/s, however, frequent saccades off-target were seen. These were usually jumps ahead of the spot; the subject would then wait for the target to reach his eye position and resume tracking. This occurred in spite of frequent and enthusiastic verbal encouragement. Such "anticipatory saccades" might be mistaken for SWJ. Thus, actual smooth pursuit and fixation were intact in all subjects and SWJ were within normal limits in 7/8, but a tracking pattern consistent with an attentional impairment was seen in 3/8. Such an abnormality in apparently normal relatives of psychiatric patients merits further study.

22.13 OCULOMOTOR FUNCTION IN ALCOHOLIC KORSAKOFF'S SYNDROME R.V. Kenyon*, J.T. Becker, H. Hermann, & N. Butters. (SPON:C.Oman` Man-Vehicle Lab., Mass. Inst. of Tech., and Boston Univ. Sch. Med.

Patients with alcoholic Korsakoff's syndrome are described as having both memory and perceptual deficits. Although the amnesic syndrome has been well studied, only a few researchers have focused on the perceptual abnormalities in this disorder. The perceptual changes include a failure to adequately scan visual stimuli, poor visual contour analysis, and a tendency to focus on irrelevant aspects of a stimulus. Many of these changes could be affected by the patient's oculomotor system. That is, the failure at visual scanning and contour analysis may reflect a problem with eye movements rather than with higher-order processing.

The purpose of the present experiment was to investigate the oculomotor functions of patients with alcoholic Korsakoff's syndrome. Four patients and one nonalcoholic age-matched control were studied. Three of the patients had been diagnosed as having Korsakoff's syndrome, while one had suffered a Werncike's encephalopathy without developing Korsakoff's syndrome. Eye movements were monitored with an infra-red detection system and both saccadic and smooth pursuit movements were studied.

The results of the study suggest that patients with Korsakoff's syndrome have significant oculomotor abnormalities. First, their latency to begin a saccadic eye movement is significantly longer than that expected for individuals in their age group. In contrast, small corrective saccades were generated with normal latency. Second, the overall saccadic velocity in two of the four of the patients was significantly slower than the norm. Third, the qualitative aspects of the saccades were unusual. Two patients had significant numbers of hypometric saccades and saccadic intrusions during fixation. Fourth, the gain of the smooth pursuit system was lower than normal.

These data suggest that significant abnormalities remain even as long as seven years after the onset of the disorder. These changes appear to be consistent with the patient's known damage to the cerebellum and to the frontal cortex functional system.

22.14 OCULAR AND MANUAL MOVEMENTS TO VISUAL AND AUDITORY TARGETS, J.D. Fisk* and J. A. Mather* (Spon: M. A. Goodale), Psychology Department, The University of Western Ontario, London, Canada N6A 5C2.

Orientation to sensory stimuli often involves the coordinated interaction of a number of muscle groups, but the manner in which the central nervous system achieves this coordination remains unclear. If the processing of sensory information is mediated by neural substrates common to a number of motor systems one would expect parallels in the performance of these systems. The present study compared the performance of the ocular and manual motor systems in a task which required subjects to orient quickly and accurately to a peripheral stimulus. Subjects looked and pointed to targets on a screen that were presented at 10° and 20° to the left and right of a central fixation target. Eye movements were recorded by DC electrooculography. Arm movements were restricted to rotation about a point directly in front of the subjects, and were recorded via a linear potentiometer. Visual targets were 1° diameter LEDs. Auditory targets were a train of clicks at 50 Hz and 70 Db. Blocks of trials were run with either 200 ms (short) or 5 s (long) target durations. The latency to initiate movement of the eyes and hand was lower when the target duration was short. This suggests that the processing time for both motor systems was affected by a common factor. The duration of movement of the eyes and hand was also lower for short targets. This result may reflect the use of additional neural systems which modify the motor output on the basis of sensory information obtained during the production of the movement. Changes in target modality from visual to auditory also affected the eye and hand movements similarly. With visual targets the latency to initiate movement and the error of final position of both the eyes and hand were lower for the 10° targets. In contrast, the latency and error with auditory targets were lower for the more eccentric 20° targets. The similarity of the effects of changes in the stimulus parameters suggest that a common neural substrate may underlie the analysis of sensory information for both systems. The relatively large decrease in accuracy of eye movements to auditory as opposed to visual targets demonstrated the specialization of the ocular motor system for orientation to visual stimuli. However, the findings of this study suggest that the sensorimotor information for orienting to a periphral stimulus may be integrated by processes common to the ocular and manual motor systems. This research was supported by Grant #T199A1 from the Natural Sciences and Engineering Research Council of Canada to Dr. J. A. Mather.

22.15 NON-VISUAL CONTROL OF FINGERTIP POSITION IN SPACE: THE USE OF RETINAL VERSUS OCULOMOTOR REFERENCE INPUTS.
O.Bock* and R.Eckmiller (SPON: W.H.Zangemeister). Div. of Biocybernetics, Univ. of Düsseldorf, D-4000 Düsseldorf, FRG.

Pursuit of visual targets by the tip of the right index finger was studied in 10 healthy human subjects. Visual cues on fingertip position were excluded by a horizontal panel below eye level, restricting possible sources of position information to proprioception and efference copy. A mechanical device restrained arm movements such as to assure fingertip motion along a horizontal arc (45 cm radius, center between axes of eye rotation) just below the cylindrical screen (45 cm diameter) for visual target display.

Static characteristics were studied by asking the subjects to point at stationary targets appearing in a random order at eccentricities between 25 deg left and right (experiment S). For dynamic characteristics subjects pursued a visual target (15' diameter) moving sinusoidally at different amplitudes (20, 10, and 5 deg) and frequencies from 0.1 to 1.2 Hz (experiment D). In both experiments subjects either fixated a point straight ahead (SR,DR: target position monitored as retinal eccentricity), or pursued the target with the eyes (SO,DO: target position monitored as ocular position plus eventual retinal error).

In experiment S subjects typically pointed past the targets. The amount of past-pointing was rather constant for a given subject (s.d. 1 to 2 deg) with individual means ranging from 6 deg left to 6 deg right. However, when pointing to two successive targets, the amplitude of fingertip movement corresponded well to target distance (with a slight tendency to overshoot at small and undershoot at large eccentricities). No unequivocal difference between SR and SO was detected.

In experiment D the gain of fingertip movement strongly depended both on stimulus amplitude and frequency. In DR the gain at 20 deg stayed close to 1 dB up to 0.3 Hz and then fell to -2 dB at 1.2 Hz. With decreasing amplitude DR gain increased and a distinct peak formed at 0.3 Hz. This peak reached 4.5 dB at 5 deg amplitude, i.e. the finger overshoot the target by 70%. In DO the gain at 20 deg was 0.5 dB below DR and changes with decreasing amplitude were similar but less dramatic, further increasing the gap to DR. The peak at 0.3 Hz reached in DO only 2.5 dB at 5 deg amplitude, i.e. the finger overshoot the target by 30%.

Our results indicate that different reference sources clearly modify the control of fingertip position in space: if a retinal rather than oculomotor signal is available at low stimulus amplitudes and frequencies, fingertip motor program deviates most from target movement. As a next step, in an inverse set of experiments, dependence of oculomotor control on non-visual reference inputs of fingertip position will be studied.

22.16 CONTRIBUTION OF VISUAL FEEDBACK TO THE CONTROL OF DIRECTIONAL AIMING IN ADULTS. C. Bard, M. Fleury and J. Paillard. Physical Activity Sciences Laboratory, Laval University, Québec, G1K 7P4, Canada. CNRS Marseille, France.

The manipulation of movement speed in directional aiming tasks and of available visual information at specific moments during limb trajectory allows the identification of visual afference modalities and the determination of their role in visuo-motor correction. The aim of this study was to investigate directional aiming accuracy according to movement speed and visual feedback. Eight adults participated in the experiment. The subject, holding a joy stick, performed an horizontal arm extension in a sagittal plane toward luminous targets at 0^0, 10^0, 20^0 or 40^0 of eccentricity in the right visual field. Twenty trials (5 repetitions for each target) were administered. For each trial, RT and MT of the arm, angular errors in aiming, eye and head latencies were measured. The independent variables were: condition of feedback, with visual feedback, without visual feedback, with initial feedback and with terminal feedback, and movement speed. Results showed that aiming accuracy deteriorates independently of movements speed when movement is performed in an open-loop condition. For all feedback conditions, accuracy is better in slow aiming. For all feedback and movement speed conditions, aiming accuracy deteriorates with target eccentricity. Relative spatial error increases according to target eccentricity. Whatever feedback condition, or movement speed, ocular latency does not vary. Head latency (HL) significantly diminishes in fast aiming - $\overline{X}_F$ = 415 msec, $\overline{X}_S$ = 468 msec, but does not vary according to other experimental conditions. Hand reaction time (RT) significantly diminishes in fast aiming; it also varies according to target eccentricity. Two main conclusions can be drawn from these results. (1) With the present directional aiming task, whatever the movement speed, performance deteriorates when subjects work in an open-loop condition, compared to other feedback conditions. This confirms that even in fast aiming (mean duration $\simeq$ 110 msec), a certain type of eye-hand correction could happen, supporting the hypothesis that certain corrections could originate from peripheral vision. (2) In a closed-loop condition, aiming performance is more precise in slow than fast aiming. The "Symmetric Impulse-Variability Model" (Meyer et al., 1982) could provide an explanation for this result, since movements with a stronger initial impulse were faster and less precise than the ones with a weaker impulse. It is worth nothing that target focussing by the eyes, in terms of ocular latency, is identical in all experimental conditions (feedback and speed), which supports the hypothesis that the motor system is more susceptible of imprecision, than the information processing system, when speed and therefore strength applied to the system are increased.

EXCITABLE MEMBRANES AND SYNAPTIC TRANSMISSION: INVERTEBRATE STUDIES

23.1 ON THE MECHANISM OF PRESYNAPTIC INHIBITION. R.M. Glantz, L.T. Wang* and B. Waldrop Biology Dept., Rice Univ., Houston, TX 77251

A previous report (Glantz, Neurosci. Abstr. 7:251, 1981) describes the features of presynaptic inhibition at the excitatory synapses between ascending and descending interneurons in the crayfish brain. Briefly, the EPSP in descending interneurons is diminished by 30-60% as a consequence of concurrent stimulation of cephalic sensory nerve roots. This stimulation elicits polysynaptic, depolarizing IPSPs in ascending interneuron terminals. The IPSP amplitude is 3-5 mV and is associated with a 30% increase in the terminal input conductance and a reversal potential of 12 mV above rest.

Here we report on the geometry, passive, and active characteristics of the ascending cell terminals. The results imply that the passive shunt hypothesis of presynaptic inhibition is probably incorrect.

The terminal region is actively invaded by the axonal spike. This conclusion follows from a comparison of action potentials in axons and terminal arborization of ascending cells, summarized below:

	Ampl. (mV)	Overshoot (mV)	Threshold current (nA)	Sensitivity to applied current (mV/nA)	Half width (ms)
Axon n=9	97.8±7.8	24.9±8.0	7.1±3.8	2.1±1.0	0.9±.2
Term. n=4	97.5±12.9	22.7±8.0	4.3±2.5	1.8±0.2	1.8±.4

The passive cable properties of the terminal region have been calculated on the basis of measurements of input resistance, membrane time constant, and physical dimensions derived from Lucifer Yellow injections. The varicosities (presumed sites of transmitter release) of the terminals are 0.1 to 0.3 λ from the active recording sites. If the intervening neurite is electrically passive then the decremented time integral of the impulse, calculated for the varicosity, will be about 96% of the active spike value.

IPSPs in the terminal increase the terminal input conductance by 30% but decrease the spike amplitude by only 5%. The spike half-width is not affected. If an electrically inactive varicosity is subjected to an IPSP the calculated spike time integral will diminish by about 5%. For transient signals (150 Hz) the length constant (λ_f) is 0.71 times the d.c. length constant. The IPSP conductance increase diminishes λ_f by about 25%. The effect on the peak spike amplitude will still be small however because the electrotonic distance to the varicosity is short and the input impedance in the terminal arbor is greater than that of the principal neurite. We conclude that the passive shunt hypothesis of presynaptic inhibition is probably not sufficient to account for the observed attenuation of EPSPs associated with presynaptic IPSPs.

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23.3 A COMPARISON OF THE EFFECT OF TEMPERATURE ON CELL R15 PACEMAKER ACTIVITY IN THE IN SITU AND EXCISED ABDOMINAL GANGLION OF APLYSIA. S.N. Treistman and G.M. Bablanian. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The bursting pacemaker activity (BPA) seen in cell R15 of Aplysia is endogenous to the cell, and biophysical studies have identified some of the ionic currents which are important to its generation. The BPA recorded from R15 in the excised abdominal ganglion is lost when the cell is cooled below 12°-14°. Since R15 appears to play an important role in osmoregulation, radical changes in the output of this cell as a function of environmental temperature would be expected to have significant effects upon the animal's ability to osmoregulate. The present study was undertaken to determine whether in the intact animal, R15 exhibits the same temperature sensitivity as in the excised ganglion, or whether the effect of temperature upon R15's biophysical properties are moderated by either synaptic inputs or factors in the hemolymph. The abdominal ganglion containing cell R15 was exposed by a small incision in the dorsal body wall, pinned to a platform, and impaled. The sides of the incision were pulled above the level of surrounding sea water preventing loss of hemolymph. A thermistor placed adjacent to the ganglion recorded hemolymph temperature, while a thermometer recorded changes in sea water bath temperature. In order to compare the temperature sensitivity of the cell in situ and in vitro, the sea water temperature surrounding the intact animal was first lowered, and the strength of BPA was monitored as a function of hemolymph temperature. The ganglion was then removed from the animal, cell R15 was reimpaled, and the temperature was changed to match that previously induced in the intact preparation. In no case did we find an increased resistance to loss of BPA when the intact animal was compared with the isolated ganglion. In fact, when differences between the in situ and in vitro cell were encountered, they showed that suppression of BPA occurred at even warmer temperatures in the in situ cell. These results led us to examine whether the ganglion might be protected from rapid cooling when the animal is exposed to cooled sea water. Such a mechanism does appear to exist. The hemolymph temperature adjacent to the abdominal ganglion was monitored while the sea water surrounding the animal was chilled. Cooling of the hemolymph proceeded along an exponential time course with a time constant of 128.2 minutes. However, when the circulatory system of the animal was disrupted by severing the aorta, reexposure of the animal to the same temperature conditions resulted in a significantly faster ($p<0.001$) cooling rate with a time constant of 50.0 minutes. Thus, although R15's thermosensitivity is present in the whole animal, it is buffered from transient temperature change by a previously undescribed mechanism.

23.4 THE EFFECT OF R15 HYPERPOLARIZATION ON HEMOLYMPH COMPOSITION IN INTACT APLYSIA. G.M. Bablanian* and S.N. Treistman (SPON: L. Kennedy). Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

R15, a bursting pacemaker cell in Aplysia, is thought to be a neurosecretory cell concerned with osmoregulation. We have used an intact preparation of Aplysia to correlate the activity in R15 with changes in the ion and metabolite concentration in the hemolymph, possibly related to its osmoregulatory role. The abdominal ganglion was exposed through an incision in the dorsal body wall, keeping synaptic inputs and circulation intact. In previous experiments, we have established that R15 shows bursting pacemaker activity (BPA) in the intact preparation, and that BPA is inhibited by a dilution of the sea water surrounding the animal. For the present studies, R15 was hyperpolarized for 30 min and hemolymph samples were taken at subsequent time points. The samples were analyzed for K^+, Na^+, ammonia, and amino acid composition. We found that K^+ rose from 11.7 ± 0.5 mM to 13.2 ± 0.3 mM in the 2.5 hours after inhibition of R15. This rise of 12.8 ± 1.7% was statistically significant, and was not found in control experiments in which R15 was impaled but not hyperpolarized or in which other cells were hyperpolarized. Na^+ values were unchanged by hyperpolarization of R15. Ammonia levels also increased 69.6 ± 16.8% from 0.14 ± 0.01 to 0.20 ± 0.03 mM. Again, control experiments did not produce this rise. Amino acid analysis revealed large changes in the amino acid composition of the hemolymph after inhibition of R15. Not all amino acids were affected. The largest changes were a 367% rise in taurine, a 211% rise in lysine, a 253% rise in glutamic acid, and a 252% rise in histidine. All values are obtained by comparison with controls. These results demonstrate wide-ranging physiological effects from a relatively short period of hyperpolarization in a single neurosecretory cell.

23.5 SODIUM PUMP INHIBITION AND DEPOLARIZATION IN HYPOXIC HYPERPOLARIZING L_2-L_6 NEURONS OF APLYSIA. Philip E. Coyer. Department of Neurology and the Neurosciences Program, University of Alabama in Birmingham, Birmingham, Alabama, 35294.

Members of an identifiable group of pacemaker neurons are known to hyperpolarize in response to hypoxia (Coyer et al., J. exp. Biol. 102, 1983). A tenable hypothesis which has been raised concerning this observation is that low oxygen conditions stimulate the sodium pump through alterations in the (ATP)/(ADP)(Pi) ratio and glycolytic feedback mechanisms. Presumably, continued production of ATP resulting from this mechanism augments the sodium pump and accounts for the increases in intracellular potassium ions associated with membrane hyperpolarization. To test this hypothesis, 10 experiments were conducted on hypoxic hyperpolarizing members of the L_2-L_6 neurons of the Aplysia abdominal ganglion. Measurements of membrane potential and intracellular potassium ion activities were made under control (normoxic), experimental (hypoxic), and return to control (reoxygenation) conditions to verify that hypoxia and normoxia results in reversible changes for these neurons. Subsequently, a pressure micro-ejection pipette filled with 5 mM sodium iodoacetate, a glycolytic inhibitor, was inserted into these 10 neurons. Membrane potential was also recorded from the ejection pipette to insure that the same neuron was impaled with both recording and pressure insertion microelectrodes. Pressure pulses of variable durations were delivered to the system thus expelling the sodium iodoacetate into these neurons. The sodium salt was chosen since increases in intracellular sodium are known to stimulate the pump and result in membrane hyperpolarization. Providing iodoacetate inhibited glycolytic feedback mechanisms as is known, the response of these neurons to hypoxia should have been just the opposite as that observed for normal cells. The irreversible depolarization of these same 10 cells, which were observed to hyperpolarize during hypoxia under normal conditions (no injection), substantiated that glycolytic feedback mechanisms were operative under normal conditions in which there was augmentation of the sodium pump. These mechanisms were inoperative under iodoacetate injected conditions in which the pump was not augmented. The results coupled with those obtained for hypoxic depolarizing neurons of the R_3-R_{13} group suggest that metabolic differences and the pump dependence of the membrane potential account for the differential selectivities of the nerve cells to hypoxia.

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23.6 AN ANTICALMODULIN DRUG, W-7, INHIBITS THE VOLTAGE-DEPENDENT CALCIUM CURRENT IN PARAMECIUM CAUDATUM. T.M. Hennessey and C. Kung*. Lab. of Molec. Biol. and Dept. of Genetics, Univ. of Wisc., Madison, Wisc. 53706.

W-7, N-(6-aminohexyl)-5-chloro-1-naphthalene sulfonamide, is a potent anticalmodulin drug. The ID_{50}, the concentration of drug which inhibits half of the calf-brain calmodulin-dependent phosphodiesterase activity, is 30 μM. Its C-5-dechlorinated analog, W-5, is much less effective (ID_{50} = 240 μM). These drugs are not specific for calmodulin, however (Schatzman et al., BBA 755:144-147, 1983).

The duration of backward swimming of Paramecium caudatum when transferred into a solution with 20 mM K^+ (a behavioral measurement of the Ca-channel activity) was reduced from 50 sec to 25 sec by the addition of 20 μM W-7 or 150 μM W-5. Backward swimming was completely blocked by 150 μM W-7 or 400 μM W-5 within 1 sec. These effects could also be reversed within 1 sec after washing the cells out of the drugs.

These drugs inhibit the voltage-dependent Ca^{2+} current in a concentration-dependent and reversible manner. This current is responsible for the Ca-action potentials and the backward swimming of Paramecium. The inward Ca^{2+} current, induced by step depolarizations controlled by a voltage clamp, was isolated after blocking the K^+ outward currents with internal Cs^+ and external TEA^+. The isolated Ca^{2+} current was reduced to half by 40 μM of W-7 or 150 μM of W-5. The voltage at which the maximal inward current is seen was also shifted by 10-15 mV less negative by 150 μM of W-7. Under the voltage-clamp conditions used (4 mM K^+, 1 mM Ca^{2+} bath, 2 M KCl electrodes) none of the other voltage-dependent currents (Kung and Saimi, Ann. Rev. Physiol. 44:519-534, 1982) were significantly affected, even at drug concentrations which suppressed over 90% of the Ca^{2+} current. The Ca^{2+}-dependent K^+ current was also decreased by these drugs, presumably due to the lowered internal Ca^{2+} concentration expected of the reduced Ca^{2+} current.

This system may be used to study the mechanism of action of W-7 on excitable membrane and to screen other potential Ca-channel blockers.

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23.7 CA DIFFUSION MEASURED DIRECTLY IN APLYSIA NERVE CELL BODIES. D. Tillotson* and E. Nasi* (SPON: P. Nasi). Dept. of Physiology, Boston Univ. Sch. Med., Boston, MA 02118.

The properties of intracellular Ca^{++} regulation allow Ca^{++} to function so effectively in the triggering of a variety of physiological mechanisms. In general, the resting internal Ca^{++} is held to extremely low levels ($<10^{-7}$ M) offering the possibility of a wide dynamic range for transient changes in internal Ca^{++} with cell activation. We have studied the mechanism(s) that handle the Ca^{++} transients which occur with membrane Ca^{++} channel activation in nerve cell bodies. After Ca^{++} crosses the cell membrane it is not freely diffusible. The Ca^{++} buffering machinery provides, as a function of its Ca^{++} binding, a resistance to free diffusion. The extent to which Ca^{++} mobility within the cytoplasm is slowed by this resistance is directly related to the strength of the buffer. We have directly measured the diffusion rate of iontophoretically injected Ca^{++} using the Ca^{++} sensitive dye Arsenazo III. This was accomplished by severely restricting the cytoplasmic region sampled by the AIII light probe system (accepting probe diameter 30u). A standard Ca^{++} injection (400na, 100msec) was delivered and the absorbance change produced within a discrete region was measured. The measuring system, and hence the measuring region, was then moved in 10u steps with respect to the injecting electrode tip and the injection was repeated. With the Ca injector tip positioned near the cell membrane, the diffusion coefficient was measured to be in the range of 40 - 170 u^2/sec, which is above the value estimated for squid axon.

23.8 LIQUID SCINTILLATION STUDIES OF CALCIUM MOVEMENTS IN MYOEPITHELIAL CELLS. J.E. Chaffee* and M. Anderson. Department of Biological Sciences, Smith College, Northampton, MA 01063.

The myoepithelial cells that make up the proventriculus of the marine polychaete worm Syllis spongiphila generate calcium action potentials which are associated with contractions. Each cell has a central, noncontractile core which contains membrane-bound vesicles of crystalline material; the predominant elements within the vesicles are Ca and P. Experiments using ^{45}Ca were performed to investigate the uptake of Ca by the whole cells and by the vesicles. Proventriculi were exposed to artificial sea water (ASW) containing a concentration of ^{45}Ca of about 20 µCi in 200 µL, rinsed 30 m in Ca-free ASW containing 10 mM La, and solubilized in a 10% deoxycholate solution. Samples were counted in a Beckman LS 7500, and protein content was determined by Lowry analysis of separate samples of the solubilized tissue. Proventriculi equilibrated ≥60 m after dissection and then exposed to ^{45}Ca for periods ranging from 5 to 60 m reached a saturation level after 15 m of about 1.7 x 10^{-10} mM Ca per µg protein (n=17). Ca uptake of control (exposed to ^{45}Ca for 15 m n=6) proventriculi was compared to that of proventriculi stimulated pharmacologically by 5 x 10^{-4} M carbamylcholine chloride (Carb, n=10) during the 15 m exposure to ^{45}Ca and to that of one proventriculus stimulated electrically to elicit action potentials (4/m) during the 15 m exposure to ^{45}Ca. The Ca influx of the Carb-stimulated proventriculi was about double, and that of the electrically stimulated proventriculus 5 times, that of the controls. D-600 (1 mM) applied alone did not affect resting Ca influx (n=2) but blocked greater than resting Ca influx when applied with Carb (n=2). Ca uptake of the vesicles was compared to that of whole cells by exposing proventriculi to ^{45}Ca under various conditions and filtering the solubilized tissue through millipore filters (0.2 µm pore size). Scanning electron micrographs of the filtered material showed spheroids similar in size (0.5 - 1.0 µm) to those seen in transmission electron micrographs, suggesting that the filtered material consisted of vesicles. Vesicular uptake was variable with Carb stimulation: ^{45}Ca in the vesicles of control proventriculi was 5.7 ± 1.7% that of the whole cells (n=5) while that of Carb-stimulated proventriculi was 11.3 ± 6.7% (n=8). Vesicular uptake of proventriculi stimulated electrically (n=6) did not differ from controls except in one preparation (13.5%) allowed to rest 60 m after stimulation prior to solubilization. This result suggests that time is required for labelled Ca to enter the vesicles. (Supported by NIH Grant NS12196 to M. Anderson).

23.9 LOCALIZATION OF A K^+-DEPENDENT INHIBITORY SYNAPSE IN LEECH CNS J. Yang and A.L. Kleinhaus. Dept. Neurology, Yale U. Sch. of Med., New Haven CT. 06510.

In leech segmental ganglia (Macrobdella), stimulation of the neurons sensitive to pressure (P-cells) evoked K^+-dependent inhibitory synaptic potentials on cells of unknown function (Nut cells) (Kleinhaus & Brand, Comp.Biochem.Physiol. 70A, 1981). Recently, Carnevale and Johnston (J. Neurophysiol. 47, 1982) described a method for characterizing remote synapses by recording at the soma. The method, based on an analysis of a generalized 2-port electrical network is independent of any specific geometrical assumptions and characterizes the synapse by two steady-state coupling coefficients K_{12} (soma-to-synapse) and K_{21} (synapse-to-soma). The coefficients are estimated experimentally from 1) post-synaptic potential (V_{psp}) vs post-synaptic cell membrane potential (V_m) and 2) apparent reversal potential recorded at the soma (V_{rev}) vs -RT/F ln {K}. Given K_{12} and K_{21}, the normalized electrical distance of the synaptic location can be obtained for any specific electrical model of the post-synaptic cell. We have applied this method, in conjunction with the transient analysis of lumped-soma short cable model parameter estimation (Jack & Redman, J.Physiol. 215,1971), to determine the location of P-Nut inhibitory synapse. Specific branching patterns and homogeneous membrane were assumed to allow the representation of the Nut cell as a lumped-soma short cable model.

From 29 V_{rev} determinations made at seven K^+ concentrations (2 - 25 mM), a value of K_{12}=.80±.06 was obtained. A lower bound of K_{21}=.104±.062 was estimated from 4 measurements of V_{psp} vs V_m in normal (4mM) K+.

The electrotonic response of Nut cells to a brief hyperpolarizing current injection showed a rapid initial phase (t<30ms) followed by a linear decay phase (40ms<t<70ms) on a log V vs t plot. Log $\sqrt{t}$V plot showed an early decay with time constant similar to that determined from the log V analysis and a later phase with slower decay. These results are consistent with the response of a lumped-soma short cable model with an open-circuit termination. Transient analysis done on 6 Nut cells gave electrical model parameters of (mean±SEM): membrane time constant=80.7±5.8 ms, D.C. cable-to-soma conductance ratio=10.8±5.0, and normalized length of the equivalent cable=.73±.11.

Based on these calculations the inhibitory synapse was localized at 0.52 length constant away from the soma. The relatively large value of K_{12} and smaller K_{21} as well as the inconsistency in the synaptic location calculated from these two steady-state coupling coefficients suggest that the synaptic contact occurs on a fine branch of the axonal arborization or on a specialized synaptic spine.

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23.10 FINE STRUCTURE OF SYNAPSES AND SYNAPTOSOMES FROM SQUID (LOLIGO PEALII) OPTIC LOBE. G.D. Pappas, N. Haghighat* and R.S. Cohen*. Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL. 60612.

Cephalopod optic lobes are a well-known source of cholinergic endings (Dowdall and Whittaker, J. Neurochem. 20:921, 1973). In order to utilize this property for subsequent analyses of cholinergic mechanisms of synaptic transmission in the CNS, we describe the ultrastructure of the entire optic lobe of the squid (Loligo pealii) and relate the morphology of synaptosomes derived from the optic lobe to endings in the intact tissue. Previously, Cohen (J. Comp. Neurol. 147:399, 1973) presented a description of the fine structure of the outer nuclear and plexiform layer (photoreceptor region) of the squid optic lobe; however, we have included, in addition to these regions, a morphological study of all other layers, including the medulla, indicating the different structural features of the synaptic junctions themselves and classifying these terminals into distinct morphological types. In the cortex, both chemical and electrical junctions were found, the former being the main kind, showing two basic forms. The first was an invaginated synapse, appearing only between presynaptic bags and spines which may originate from the trunks of amacrine cells of the outer granular layer. The width of the postsynaptic thickening was less than that seen in typical mammalian synapses. A clustering of vesicles is seen in the presynaptic process, particularly around the junction, in distinction to those more evenly distributed vesicles seen in the rest of the terminal. The second obvious basic form of junction was that of a typical chemical synapse, found in almost all layers except the upper portion of the first radial layer. Here, too, paramembranous densities were thinner than those found in typical mammalian synapses, even when stained with E-PTA to enhance their contrast. Synapses in the medulla were predominantly of the second type, although a few photorecepter endings extended to this region as well. The different types of terminals observed in the intact squid optic lobe corresponded to the different types of endings recognized in a synaptosome fraction derived from these lobes. Because of its high content of cholinergic endings and distinct synaptic types, the squid optic lobe may provide an interesting and viable model for the purification of cholinergic synaptosomes and synaptosomal plasma membranes for the elucidation of cholinergic mechanisms of transmission in the central nervous system. Supported by NIH grants NS 15889 and NS 16610 and NSF.

23.11 SYNAPTIC ORGANIZATION IN THE CARDIAC GANGLION OF THE AMERICAN LOBSTER. P. M. Morganelli* and R. G. Sherman. Dept. of Zool., Miami Univ., Oxford, OH 45056.

The nerve terminals in the cardiac ganglion of Homarus americanus are situated in regions of neuropil which are invested by glia. Each neuropil region contains several terminals representing from one to three different types. Seven different types of nerve terminal were distinguishable by transmission electron microscopy. Four form chemical synapses (Types 1, 2, 5 and 6) and three represent neurosecretory neurons (Types 3, 4 and 7). Type 1 is the most frequently observed and occurs in both pacemaker and follower cell regions. These terminals are packed with spherical, clear-cord vesicles (CCV) of 40-50 nm diameter, with a few dense-cored vesicles (DCV) of 70-130 nm interspersed. Type 5 terminals are a special case of Type 1 which form symmetrical synapses with Type 1 terminals. Type 2 terminals occur in the follower cell region and contain flattened CCV (17-27 X 80-160 nm) with a few DCV (100 nm) interspersed. Type 6 terminals occur near to pacemaker somata, contain mostly spherical CCV (40-80 nm), but also some DCV (100 nm), and have a granular cytoplasm. Based on vesicle morphology, Types 1, 5 and 6 are presumed to form excitatory synapses, while Type 2 is presumed to be inhibitory.

The three types of neurosecretory terminals show no release site morphology. Type 3 terminals occur in both follower and pacemaker regions, and contain a large number of both spherical CCV (30-40 nm) and DCV (70-120 nm). Type 4 terminals occur in the pacemaker region and contain primarily DCV, but also some CCV. Type 7 also has been seen only in the pacemaker region, and some of these terminals lie close to pacemaker somata. They possess a pleomorphic population of CCV (40-90 nm) along with many DCV (100 nm). The axoplasm of Types 4 and 7 is granular.

Axoglial synapses have been observed in a few instances, involving nerve terminals that contain mostly spherical CCV (30-40 nm) and a few DCV (100 nm). Electrotonic junctions were seen in the follower cell region. The nerve terminals involved were spherical processes of 4-5 um diameter which were situated in close apposition to one another. In these cases, regions were apparent where fusion of adjacent plasma membranes had taken place.

23.12 EXPOSURE AND ISOLATION OF FUNCTIONAL, SYNAPTICALLY CONNECTED NEURONS OF THE MOTOR NERVE NET OF A JELLYFISH. P. A. V. Anderson. C. V. Whitney Laboratory and Department of Physiology, University of Florida, St. Augustine, FL 32084.

The motor nerve net of the jellyfish Cyanea capillata is a two dimensional network of neurons that innervates the swimming musculature. The neurons are bipolar and are connected by chemical synapses. The cable properties of the neurons and the arrangement of the synapses are such that a microelectrode in the soma of a cell will record all that occurs in the synaptic terminal. Because the synapses are structurally symmetrical, and apparently functionally so, each terminal alternately serves a pre- and post-synaptic function. Since two synaptically linked cells can be impaled simultaneously, this preparation affords the opportunity for intracellular recordings from pre- and post-synaptic terminals.

The neurons lie between the cell bodies and muscle tails of the myoepithelial cells that form the bulk of the tissue. The myoepithelial cells are connected by septate desmosomes and, consequently, constitute a diffusion barrier and limit access to the neurons. A technique has been developed whereby these epithelial cells can be removed, with minimal damage to the neurons. The neurons remain in place and attach to the underlying, transparent mesoglea. The result is a preparation that very much resembles a nerve tissue culture preparation. Individual neurons retain their normal appearance and form connections with one another. The number of nerve cells remaining in place depends on the severity of the stripping procedure and, by carefully controlling the variables, the result can be an almost intact nerve net, widely separated single cells or synaptically connected, paired cells. In the saline prepared following an analysis of the free ion content of the mesoglea, the cells retain their normal resting potentials and produce normal action potentials. Furthermore, the synapses continue to function quite normally and in preparation that contain large numbers of neurons activity will spread over wide areas and activate any muscle remaining at the edges of the preparation. By supplementing the saline, these preparations can be maintained in good condition for several days. Under these culture conditions, neurons damaged during isolation produce growth cones and appear to reform connections with other neurons. Because the cells remain in contact with a native extracellular matrix, the mesoglea, the preparation can almost be considered as an in vivo tissue culture preparation.

The advantages offered by this preparation to the study of the synapses are numerous. Most important is the unrestricted access, but because the cells and their synapses remain functional, it may be possible to apply the patch clamp/whole cell voltage clamp technique and quantify the currents involved in synaptic transmission. (Supported by NSF Grant BNS 82-0949)

TRANSMITTERS IN INVERTEBRATES

24.1 ISOELECTRIC FOCUSING AND IMMUNOBLOT STAINING of DROSOPHILA CHOLINE ACETYLTRANSFERASE. P. M. Salvaterra, G. D. Crawford* and L. Correa*. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

We have recently shown purified Drosophila melanogaster choline acetyltransferase (ChAT, E.C. 2.3.1.6) consists of three major structurally related polypeptides with molecular weights of 67K, 54K and 13K daltons after SDS gel electrophoresis (Slemmon et al., in press). In contrast to this complex polypeptide composition, gel filtration or sucrose gradient centrifugation of native enzyme shows only a single symmetrical activity peak at 67 K daltons. From these results, it is likely that the enzyme activity exists in two forms: a single polypeptide with a molecular weight of 67K daltons and a non-covalent complex of the 54K and 13K dalton polypeptides. Consistent with this structural model is the observation of two enzyme activity forms noted after isoelectric focusing of homogenates or partially purified ChAT from wild type (Oregon R or Canton S) on thin layer polyacrylamide gels. Also supporting this structural model is the observation that monoclonal anti-Drosophila ChAT antibodies stain both the 67K and 54K dalton polypeptides in an immunoblotting procedure. These antibodies are directly inhibiting and probably bind determinants at or near the active site of the enzyme (Crawford, G.D. et al., J. Biol. Chem., 257:3853, 1982). We have also examined the isoelectric focusing patterns of two temperature-sensitive presumed structural gene mutants of ChAT described by Greenspan and Hall (J. Comp. Physiol., 137:83, 1980). The Cha ts^1 mutant has only one pI form coincident with the higher pI form of wild type, while Cha ts^2 has two pI forms, both slightly different from wild type. Monoclonal anti-ChAT antibodies react with the Ch ts mutant forms to directly inhibit their activity in a manner similar to reaction with the wild type form. This suggests that the determinant(s) are preserved on the mutant forms. Interestingly, immunoblots of partially purified Cha ts^1 show antibody staining of only one polypeptide at 67K daltons. Supported by NS18858.

24.2 CHOLINERGIC RECEPTORS IN THE APLYSIA GILL. S. Weiss*, J.I. Goldberg*, J.P. Edstrom* and K. Lukowiak* (SPON: B. MacVicar). Departments of Chemistry and Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Several lines of evidence suggest that acetylcholine (ACh) is a putative neurotransmitter in the Aplysia gill involved in the regulation of contractile behavior. Although receptor mechanisms have been characterized in other Aplysia tissues, the cholinergic pharmacology of the Aplysia gill has not been detailed. The perfused gill, isolated from the abdominal ganglion (CNS), exhibits periods of spontaneous phasic movements which are mimicked by infusing the neuropeptide FMRFamide (EC_{50}, 0.1 μM), dopamine (DA) (EC_{50}, 1 μM) and membrane-permeable analogues of cyclic AMP through the gill. The effects of ACh and several cholinergic agonists and antagonists on quiescent, spontaneously-active and pharmacologically-activated gills were examined. At concentrations greater than 1 μM, ACh rapidly elicited a slowly developing tonic contraction of the afferent vein that reversed immediately upon washout. This effect was observed on both quiescent and active preparations. At concentrations less than 1 μM, ACh perfusion resulted in a reduction of gill tone. The excitatory effect of ACh (10 μM) was reduced 70% and 60% by the muscarinic antagonists atropine (10 μM) and hexamethonium (10 μM), respectively. The ACh-evoked contraction was potentiated 2.5-fold when curare (10 μM) was co-infused.

Perfusion of carbachol (10 μM) did not mimic the excitatory effects of ACh. In fact, at all concentrations examined (1-100 μM), carbachol infusion reduced baseline tension, the amplitude of spontaneous contractions and those evoked by FMRFamide and DA. Contractile movements elicited by perfusion of p-chlorophenylthio-cyclic AMP were dramatically reduced when carbachol (10 μM) was added to the perfusate. Further addition of curare (10 μM) reversibly blocked carbachol inhibition of the cyclic AMP-evoked contractions.

These findings suggest that excitatory (muscarinic) and inhibitory (nicotinic) cholinergic receptors are involved in the regulation of gill contractile behavior by ACh.

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24.3 DOPAMINE REDUCES ARSENAZO III ABSORBANCE CHANGE DURING DEPOLARIZATION OF BURSTING NEURON. D. V. Lewis, W. A. Wilson, Lab. of Neurophysiology, Depts. of Pediatrics and Pharmacology, Duke University, Durham, N.C. 27710.

The bursting neuron R15 of Aplysia californica exhibits a voltage sensitive calcium conductance. Using the calcium indicator, Arsenazo III, increases in cytoplasmic calcium have been observed during voltage clamp depolarizations activating the persistent slow inward current (SIC). Dopamine applied to R15 blocks the bursting, eliminates the negative slope resistance region of the current voltage curve and hyperpolarizes the cell. One explanation for the effect of dopamine would be blockade of the SIC. If the SIC were mediated by calcium and if dopamine blocks the SIC, then dopamine should block voltage dependent calcium influx. We have monitored calcium influx into R15 by injecting Arsenazo III intracellularly and measuring absorbance changes of the cell using two fiber optic probes to deliver light to and collect light from the cell. When absorbance changes were very small, signal averaging was used to enhance the signal to noise ratio. The absorbance changes were measured only in the soma in early experiments by placing the fiber optics on either side of the exposed soma. Increased Arsenazo III absorbance (indicating increased cytoplasmic calcium activity) occurred during the SIC activated by 10 sec voltage clamp steps from -50mV to between -35 and -25mV. Bath applied dopamine, which clearly eliminated negative slope resistance, did not reduce the Arsenazo absorbance changes. In another series of experiments, the fiber optics were placed immediately below the soma, transilluminating the pedicle of neuropil containing the proximal axon of R15. The axon can be seen to fill with dye when the soma is injected with Arsenazo III. Absorbance increases can be detected in the axon during the small depolarizations presumably due to calcium influx into the axon. Dopamine reduces these absorbance rises in contrast to its lack of effect on somatic calcium influx. To demonstrate that voltage control of the axon was not altered by dopamine application, a second microelectrode was used to monitor voltage changes in the axon. We hypothesize that dopamine reduces voltage sensitive calcium influx into the proximal axon and that this effect may contribute to the loss of negative slope resistance seen with dopamine. An effect of dopamine on axonal but not somatic calcium conductance is consistent with local iontophoretic application of dopamine being effective on the axon but not on the soma (Ascher, P., J. Physiol. 255:173, 1972).

24.4 DOPAMINE AND SEROTONIN IN LIMAX FEEDING: DISTRIBUTION AND METABOLISM. S. J. Wieland, H. Zaininger*, E. G. Jahn*, and A. Gelperin. Department of Biology, Princeton University, Princeton, New Jersey 08544 and Bell Laboratories, Murray Hill, New Jersey 07974.

Both dopamine and serotonin produce profound effects on neural elements of the feeding control system in Limax maximus. To characterize the roles of neurons which contain these monoamines, we mapped the location and projection of aminergic neurons in the cerebral and buccal ganglia, endogenous levels of precursors, the amines, and their possible metabolites.

Histochemistry for catecholamines and serotonin revealed fluorescent amine products in all three lip nerves of the cerebral ganglion and the cerebral-buccal connective. Simultaneous retrograde labeling with the red fluorescent dye propidium iodide showed lip-projecting dopaminergic neurons clustered next to non-amine containing neurons projecting out the same nerve. The buccal ganglia contained several small amine fluorescing neurons and the buccal roots all contained several fluorescent fibers.

Quantitation by HPLC-EC of endogenous dopamine and serotonin showed 2.4 (± 1.09 SEM, n=6) pmols dopamine and 2.18 (± 0.23 SEM, n=6) pmols serotonin in the pair of cerebral buccal connectives, compared to 133 (± 3.6 SEM, n=29) pmol dopamine and 91 (± 3.2 SEM, n=29) pmol serotonin in the cerebral and 32 (± 5.8 SEM, n=9) pmol dopamine and 14 (± 2.5 SEM, n=9) pmol serotonin in the buccal ganglia. The subesophageal ganglia contain 303 (± 13.1 SEM, n=19) pmol dopamine and 191 (± 9.9 SEM, n=19) pmol serotonin. Accumulation of ^{3}H-dopamine and ^{3}H-serotonin during incubation with ^{3}H-tyrosine and ^{3}H-tryptophan indicates both monoamines were actively synthesized from their respective amino acids. Incubation with α-methyl-p-tyrosine selectively blocked ^{3}H-dopamine accumulation without interfering with ^{3}H-serotonin accumulation, and conversely, p-Cl-phenylalamine selectively blocked ^{3}H-serotonin synthesis. These results suggest dopamine and serotonin are synthesized through the same pathways as in the vertebrates. While α-methyl-p-tyrosine blocked ^{3}H-dopamine accumulation over a 24 hour period, total endogenous levels of dopamine did not decline over the same period in the presence of this blocker, even with the addition of benztropine, which blocks dopamine re-uptake in the vertebrate CNS. This suggests that either re-uptake by Limax is not blocked by benztropine, or that multiple pools of dopamine may exist with very different turnover rates.

Results of neurophysiological experiments testing the effects of dopamine synthesis and re-uptake blockers on the neural control system for feeding will also be discussed. Supported in part by NSF Grant BNS 8005822.

24.5 STUDIES OF CATECHOLAMINE NEURONS IN APLYSIA: LIGHT AND ELECTRON MICROSCOPY. Ronald S. Goldstein*, (SPON: M. Klein) Center for Neurobiology & Behavior, College of Physicians & Surgeons of Columbia University and The New York State Psychiatric Institute, New York, N.Y. 10032.

The cell biology and ultrastructure of serotonergic, cholinergic, histaminergic and peptidergic neurons have been studied in Aplysia. Formaldehyde and glyoxylic acid-induced fluorescence have shown that catecholamine neurons exist in Aplysia, but their small size and apparent rarity have prevented their identification for further study.

The FaGlu catecholamine histofluorescence method of Furness and colleagues (Histochemistry 57:285) was modified for use in Aplysia tissue. Wholemounts and sections of juvenile and adult ganglia and peripheral tissues were examined. In serially sectioned cerebral ganglia, 70-80 positive neurons were observed, many in clusters of 10-20 cells. This is 10 times as many cells as were found using glyoxylic acid, because of improved preservation of structure with the FaGlu method. It should now be easy to find these clusters in live ganglia and, in conjunction with intracellular marking, this technique should facilitate the future identification of catecholamine neurons in Aplysia.

Catecholaminergic processes were densely packed in localized portions of the neuropil of all central ganglia. Many of the axons were smooth, some were seen to bear fine varicosities. In contrast, serotonin has been shown by immunocytochemical studies in this laboratory (Neuroscience, in press) to be both widespread throughout the neuropil and to form an extensive plexus of fibers and varicosities in the cell body layer of the ganglia.

The FaGlu technique, which produces water-insoluble fluorophores, also allowed ultrastructural study of catecholaminergic neurons in Aplysia. In the electron microscope, catecholaminergic neurons were seen to contain large numbers of 70-80 nm dense cored vesicles, in contrast to the vesicles found in serotonergic neurons receiving the same treatment. Since the FaGlu technique can be combined with radioautography, studies of the specificity of transmitter metabolism and association of transmitter with subcellular organelles that have been performed on serotonergic and histaminergic neurons in Aplysia can now be extended to catecholaminergic neurons as well.

24.6 SEROTONIN-, PROCTOLIN-, AND BOMBESIN-LIKE IMMUNOREACTIVITY: HISTOCHEMICAL LOCALIZATION IN LIMULUS NERVOUS SYSTEM. G.A. Wyse, Dept. Neurobiol., Harvard Med. School, and Dept. Zoology, U. of Massachusetts, Amherst, MA 01003.

Neurons immunoreactive to serotonin and to the peptides proctolin and bombesin were localized by fluorescence microscopy of 20 μ cryostat-sectioned Limulus tissues. Methods followed Beltz and Kravitz (J. Neurosci. 3:585-602, 1983). Serotonin-like immunoreactivity (SLI) occurs in a consistent pattern in the CNS. In the brain, somata with SLI are associated with the central body and with protocerebral neuropil (Chamberlain et al., Neurosci. Abstr., this volume). Clusters of somata with SLI are associated with each of the fused prosomal ganglia forming the circumesophageal ring: five pairs of pedal (thoracic) ganglia and paired chilarial and opercular ganglia. Serotonin-immunoreactive fibers enmesh the neuropil of each of these regions and of the optic medulla and optic lamina. Serotonin immunoreactive cells and fibers also occur in each of the abdominal (opisthosomal) ganglia. Peripheral nerves, heart, cardiac ganglion, and gut appear to lack SLI.

Proctolin. Somata exhibiting proctolin-like immunoreactivity (PLI) occur in the central body of the brain, in chilarial and opercular ganglia at the posterior end of the prosomal circumesophageal ring, and in abdominal ganglia. Fibers with PLI ramify sparsely within neuropil of protocerebral, pedal, chilarial, and abdominal ganglia. The corpora pedunculata and optic lobes appear to lack PLI. Peripherally, fibers with PLI occur in the cardiac ganglion and in muscle layers of the gut, areas in wich proctolin enhances muscle contraction.

Bombesin. Bombesin-like immunoreactivity (BLI) appears confined to a few (2-4) neurons with somata in the central body of the brain. Fibers with BLI are rare within the CNS itself, but an extensive meshwork of processes with BLI surrounds the circumesophageal ring of fused prosomal ganglia. These processes appear to end in hemolymph space under the sheath. No BLI appears in abdominal ganglia, heart, cardiac ganglion, or gut.

These results demonstrate that specific Limulus neurons have serotonin-like, proctolin-like, and bombesin-like immunoreactivities. The anatomical distributions of these neurons suggest that serotonin and proctolin (or a proctolin-like peptide) act as transmitters or local modulators in the nervous system; that proctolin may act locally at the heart and gut; and that bombesin (or a bombesin-like peptide) is released into hemolymph from superficial endings and acts as a neurohormone.

Supported by NIH grants to E.A. Kravitz.

24.7 LOCALIZATION OF SEROTONIN-LIKE IMMUNOREACTIVITY IN THE LIMULUS PROTOCEREBRUM. S. C. Chamberlain, B.-A. Battelle and G. A. Wyse. Syracuse Univ., Syracuse, NY 13210; NEI, NIH Bldg. 6, Rm 224, Bethesda, MD 20205; and Univ. Mass., Amherst, MA 01003.

Neurons with serotonin-like immunoreactivity (SLI) were localized using a primary serum anti-serotonin-bovine serum albumin conjugate antibody from rabbit, a fluoroscein-conjugated antirabbit IgG secondary antibody, and fluorescence microscopy. Both cryostat and vibratome sections of 4% paraformaldehyde fixed tissue were used. Preabsorption of the primary antibody with 500 µg/ml synthetic serotonin creatinine sulfate did not completely block staining, however, remnant staining was rare, and in repeated control experiments formed an apparently random subgroup of the stained population.

The neuropils of the visual lamina, medulla, and ocellar ganglion all contain small-diameter beaded fibers with SLI; however no immunoreactive somata were observed in the adjacent ganglion cell layers. The neuropil of the central body contains a sparse network of fibers with SLI which arise from a band of large-diameter fibers along its medial edge. The somata of these neurons lie within the curve of the central body. The central neuropil of the protocerebrum is richly invested with fibers with SLI, some of which arise from somata in the protocerebrum and some of which may come from other parts of the circumesophageal ring. No fibers with SLI were observed in any of the optic nerves.

Somata with SLI are found in four distinct places in the protocerebrum: i) Within the curve of the central body (20-50 somata); ii) A small lateral cluster of cells between the corpora pedunculata and the central neuropil (4-9 somata); iii) A posterior midline ganglion (7-12 somata); and iv) a small ventral cluster of cells within the corpora pedunculata (3-4 somata). This last group of neurons gives rise to a small number of beaded fibers which innervate a glial strand in the center of the ipsilateral corpora hemisphere.

Supported in part by NEI grants.

24.8 EVIDENCE FOR PROCTOLIN-LIKE SUBSTANCES IN THE CENTRAL NERVOUS SYSTEM OF THE LEECH. C. Li* and R. L. Calabrese. The Biological Laboratories, Harvard University, Cambridge, MA 02138.

The neuropeptide proctolin has been described in many arthropods. With immunohistochemistry on whole mount preparations, we have found cells with proctolin-like immunoreactivity (PLI) in the ganglia of the leech (Hirudo medicinalis). Three different anti-proctolin antisera were used; a similar staining pattern was seen with all of the antisera. Two to four bilaterally symmetric pairs of cells with PLI appeared in the anterior packets of segmental ganglia 1-20; an unpaired medially located cell with PLI appeared only in ganglia 5-7. Two fixatives (4% paraformaldehyde and Bouin's fixative) and two antibody labelling techniques (indirect immunofluorescence and peroxidase-antiperoxidase procedure of Sternberger) were tried; the same results were always obtained. Staining could be eliminated by addition of synthetic proctolin to antisera. This work provides strong evidence that proctolin or proctolin-like substances exist in these cells.

Homogenates of leech ganglia showed proctolin-like activity as measured by the bioassay of O'Shea and Adams (Science 213: 567, 1981). In this assay, proctolin causes an increase in the frequency of myogenic contractions of the locust leg extensor muscle. We estimate by this method that there are approximately 0.27 femtomoles of proctolin or proctolin-like substances per midbody ganglion. These assay results corroborate our work with the antisera and further indicate the presence of proctolin or proctolin-like substances in these cells. Physiological experiments are planned to study the possible function of these cells.

We wish to thank the Taghert/Goodman, Schwartz/Siwicki/Kravitz, and Bishop/O'Shea groups for generously donating their anti-proctolin antisera. The bioassays were kindly done by O'Shea and colleagues.

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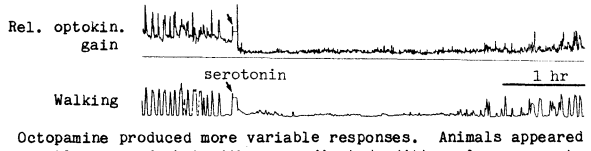
24.9 SEROTONIN AND OCTOPAMINE AFFECT WALKING AND OPTOKINETIC RESPONSES IN CRAYFISH. Stacey J. Arnesen* and Richard F. Olivo. Dept. Biological Sci., Smith College, Northampton MA 01063.

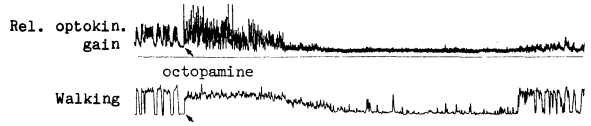
Recent work shows that serotonin and octopamine modulate the crayfish's posture and escape response, and that octopamine inhibits swimmeret beating. We investigated the effects of these amines on two responses that are normally enhanced by spontaneous arousal: locomotion and the optokinetic response.

Crayfish were suspended above a floating foam rubber ball on which they could walk freely, and were surrounded by a striped drum that oscillated sinusoidally to elicit continuous eye movements. Leg and claw movements were detected by a video camera and column-scan digitizer (Olivo & Thompson 1982, Soc. Neurosci. Abstr. 8: 735); eye movements were measured by a wire wand and capacitative transducer. Each experiment began with a control period of about 1 hour, during which spontaneous periods of active walking and enhanced eye movements alternated with quiescent periods. We then injected 1 mg (or occasionally more) of one of the amines into the pericardial sinus, and recorded its effects and the subsequent recovery during the next 5 to 6 hours. Records on FM tape were processed by computer to yield walking activity and the peak-to-peak amplitude of eye movements (relative optokinetic gain).

Injection of serotonin (at the arrows in the figure) invariably suppressed spontaneous walking and eliminated the optokinetic response. Recovery began after several hours.



Octopamine produced more variable responses. Animals appeared constantly aroused, but with uncoordinated, jittery leg movements. In the example shown, the optokinetic response increased and then decreased before recovering, but in some cases it decreased immediately.



Thus, serotonin suppresses the two systems, while octopamine has more variable effects but often enhances the two responses.

24.10 THE SEROTONERGIC INNERVATION AND MODULATION OF THE STOMATOGASTRIC GANGLION OF DECAPOD CRUSTACEANS. B.S. Beltz, E. Marder, S. Hooper*, J.S. Eisen, R. Flamm*, and R. Harris-Warrick. Neurobiol., Harvard Med. Sch., Boston, MA 02115; Biol., Brandeis Univ., Waltham, MA 02254; and Neurobiol and Behavior, Cornell Univ., Ithaca, NY 14853.

Serotonin serves as a neurotransmitter and neuromodulator in invertebrate and vertebrate nervous systems. The stomatogastric ganglion (STG) of decapod crustaceans is a well-studied central pattern generator that could provide a model system for examining the effects of amines on neuronal circuits. Therefore, we analyzed the distribution and physiological effects of serotonin in the stomatogastric system of three species of decapod crustaceans (Cancer irroratus, Homarus americanus, and Panulirus interruptus). Serotonin-like immunoreactivity was mapped in whole mounts of the complete stomatogastric system using the method of Beltz & Kravitz (J Neuroscience 3: 585-602, 1983). Staining was seen in the neuropile of the commissural ganglia of all three species. Furthermore, in Cancer and Homarus, but not in Panulirus, serotonin-like immunoreactivity was found (1) in fiber tracts connecting the commissural ganglia and the stomatogastric ganglion, (2) in a densely staining neuropile within the stomatogastric ganglion, and (3) in fibers in the motor nerves leaving the stomatogastric ganglion. In Cancer, these motor nerves also contain several brightly staining bipolar neurons. Endogenous serotonin levels in extracts of STG from all three species were measured by High Performance Liquid Chromatography (HPLC). Serotonin levels (fmoles) were as follows: Cancer, 350+230 (n=7); Homarus 811+310 (n=5); Panulirus, non detectable (< 5) (n=4). Concentrations of serotonin (normalized to tissue volume) are as follows: Cancer 35µM; Homarus, 20µM; Panulirus, not detectable (<.03µM). Therefore both immunohistochemical and HPLC techniques indicate that the Panulirus stomatogastric system contains much less serotonin than comparable regions in Cancer and Homarus. However, bath application of serotonin (10^{-5}M to 10^{-4}M) produced alterations of motor output in isolated STG of all three species. Dramatic increases in the number of lateral pyloric (LP) action potentials/burst were usually seen and the cycle frequency was usually reduced. In Cancer, and occasionally in the other species, serotonin produced a motor pattern qualitatively different from the normal pyloric rhythm. Further physiological experiments characterizing these effects are in progress in order to distinguish between hormone-like and transmitter-like actions of serotonin in these three species. B.S.B. was supported by a NIH Postdoctoral Fellowship. Research supported by NS-17813 to E.M., NS-17323 to R.H-W. and NS-07848 to E. Kravitz.

24.11 **ORCHESTRATING BEHAVIORS: A CENTRAL MODULATORY ROLE OF OCTOPAMINE IN THE LOCUST.** Sompong Sombati*. {SPON: G. Streisinger}, Inst. of Neuroscience, University of Oregon, Eugene, OR 97403

The locust metathoracic ganglion contains a cluster of about 30 dorsal unpaired (DUM) neurons. Some are confined to the CNS, of unknown functions, others have axons terminating near skeletal muscles. These neurons tend to fire intensely, when the animal is disturbed. They synthesize and release octopamine (OA) which blocks intrinsic rhythmicity and potentiates neuromuscular transmission. All have central branches, some of which may exert effects on central synapses. Possible central actions were tested by iontophoresis of OA into discrete regions of neuropil known to be associated with various behaviors.

The fast extensor tibiae (FETi) motorneuron (MN) makes central excitatory connections with many flexors tibiae MN's. This is necessary for co-contraction of antagonistic muscles prior to a locust jump. Intracellular recordings were made from identified flexor MN's, while simultaneously antidromically stimulating FETi and iontophoresing OA on flexor neuropil. This potentiated the central transmission from FETi to flexor MN's by up to 100%. FETi spikes then, elicited spikes in flexor MN's, creating "fictive" co-contraction phase. OA effect outlasted the cessation of iontophoresis by about 1 min. Some DUM neurons showed similar effects on these pathways when intracellularly stimulated.

In 3 defined locations, OA iontophoresis generated long-lasting bouts of rhythmic motor activity. One site caused flexing/extending of the tibia at about 1 Hz, resembling that seen in walking or running. At another, flight rhythm of the flight muscles on both sides was evoked with a frequency of about 10 Hz. This lasted up to 25 min. The activity duration was dose-dependent. Simultaneously, the respiratory rate was also increased. At a different location, OA could increase the respiratory rate, independent of flight activity.

The experiments were repeated on the last abdominal ganglion of the female locust, known to contain the central pattern generator for oviposition digging (Thompson, Soc. Neurosci. Abstr. Vol.8, 1982). The activity is inhibited by higher centers and can be released by severing the connectives, stimulation of which can re-inhibit the activity. OA iontophoresis into a specific neuropil region during oviposition, inhibited the behavior as did stimulation of the severed connectives.

These observed effects of OA are consistent with the idea that OA may be released centrally when the animal is disturbed. This may orchestrate the appropriate expression of behaviors for an escape, in this case, by enhancing running, jumping or flying while suppresing conflicting behaviors such as oviposition.

{Supported by NSF Research Grant BNS 82-41884 to Dr. G. Hoyle}

24.12 HISTAMINE UPTAKE IN THE OPTIC LOBE OF THE LOCUST: AUTORADIOGRAPHIC STUDIES. Michael S. Elias* and Peter D. Evans. ARC Unit of Insect Neurophysiology and Pharmacology, Dept. of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ. U.K.

The retina and optic lobe of the locust, (Schistocerca americana gregaria), contain very high levels of endogenous histamine, as well as the enzymes necessary for the biosynthesis and metabolism of histamine (Elias, M.S. and Evans, P.D., J. Neurochem, 1983, in press). The presence of a histamine uptake system, of potential use in the inactivation of histamine, was therefore investigated.

Locust optic lobes and retinae were incubated in physiological saline containing (2,5-^{3}H)-histamine (21.5 μM, 4.64 Ci/mmol) for two hours at 21° C. At the end of the incubation >90% of the radioactivity in the tissue was present as histamine. Tissues were then processed for conventional light microscope autoradiography and covered with Kodak AR-10 stripping film. Dense accumulations of silver grains were seen in several regions of the optic lobe. Grains were most highly concentrated along the medial border of the medulla neuropil and around the entire periphery of the lobula. Small aggregations of grains were occasionally seen immediately medial to the basement membrane situated between the retina and lamina. No significant accumulations of grains were present within the retina or the neuropil zones of the lamina, medulla or lobula.

Along the medial border of the medulla histamine appeared to be taken up into cell bodies, though it was unclear whether the cells were glia or neurons. To resolve this question further autoradiographic experiments were carried out at the electron microscope level (method of Salpeter, M.M. and Bachmann, L. J. Cell Biol. 22: 469, 1964). E.M. autoradiographs showed histamine-accumulating cells along the medulla border to exhibit characteristics of electron density and morphology typical of glial cells. Labelled histamine was present within both glial cell bodies and their processes. In the region surrounding the neuropil of the lobula histamine was concentrated within fine glial processes wrapped around neuronal cell bodies and their axons. No neurons or axons showed any accumulation of silver grains above background.

These results are in concordance with previous studies showing the active glial uptake of amino acid and biogenic amine putative neurotransmitters. They suggest that glial cells may play a role in the inactivation of histamine released as a neurotransmitter or neuromodulator in the optic lobe of insects.

M.S. Elias was the recipient of a Marshall Scholarship.

24.13 CALCIUM/PHOSPHOLIPID-DEPENDENT PROTEIN PHOSPHORYLATION IN APLYSIA NEURONS. S.A. DeRiemer, L.K. Kaczmarek, K.A. Albert, and P. Greengard. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Electrical stimulation of the bag cell neurons of Aplysia generates a long-lasting afterdischarge triggered by a rise in intracellular cAMP and associated with changes in the phosphorylation state of at least two proteins (BCI and BCII). Evidence indicates that calcium entry, as well as cAMP levels, may regulate bag cell electrical activity and protein phosphorylation. We have previously shown that a calcium-calmodulin dependent protein kinase very similar to one found in the mammalian brain exists in the Aplysia nervous system. A second class of calcium-dependent protein kinase regulated by phospholipids (Ca/PS-PK, C kinase) has also been found in both vertebrate and invertebrate nervous systems (Kuo et al., PNAS 77:7039, 1980). We have now determined that an enzyme of this type exists in the Aplysia nervous system. Protein phosphorylation was examined in cell free preparations of the Aplysia nervous system using SDS-PAGE. Homogenates were also fractionated by centrifugation at 100,000xg to yield particulate and cytosolic fractions. Addition of phosphatidyl serine (50 mg/ml) and 1,3 diolein (5 mg/ml) to cytosolic fractions in the presence of calcium caused an increase in phosphate incorporation into protein bands with M_r's of 87K, 72K, 59K, 48K, 38K. These proteins were not phosphorylated upon addition of calcium plus calmodulin. In particulate fractions additions of Ca-PS plus diolein had little or no effect beyond that of calcium alone, presumably due to the presence of endogenous lipids. In addition to observing endogenous substrates for Ca/PS-PK, we found that Aplysia nervous system homogenates could phosphorylate an M_r 87K protein purified from mammalian brain. This protein appears to be a specific substrate for Ca/PS-PK. An increased phosphorylation of this protein was observed in crude homogenates and in particulate and cytosolic fractions in the presence of calcium, PS and diolein suggesting that Ca/PS-PK is present in membranes and in cytosol.

Calcium entry into Aplysia neurons may therefore activate at least two distinct phosphorylating enzymes, Ca/CaM PK and Ca/PS-PK, with differing substrate specificities. We are now determining whether protein substrates for Ca/PS-PK change their phosphorylation state during or after a bag cell afterdischarge.

24.14 FUNCTIONAL MORPHOLOGY OF THE ATRIAL GLAND AND THE LARGE HERMAPHRODITIC DUCT OF APLYSIA CALIFORNICA. S. D. Painter, R. A. Zuckerman*, G. T. Nagle and J. E. Blankenship. Marine Biomedical Institute, Univ. Tx. Med. Br., Galveston, TX 77550.

The atrial gland (AG) is located in the distal end of the large hermaphroditic duct (LHD) of the reproductive tract of A. californica. This gland contains peptides which can induce bag cell discharge and egg-laying activity, and it has been suggested that these peptides are released into the blood during copulation to ensure that the animal will subsequently lay eggs. Yet the AG appears to be an exocrine gland, secreting into the lumen of the LHD rather than into the hemocoel. Its secretions have, therefore, also been ascribed a prostatic, lubricating or pheromonal function.

To gain insight into the possible functional roles of the AG, we have examined the structure of the LHD in serial thick and thin sections during various phases of reproductive activity. The duct is complex, composed of two functionally separate compartments which can be easily distinguished: the red hemiduct (RHD) and the white hemiduct (WHD). They form two separate, though closely apposed, ducts near the base of the accessory genital mass (AGM). They fuse, about 1 cm from the AGM, to form a single duct bisected by a tissue septum. This septum becomes a series of typhlosoles in the distal duct which keep the compartments functionally, if not physically, separate. The AG arises as a small group of cells adjacent to the RHD on a minor typhlosole. The gland rapidly enlarges and assumes a more peripheral position; the RHD coordinately regresses and assumes a more central position. Large vesicles (d = 1-2μ) in the AG columnar epithelial cells stain immunocytochemically with monoclonal antibodies directed against biologically active AG peptides.

The egg cordon passes through the RHD and in close proximity to the AG; it is not associated with the WHD. In contrast, the penis and sperm (both homotypic and heterotypic) pass through the WHD and are never in direct contact with either the RHD or the AG. Occasionally, stray sperm and unpackaged eggs are also observed in the WHD, presumably in transit to the gametolytic gland for destruction. These observations suggest that the AG is unlikely to be involved in male copulatory functions. Since it contains peptides that can induce egg release and is intimately associated with the oviduct, it is more likely that the atrial gland is involved in the female function of egg laying. Supported by NIH NS07010 (S.D.P.), NS07025 (G.T.N.), NS 11255 and NSF PCM 82-15185 (J.E.B.).

24.15 HIGH-PRESSURE LIQUID CHROMATOGRAPHY ANALYSES OF GANGLIA EXTRACTS FROM INSECT AND SNAILS. M.K. Leung*, G.B. Stefano, P. Assanah* and W. Burrowes*. (Spon: E. Catapane) Depts. Chemistry and Biological Sciences, SUNY/College at Old Westbury, New York 11568

Previous studies have demonstrated the presence of mammalian-like opioid mechanisms in various invertebrate nervous systems. Recently, Met-and Leu-enkephalin and the heptapeptide Met-enkephalin precursor in ganglia extract of M. edulis have been isolated by high-pressure liquid chromatography (HPLC) and identified by amino acid sequence determination. Similar HPLC analyses were used in the study of ganglia extracts of insect (Leucophaea maderae) and snail (Helix aspersa). Ganglia from these organisms were extracted separately in the cold with 0.5M acetic acid in the presence of phenylmethylsulfonyl fluoride and pepstatin A. High molecular weight proteins and lipids were removed from the extracts by trichloroacetic acid precipitation and ether extraction respectively. The extracts were lyophilized and redissolved in 10 mM acetate fuffer pH4·0. They were then subjected to HPLC analyses on a Brownlee RP-300 reverse-phase column (4·6 X 250 mm) using the acetate buffer with a 2-propanol linear gradient of 5-25% in 15 min. The results showed the presence of peptide with the same R_t as authentic Met-enkephalin in extracts of both organisms. The level of this peptide in the insect extract is about 2X higher than that in the snail. This is in direct correlation with earlier studies demonstrating higher Met-enkephalin binding in L. maderae ganglia than in snail. Identification of this peptide is now in progress. The results also showed that little or no peptide with R_t similar to Leu-eukephalin was detected in either ganglia extract.

Supported by NIH-MBRS Grant RR 08180

24.16 THE DISTRIBUTION AND ACTIONS OF A FMRFamide-LIKE PEPTIDE. H. K. Lehman* (SPON: M. J. Greenberg). C. V. Whitney Marine Laboratory, Rt. 1, Box 121, St. Augustine, FL 32084.

FMRFamide-like peptides occur in the tissues of the snail, Helix aspersa. They have been characterized by HPLC, radioimmunoassay and bioassay. In addition, the major peptide has been characterized by its amino acid composition. The physiological role for no FMRFamide peptide is known. Therefore, I have been examining the tissue distribution of the major FMRFamide homolog in Helix as an approach to possible sites of release and action.

Twenty-three tissues in Helix aspersa were extracted and purified by HPLC, and the concentration of FMRFamide-like peptide was estimated with sensitive (0.1 pmole) radioimmuno- and bioassays. These tissues were also examined by immunocytochemical methods. The immunoreactive FMRFamide-like peptide has a wide but uneven distribution throughout the digestive, reproductive, circulatory, secretory and nervous systems. Tissues with greater than 20 nmoles of immunoreactivity per gram wet weight include the penis-vas deferens complex, the subesophageal and cerebral ganglia, the collar, and the tentacles. Nerves and nerve varicosities are enriched in these tissues, particularly in the muscle layers; cell bodies were only found in the ganglia. Tissues with lesser amounts of immunoreactive FMRFamide (2-15 nmoles) include the digestive tract, salivary glands, ventricle and auricle, and portions of the female reproductive tract. Nerve fibers are only sparsely distributed in all of these tissues; cell bodies were observed in regions of the digestive tract.

The notion that activity should be correlated with distribution was tested by examining the effects of purified extracts, FMRFamide, and FMRFamide analogs on the contractility and rhythmicity of those muscular organs in Helix which had been shown to contain immunoreactive FMRFamide. A correlation seems to occur: the richly innervated penis is about ten times more sensitive to these peptides than is the less innervated crop; and the radula retractor muscle, which seems to contain no FMRFamidergic innervation, does not respond.

I suggest that the Helix FMRFamide-like peptide is a neuromuscular effector; but further experiments are needed to determine if it is primarily a transmitter or modulator.

24.17 L-GLUTAMIC ACID, A POSSIBLE NEUROTRANSMITTER TO THE APLYSIA ANTERIOR AORTA. M. Sawada,* D. Gibson and D. McAdoo. Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX. 77550.

L-Glutamic acid is a strong candidate to be an excitatory neurotransmitter in the vertebrate central nervous system and at the arthropod neuromuscular junction. Observations that arteries of the marine gastropod Aplysia are very sensitive to L-glutamate led us to systematically characterize the effects of L-glutamate on muscle fibers in the Aplysia anterior aorta. Iontophoretically applied L-glutamate caused depolarizations at only 14% of the sites penetrated, indicating that there are highly localized regions of sensitivity to L-glutamate on muscle fibers in the Aplysia artery. Muscle fibers were insensitive to iontophoretically applied D-glutamate. Responses to iontophoretically applied glutamate desensitized very rapidly. The L-glutamate antagonist, 2-amino-4-phosphonobutyric acid antagonized the responses to iontophoretically applied L-glutamate and EJPs in the anterior aorta. Iontophoretically applied L-glutamate caused strong conductance increases at the sensitive spots on the muscle fibers. Bath applied L-glutamate also depolarized anterior aorta muscle fibers. However, this response did not desensitize, was not blocked by 2-amino-4-phosphonobutyric acid, and was not accompanied by a significant change in membrane conductance. Thus bath applied and iontophoretically applied L-glutamate appear to act on the muscle fibers by different mechanisms. The observations reported here make L-glutamate a strong candidate to be an excitatory neurotransmitter to mollsucan circulatory muscle. Supported by DHEW NS 13311 and NS 11255.

24.18 BEHAVIORAL AND ELECTROPHYSIOLOGICAL RESPONSES TO 5'-AMP INDICATE CRUSTACEANS HAVE EXTERNAL CHEMORECEPTORS RELATED TO INTERNAL PURINERGIC RECEPTORS OF VERTEBRATES. W.E.S. Carr*, C. D. Derby, P. L. Linser*, R. A. Gleeson* and B. W. Ache* (SPON: L. A. Wilkens). C. V. Whitney Laboratory of University of Florida, St. Augustine, FL 32084

In vertebrate animals, adenosine and its non-cyclic nucleotides are known to function as transmitters and/or modulators in brain, heart and elsewhere. Using both behavioral and electrophysiological procedures, we have found that both a shrimp and a spiny lobster have external chemoreceptors for the nucleotide, adenosine 5'-monophosphate (AMP). Moreover in both cases, structure-activity relationships and the effect of an antagonist indicate these chemoreceptors have marked similarities to internal purinergic receptors.

Behavioral studies in the shrimp show that AMP is a potent chemoattractant. Response increases with dose up to 10 μM and then declines at higher concentrations. AMP is about 150 times more effective than ADP. ATP and adenosine are not attractants. The response is antagonized by theophylline. Bioassays of about 30 substances structurally related to AMP reveal that the integrity of both the adenine and the ribose phosphate moieties are required for maximal activity. However, the integrity of the ribose phosphate is of special importance since most modifications here completely inactivate the molecule. These results suggest the shirmp has external chemoreceptors similar to the P_1-type (= R-type) purinoceptors found in vertebrate tissues.

Recordings from single olfactory cells in the antennule of the spiny lobster reveal the existence of receptor cells with the type of specificity implied by the behavioral studies. The potency sequence in these cells is AMP > ADP > ATP >> adenosine. The response to AMP is antagonized by theophylline. Further studies of the structure-activity relationships of these cells are in progress. Also in progress are studies of AMP binding to receptors on excised sensilla from antennules of the spiny lobster.

Collectively, our studies indicate that crustaceans may serve as valuable model systems for studying purinergic receptors, as well as several other receptor types, at levels bridging the gap from behavior to electrophysiology to receptor characterization.

24.19 EVIDENCE FOR GABA AS AN INHIBITORY NEUROTRANSMITTER IN THE LEECH. H.T. Cline, M.P. Nusbaum, W.B. Kristan, Jr. Graduate Group in Neurobiology, UCB, Berkeley, CA 94720, and Dept. of Biology, UCSD, La Jolla, CA 92093.

The isolated leech ventral nerve cord has the capacity to synthesize GABA from glutamate, indicating the presence of the enzyme glutamic acid decarboxylase (GAD). In addition, 30-35 identifiable neurons per ganglion accumulate exogenous ^{3}H-GABA by a high affinity uptake system.

The presence of GAD activity and specific accumulation of GABA by a reproducible set of neurons suggest that GABA may be a transmitter in the leech. In order to test this possibility we identified two pairs of the GABA-accumulating neurons, and are studying the pharmacological and physiological properties of the synapses they make on other neurons.

We identified two of the GABA-accumulating neurons as the inhibitory motor neurons, cells 1 & 2, which innervate the longitudinal muslces in the body wall. The inhibitory motor neurons were identified electrophysiologically, filled with Lucifer Yellow and incubated in ^{3}H-GABA. Autoradiographic processing reveals both Lucifer Yellow and silver grains in the same cell body. Lucifer Yellow itself does not cause neurons to accumulate GABA, because neither mechanosensory cells nor excitatory motor neurons which have been filled with dye accumulate ^{3}H-GABA.

The inhibitor of the dorsal longitudinal muscles, cell 1, makes intraganglionic synapses onto the excitatory motor neurons of the dorsal longitudinal muscles, cells 3, 5 & 7. Intracellular stimulation of cell 1 produces a hyperpolarization in cell 3 accompanied by increase in membrane conductance. Bath application of GABA (100 µm) in normal Leech Ringer (1.5 mM Mg) causes a rapid hyperpolarization in cell 3 and an increase in membrane conductance. The original membrane potential and conductance are rapidly recovered when GABA is replaced by normal Ringer. 15 mm Mg^{++}, which is sufficient to block the synapse between cell 1 and 3, does not block the response to bath-applied GABA. Therefore it is likely that GABA is acting directly on cell 3.

The response to bath-applied GABA is blocked by muscimol (100 µm), a rigid analog of GABA. Nipecotic acid (100 µm), another GABA analog, also induces a hyperpolarization in cell 3.

In summary, identified inhibitory motor neurons accumulate GABA, and their inhibitory effect on other neurons is mimicked by GABA. Muscimol appears to be an antagonist and nipecotic acid appears to be an agonist for the GABA response.

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TRANSMITTER IMMUNOCYTOCHEMISTRY

25.1 IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN DEVELOPING RAT SPINAL CORD. P.E. Phelps, R.P. Barber*, G.D. Crawford*, P.M. Salvaterra, and J.E. Vaughn. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

The specificity of an immunocytochemical method for identifying cholinergic neurons with a monoclonal antibody to choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine, has been demonstrated in the adult rat CNS (Houser et al., Brain Res. 266:97-119). This method has been used to identify cholinergic neurons in developing cervical spinal cord of rat pups ranging in age from 1-28 days postnatal (dpn), as well as in adult specimens. The postnatal development of four types of ChAT-positive neurons, motoneurons, and three previously unidentified groups of cholinergic cells, has been studied.

Large and small motoneurons (MNs) in newborn rats contained a moderate amount of ChAT-positive reaction product in their somata, dendrites, and axons. The intensity of ChAT-positive staining increased by 8 dpn, and was near adult levels by 14 dpn. Intensely ChAT-positive MN dendrites formed prominent bundles in specimens 14 dpn and older. Large MNs in adult spinal cord received synapses from ChAT-positive terminals. A few similar structures were located on MN's at birth and increased substantially in number by 8-11 dpn.

The most intensely ChAT-positive group of neurons in 1-5 dpn specimens were named partition cells because they occupied a region extending from the central canal to an area just dorsal to the lateral MNs, and thereby formed a division between dorsal and ventral horns. These partition cells were medium to large in size and had 5-7 primary dendrites that displayed mediolateral and rostrocaudal orientations. Axons of these cells could be traced into the ventral funiculus, MN pools and the ventral commissure. Another ChAT-positive cell group encircled the central canal. Although detectable at birth, processes of these small, central gray cells were not well delineated until 14 dpn, and were intensely ChAT-positive by 21 dpn. The fourth group of ChAT-positive neurons was located in the dorsal horn (laminae III-IV and adjacent to the dorsal columns). These cells were present at early ages, but it was not until 11-14 dpn that their dendrites could be observed to project dorsally into laminae II-III. A band of ChAT-positive punctate structures also was prominent in lamina III of adult dorsal horn, but was not detectable until 14 dpn in developing spinal cord. By 21-28 dpn, the density of this band gradually approximated that observed in adult specimens. This study is being extended to include embryonic stages of development. All four ChAT-positive neuronal groups described above have been identified as early as embryonic day 17. Supported by USPHS Grant NS18449.

25.2 DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE POSITIVE NEURONS AND TERMINALS IN HIPPOCAMPUS. D. A. Matthews, P. M. Salvaterra, G. D. Crawford*, C. R. Houser and J. E. Vaughn. Division of Neurosciences, City of Hope Research Inst., Duarte, CA 91010.

It is generally accepted that the septohippocampal pathway is the sole source of cholinergic innervation to the hippocampus. However, some confusion persists regarding the distribution of cholinergic terminals and the source of the residual choline acetyltransferase (ChAT) activity that persists following septal lesions. This is due, in part, to a lack of information about the cellular localization of ChAT, the synthesizing enzyme for acetylcholine and a definitive cholinergic marker. Therefore, a monoclonal antibody to ChAT has been used to study cholinergic structures in normal rat hippocampus and in hippocampus deprived of its septal innervation.

Small numbers of previously undetected ChAT-positive (ChAT+) neuronal somata were found scattered throughout the septotemporal extent of the hippocampus. They were most common in stratum lacunosum-moleculare of regio superior, but were also found in various layers of the dentate gyrus and occasionally in the remaining hippocampal laminae. In electron micrographs, ChAT immunoreactivity was most often observed within synaptic vesicle-laden profiles that did not exhibit definitive synapses. However, some ChAT+ profiles formed asymmetric synapses with small dendrites or spines, and other possible synapses were either symmetric or of an intermediate type. Light microscopy demonstrated that ChAT+ terminal fields were organized in discrete bands and laminae. Pronounced bands were observed: 1) immediately superficial to stratum granulosum, 2) deep to stratum pyramidale, and 3) on the border between stratum radiatum and stratum lacunosum-moleculare. In the dentate gyrus, ChAT+ staining was pronounced in the hilus at the temporal levels, but only moderate staining occurred in the anterior hilus and throughout the molecular layer. A close correspondence was observed in the density and distribution of ChAT+ terminals and acetylcholinesterase staining. Electrolytic lesions of the medial septal nucleus had no effect on ChAT+ somata, but virtually abolished the ChAT+ laminar pattern and eliminated all but occasional small patches of ChAT+ terminals. These results confirm that the vast majority of hippocampal cholinergic terminals originate either from medial septal neurons or from fibers of passage. The newly observed intrinsic neurons can account for at least some of the ChAT activity remaining after septal lesions, and they apparently also contribute to the cholinergic innervation of the hippocampus. Supported by USPHS Grant NS18858.

25.3 CHOLINERGIC NEURONS IN RAT CEREBRAL CORTEX IDENTIFIED BY IMMUNOCYTOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE. C.R. Houser, G.D. Crawford*, P.M. Salvaterra and J.E. Vaughn. Division of Neurosciences, City of Hope Research Inst., Duarte, CA 91010.

Choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine and a definitive marker for cholinergic neurons, has been localized immunocytochemically in sensory-motor regions of rat cerebral cortex with a monoclonal antibody to ChAT. The specificity of the monoclonal antibody and immunocytochemical methods has been demonstrated previously in several well characterized cholinergic systems (Houser, C.R. et al., Brain Res. 266: 97, 1983). ChAT-positive punctate structures and fine fibers with periodic varicosities were distributed in a loose network throughout the cortex. Some of these fibers were continuous with positively stained fibers in the white matter underlying the cortex, many of which presumably originate from subcortical cholinerigic neurons. ChAT-positive fibers appeared to be rather evenly distributed throughout all layers of motor cortex, but a slight laminar pattern was evident in sensory cortex, where lower densities of fibers were observed in regions of granule cell aggregates in layer IV. Electron microscopy demonstrated that ChAT-positive reaction product was concentrated in small vesicle-filled profiles throughout the cortex, and these structures were occasionally observed in continuity with lightly-stained axons. Although many of the vesicle-filled profiles did not exhibit definitive synaptic junctions in single thin sections, some of these profiles formed synaptic contacts, and preliminary findings suggested that they were of the symmetric type. ChAT-negative dendrites of various sizes were the most common postsynaptic elements of these synapses.

In addition to fibers, ChAT-positive cell bodies also were present in cortical layers II-VI, and were most numerous in layers II-III. These somata were small in size (12-18μm in major diameter) and exhibited the characteristics of nonpyramidal neurons. The majority of ChAT-positive neurons were bipolar in form, displaying vertically-oriented dendrites that often extended across several cortical layers. ChAT-positive neurons with multipolar and bitufted dendritic patterns also were observed, but were less common than bipolar forms. Electron microscopy confirmed the presence of ChAT-positive reaction product within the cytoplasm of small neurons. These neurons received both symmetric and asymmetric synapses on their somata and proximal dendrites, and such observations support their identification as nonpyramidal, intrinsic neurons. Thus the present findings indicate that there is an intrinsic source of cholinergic innervation of the cerebral cortex in addition to previously described extrinsic sources. Supported by USPHS Grant NS18858.

25.4 A COMPARISON OF THE DISTRIBUTION OF CHOLINE ACETYLTRANSFERASE AND TYROSINE HYDROXYLASE IMMUNOREACTIVITIES IN RAT RETINA. E. Eckenstein*[1], R.W. Baughman[1], M.V. Sofroniew[2] and J. Thibault[3]. [1]Harvard Medical School, Boston MA 02115; [2]University of Oxford, Oxford, England; [3]College de France, Paris, France.

The neurotransmitter synthesizing enzymes, choline acetyltransferase (ChAT) and tyrosine hydroxylase (TH) represent specific markers for cholinergic and catecholaminergic neurons, respectively. We have studied the distributions of these enzymes in the rat retina with immunohistochemical methods. The specificity of the TH staining observed was established by preabsorbing the TH-antiserum with purified TH. For ChAT, a previously characterized antiserum was used and the staining pattern was confirmed with two different monoclonal antibodies to ChAT. The ChAT-antiserum labelled numerous small (10 micron diameter) neurons in the amacrine and ganglion cell layers with similar numbers of cells in each layer. These cells lay immediately adjacent to the inner plexiform layer (IPL). Laminae 2 and 4 (of 5) in the IPL showed strong staining for ChAT, probably representing cholinergic terminals. Confirmation of the stained neurons as amacrine cells awaits identification, in the same section, of the ganglion cells by retrograde labelling. TH-immunoreactivity was found in large neurons (>20 micron diameter) present in the amacrine cell layer, and very rarely, in the ganglion cell layer. These cells were over 10x less numerous than the ChAT-positive cells. In the IPL, a dense plexus of fibers and varicosities was stained in Lamina 1 and a low density was seen in lamina 3; occasional labelled processes were also observed in the outer plexiform layer. The present results are in good agreement with previous studies on the localization of specific neurotransmitter uptake. The immunohistochemical techniques used here, however, make possible simultaneous double staining for different antigens. In addition, these techniques may permit study of the synaptic connections of the neurons described here, in particular, the cholinergic neurons, at the electron microscopic level.

25.5 ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL CHARACTERIZATION OF CHOLINERGIC NEURONS IN THE RAT BRAIN. D. M. Armstrong, Y. Kress*, and R. D. Terry*. Department of Neuropathology, Albert Einstein College of Medicine, Bronx, NY 10461.

The electron microscopic immunocytochemical localization of choline acetyltransferase (ChAT), the acetylcholine biosynthetic enzyme, was employed to examine the ultrastructural morphology and synaptic interactions of cholinergic neurons in the nucleus of the diagonal band of rat brain.

The details of the production and characterization of monoclonal antibodies to ChAT have been described (Levey et al., J. Neurosci., 3:1-9). Antisera were localized by the peroxidase-antiperoxidase method in 50um Vibratome sections of brains fixed by vascular perfusion with a mixture of ice cold 4% paraformaldehyde and 0.15% glutaraldehyde in 0.1M phosphate buffer.

Electron micrographs were taken throughout the horizontal and vertical limbs of the nucleus of the diagonal band. In all regions, peroxidase immunoreactivity for ChAT was distributed throughout the cytoplasm of selectively labeled neuronal perikarya and processes. The cholinergic neurons have a prominent nucleolus, infolded nuclear membrane, and numerous cytoplasmic organelles. The majority of the labeled processes were dendrites which contain ribosomes, microtubules, and mitochondria. Within labeled dendrites peroxidase immunoreactivity was distributed throughout the cytoplasm with somewhat dense accumulations associated with microtubules and mitochondria. Labeled dendrites were postsynaptic to unlabeled axon terminals characterized by numerous small clear vesicles. In addition to asymmetric synapses with unlabeled axon terminals, labeled dendrites often formed membranous appositions with other labeled dendrites and/or perikarya. The relative absence or paucity of ChAT-labeled axons or axon terminals may reflect that the majority of these cells are projection neurons or technical limitations such as accessibility of the applied antisera to the enzyme in axon terminals. We conclude that in the nucleus of the diagonal band, reaction product for ChAT was distributed throughout the cytoplasm of selectively labeled neuronal perikarya and dendrites which formed synaptic contacts with unlabeled axon terminals.

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25.6 A COMPARISON OF THE DISTRIBUTION OF CENTRAL CHOLINERGIC NEURONS AS DEMONSTRATED BY ACHE-PHARMACOHISTOCHEMISTRY AND CHAT-IMMUNOHISTOCHEMISTRY. K. Satoh, D.M. Armstrong and H.C. Fibiger, Div. Neurological Sciences, Univ. British Columbia, Vancouver, B.C.; Dept. Neuropathology, Albert Einstein College of Medicine, Bronx, NY.

Pharmacohistochemistry for acetylcholinesterase (AChE) has been introduced as a neuroanatomical tool to detect perikarya in the CNS that may be cholinergic. In the present study, the reliability of this modified histochemical method in identifying cholinergic cell bodies was assessed. Complete series of frontal sections of the rat brain and spinal cord were processed either for AChE histochemistry 4-8h after systemic administration of diisopropylfluorophosphate (DFP, 2.0 mg/kg) or for choline acetyltransferase (ChAT) immunohistochemistry (Levey et al., J.Neurosci., 3:1,1983). The AChE-rich and ChAT-containing cell bodies were mapped on an atlas of the brain and the spinal cord, and a detailed comparison of these two cell populations was conducted with respect to their morphology and topographical distribution.

The present observations revealed an excellent correspondence between the distribution of ChAT-containing and intensely stained AChE cells in many regions of the CNS. In the forebrain, these regions include the striatum, nucleus accumbens, olfactory tubercle, medial septum, nucleus of the diagonal band, lateral preoptic region and nucleus basalis magnocellularis. Very similar distributions were also seen in hindbrain and spinal cord structures; i.e. somatic and visceral efferent cranial nerve nuclei (III-VII, IX-XII), ventral horn and intermediomedial zone of the spinal cord, midbrain and pontine tegmentum (pedunculopontine nucleus, laterodorsal tegmental nucleus). ChAT-immunoreactivity and a high content of AChE were also observed in perikarya located in restricted areas of the reticular formation in the lower brain stem.

Distinct incongruencies between the results of the two techniques were noted in several regions which contained cells that were stained intensely for AChE but where there was an absence of ChAT-containing neurons. These areas were the lateral hypothalamus, subthalamus, locus coeruleus, flocculus (cerebellum), nucleus raphe magnus, some regions of the bulbar reticular formation and the nucleus cervicalis lateralis. In no instance did we observe an area that contained ChAT-positive cells but which lacked AChE-rich perikarya. According to both histochemical techniques no cholinergic cell bodies are located in the cerebral cortex, hippocampus, nuclei of the amygdala, dorsal diencephalon, and the cerebellar nuclei.

The present observations indicate that AChE-pharmacohistochemistry can be used as a reliable method to detect cholinergic neurons in many, but not all regions of the CNS.

25.7 DIRECT IMMUNOCYTOCHEMICAL METHOD VISUALIZES GLUTAMATE IN PYRAMIDAL CELLS AND GABA IN INTERNEURONES IN THE CEREBRAL NEOCORTEX.
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A direct and specific method for visualizing amino acids in tissue sections has long been needed. Radioactive amino acids taken up into brain tissue are retained to the extent of 50-65% on fixation by glutaraldehyde (G). Recently we showed that amino acids thus fixed can be visualized immunocytochemically (Nature, 301, 1983, 517-520). Here we use this method to study the cellular localization of glutamate (Glu) and GABA in the cerebral neocortex. Briefly, antisera against Glu or GABA fixed to bovine serum albumin by G were raised in rabbits and purified by immunosorbent chromatography. The sera showed good specificities in a model system in which various amino acids and peptides fixed to brain proteins were visualized under conditions similar to those used for processing fixed tissue sections. Vibratome sections (20-100 um) were cut from rat or mouse brain, perfusion fixed with 5% distilled G in NaPi buffer, and processed with the PAP method. The following description applies to all neocortical areas.

The **GABA-antiserum** stained nonpyramidal perikarya and boutonlike structures. The perikarya were mostly stellate and showed no clear lamination. The terminals were often in contact with unstained pyramidal perikarya and dendrites. These results agree closely with published data on the immunocytochemical localization of GAD, and with the notion that a population of nonpyramidal cortical neurones is GABA-ergic.

The **Glu-antiserum** stained pyramidal perikarya (which are believed to send Glu-ergic axons to various cortical and subcortical targets). Dendrites could often be followed through several cortical layers. The intervening neuropil was moderately stained. If the tissue was poorly perfused or the fixation delayed until after slicing in isotonic sucrose, cell bodies and dendrites were unstained, but the neuropil contained boutonlike stained structures.

Both antisera stained the nuclei, as well as the cytoplasm, of immunoreactive somata, indicating that the nuclear membrane is penetrable to the amino acids. None of the sera visualized glial perikarya, consistent with the notion that in these cells Glu and GABA are rapidly converted to glutamine.

Preimmune sera, sera raised against aldehyde treated wheat germ agglutinin, or immune sera preabsorbed with antigen-bearing Sepharose beads, produced only weak, diffuse staining.

This represents the first direct demonstration of Glu and GABA in neocortical neurones. The present approach should be applicable also to other free amino acids.

Supported by NAVF.

25.8 MEDULLARY RAPHE NUCLEI IN THE PRIMATE: A COMBINED CYTOARCHITECTURAL, IMMUNOCYTOCHEMICAL AND QUANTITATIVE STUDY.
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The medullary raphe nuclei play important roles in modulatingvarious sensori-motor and autonomic functions within the nervous system, as well as different aspects of the sleep-arousal continiuum. In recent years immunocytochemical studies have demonstrated the presence of many serotonergic and peptidergic neurons within these raphe nuclei. In the present study, the distributions of chemically-identified neurons were determined in relationship to the cytoarchitectural borders of the medullary raphe nuclei of the primate. The transmitter specific neurons were then quantitatively analyzed at the various levels of the raphe nuclei in terms of the relative population of neurons. In the non-human primate (squirrel (Saimiri sciureus) and rhesus (Macaca mulatta) monkeys) the brainstems were serially sectioned at 20 um with every fourth section being stained for Nissl substance and the remaining tissue sections being processed for immunocytochemistry, employing the avidin-biotin method of Hsu et al (1981). The cytoarchitecture of the medullary raphe nuclei wereadapted from the descriptions of Olszewski and Baxter (1954) and Taber et al (1960). Quantitative analyses of chemically specific and non-specific neurons were performed at nine different levels of raphe nuclei. In the medulla of the non-human primate the raphenuclei can be subdivided into three nuclei - the nucleus raphe obscurus, the nucleus raphe magnus and the nucleus raphe pallidus. The latter cell group appears to be a relatively small component, or absent, in primates in comparison to other animal species. Nucleus raphe obscurus is composed of small and medium sized neurons, while the more anterior nucleus raphe magnus contains large and giant sized cells. A few large and giant neurons can beseen caudally in nucleus raphe obscurus. The nucleus raphe pallidus in squirrel monkeys is located at anterior levels of the inferior olive and appears to be comprised of a small, but homogeneous, cell group. Serotonin immunoreactive neurons were localized in each of the three raphe nuclei, but were not evenly distributed throughout each cell group. The serotonin cells are concentrated in the nucleus raphe obscurus and raphe pallidus. Quantitative data revealed that the percentages of serotonin immunoreactive neurons decreased in a caudal to rostral direction within the raphe nuclei at each of the nine levels. These data indicate that the serotonin-like neurons in the medullary raphe represent only a small fraction of the total raphe cell populationand suggest that in the primate, other transmitter agents may have a role in modulating the different raphe functions. (Supported byNS19379-01).

25.9 ON THE SEROTONIN INNERVATION OF THE RAT PITUITARY INTERMEDIATE LOBE.
E. Mezey*, Cs. Leranth*, E. Friedman, D. T. Krieger, M. J. Brownstein*, M. Palkovits*. (Spon: W. W. Alberts). NIMH, LCB, Bethesda, Md.20205.

Serotonin containing nerve fibres and terminals were detected in the rat pituitary intermediate lobe (IML) by light and electron microscopic immunohistochemistry. Transecting the pituitary stalk caused a marked (50%) decrease in IML serotonin (5-HT) and 5 - hydroxyindoleacetic acid (5-HIAA) content and degeneration of the majority of the 5-HT positive nerve terminals in the IML. This indicates that most of the 5-HT containing nerve fibres in the IML come from the central nervous system. To locate the cell bodies that give rise to these fibers, we measured 5-HT in the IML after performing several surgical procedures: 1) transection of the ascending axons from the raphe nuclei, 2)total hypothalamic deafferentation or 3)electrolytic or mechanical lesions of the dorsomedial nuclei. Some animals underwent both 2) and 3) above. The effects of dorsomedial nucleus lesions and destruction of fibers from the raphe nuclei seemed to be additive; combining lesions 2) and 3) had the same effect as stalk transection - a 50% fall in 5-HT content. This result and our others indicate that tha 5-HT in IML nerve endings is provided by cells in the midbrain raphe and hypothalamic dorsomedial nuclei.

29.10 PROJECTION FROM THE AREA POSTREMA TO THE PARABRACHIAL NUCLEI. A NEW SEROTONERGIC PATHWAY.
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The area postrema (AP), is a circumventricular organ classically reported as a neuron-poor structure. However, there have been recent reports of efferent projections of this region to the parabrachial nucleus (PBN) and the dorsal division of the nucleus tractus solitarius, as well as direct neuronal inputs from the vagus nerve and the hypothalamus to the AP. The AP has also been suggested as a chemoreceptor area for intracerebroventricular and blood-born information (because of its weak blood-brain barrier). The identification of the neurotransmitter present in the projection to the PBN (the main efferent pathway of the AP) was investigated. A combined retrograde tracing-immunofluorescent technique was used to identify the relationships between the cellular population projecting to the PBN and the serotonin immunoreactive population of the AP in rats. The retrograde fluorescent tracer True Blue was injected in the parabrachial region and 3 days later the animals were perfused. In some rats pargyline was injected 2 hours before perfusion. Sections were processed for serotonin immunofluorescence.

Under the fluorescent microscope three different groups of cells were identified in the AP. First, True Blue positive cells (up to 250/section) that project to the PBN were observed distributed throughout the AP. Second, in the pargyline (MAO-inhibitor) treated animals a high number of serotonergic cells (125/section) was observed distributed throughout the AP. However, there was a slight tendency for a heavier distribution of serotonin immunoreactive cells in the dorsal two thirds of the AP. Third, double labelled cells were also seen. 20% of the True Blue labelled cells projecting to PBN were serotonin immunoreactive. 39% of serotonin immunoreactive cells were retrogradely labelled with True Blue. Thus a new serotonergic pathway from the area postrema to the parabrachial nucleus is described. The results suggest an active role played by serotonergic neurons in the transmission and modulation of chemical and visceral sensory input.

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25.11 AN IMMUNOHISTOCHEMICAL AND ELECTRONMICROSCOPIC STUDY OF SEROTONIN NEURONAL ORGANIZATION IN THE DORSAL RAPHE OF THE MONKEY. S.E. Kapadia,* N.C. de Lanerolle and C.C. LaMotte (Spon: E.E. Manuelidis). Sections of Gross Anatomy, Neurosurgery, and Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510.

The PAP method of Sternberger was used with an antibody to a 5-HT-BSA conjugate in order to identify serotonin neuronal structures in the dorsal raphe. Ultrathin serial sections revealed heavily immunostained 5-HT perikarya (18x22 um). Their nuclei showed multiple deep indentations. The cells had several mitochondria, a well formed Golgi body, and numerous RER cisternae, some in conspicuous laminar stacks. Dense cored vesicles (DVC) and lysosomal bodies were usually present. Immunoreactivity was intense in the cytoplasmic matrix. Outer membranes of mitochondria, RER and DCV were also stained. The immunoreactive beaded axonal varicosities contained round vesicles (18-35 nm) with large numbers of DCV (50-90 nm). Clusters of 5-HT unmyelinated (0.2-1.2 x 0.3-1.2 um) and myelinated (0.8-1.6 x 0.8-2.8 um) fibers were also observed. These 5-HT fiber bundles may provide evidence for the origin of both myelinated and unmyelinated fiber tracts from 5-HT somata within the dorsal raphe.

The 5-HT somata and dendrites were post synaptic to a variety of unlabelled terminals. These terminals contained (i) round vesicles (18-25 nm) and many small DCV (30-50 nm), or (ii) round vesicles (18-30 nm) with large DCV (90-110 nm), or (iii) small round vesicles (18-25 nm) with or without flattened vesicles. Simple synapses of these three terminal types with 5-HT structures were common. Two specialized relationships of unlabeled terminals and 5-HT targets were also observed: (i) 5-HT somatic and dendritic spines occasionally associated with crest synapses. (ii) 5-HT dendrites as the final postsynaptic element of a series of unlabeled synaptic terminals. Also observed was a 5-HT dendritic profile in apposition with another 5-HT dendritic profile, showing paramembranous thickening at the site of contact. This form of "dendritic bonding" was seen as part of a larger group of terminals and dendrites. This variety of terminals presynaptic to 5-HT structures suggests that several neuronal systems, perhaps with different neurochemicals, may modulate the 5-HT raphe neurons.

Occasionally, a 5-HT terminal was presynaptic to a 5-HT dendritic process. This relationship may be the anatomical basis for the physiological observation that 5-HT can inhibit 5-HT neurons. (Supported by NIH grant NS 13335).

25.12 COMBINED IMMUNOCYTOCHEMISTRY AND AUTORADIOGRAPHIC RETROGRADE AXONAL TRACING FOR IDENTIFICATION OF SEROTONERGIC RAPHE PROJECTION NEURONS. L. G. Isaacson*, D. A. Steindler* and B. K. Trosko* (SPON: W. M. Falls), Department of Anatomy, Michigan State University, E. Lansing, MI 48824.

A technique combining unlabeled antibody PAP immunocytochemistry and autoradiographic retrograde axonal tracing has been utilized for identification of serotonin-containing raphe projection neurons. Unilateral pressure injections (0.2 µl) of wheat germ agglutinin (WGA), N-[acetyl-^{3}H] (1.5 mCi/mg, 0.4% protein w/v, New England Nuclear) were stereotaxically placed within the caudate/putamen, thalamus, or hippocampus of six adult Hsd(ICR)BR white mice. Survival times ranged from 24-36 hours. One hour prior to sacrifice, the mice were injected with the MAO inhibitor pargyline (4 mg/ml, 75 mg/gm). Following cardiac perfusion with 4% formaldehyde, brains were removed, blocked and embedded in paraffin. Sections were cut at 4-5 µm in the coronal plane, mounted on gelatinized slides, dried and then incubated in anti-serotonin antiserum (1:100 dilution) for 24-48 hours at 4°C. They were subsequently processed using the PAP methods of Sternberger and a glucose oxidase-diaminobenzidine histochemical procedure. These sections were dried, coated with Kodak NTB-2 nuclear emulsion and exposed for 4-16 weeks at 4°C. As a control, sections were treated exactly as above, replacing primary antiserum with normal rabbit serum.

Immunoreactive, serotonin-containing cells were characterized by brown, granular reaction product within the somata and dendrites. Serotonin neurons were distributed throughout the dorsal raphe, median raphe, raphe pontis, magnus and obscurus similar to that described by Steinbusch (Neurosci. 6:557-618, 1981). No immunoreactivity was observed in control sections. Retrogradely labeled raphe projection neurons were identified by the presence of numerous silver grains over their somata and proximal dendrites. Double labeled neurons, exhibiting both immunoreactivity and autoradiographic retrograde labeling, were identified as serotonergic projections neurons. For example, serotonergic raphe-striatal projections neurons were distributed mainly within the rostral portions of the dorsal and median raphe nuclei. A few projection neurons, labeled from the striatal injection, stained negative for serotonin. These neurons most likely represent a smaller non-serotonergic raphe-striatal projection previously described (Steinbusch et al., Neurosci. Lett. 19:137-142, 1982).

The transmitter-specific neuronal tracing method described here utilizes immunocytochemistry in combination with WGA, N-[acetyl-^{3}H] axonal tracing. The high degree of sensitivity displayed by both of the labeling methods affords a permanent and accurate means of identifying neurotransmitters of projection neurons.

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25.13 LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY OF A DOPAMINE AND CYCLIC AMP-REGULATED PHOSPHOPROTEIN (DARPP-32) IN RAT BRAIN. C. C. Ouimet, H. C. Hemmings, Jr.* and P. Greengard. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

DARPP-32, a dopamine and cyclic AMP-regulated phosphoprotein with an apparent molecular weight of 32,000, has been studied in the brain by light and electron microscopic immunocytochemistry. The results suggest that DARPP-32 is enriched in a subclass of neurons that receive a dopamine input, and that it is absent from dopaminergic neurons themselves. Biochemical evidence (Walaas and Greengard, 1983) that the phosphorylation of DARPP-32 is regulated by dopamine and by cyclic AMP suggests that the DARPP-32 neurons belong to that subclass of dopaminoceptive cells possessing the D_1 receptor.

The indirect immunofluorescence technique was used for light microscopy and the peroxidase-anti-peroxidase technique of Sternberger was used for both light and electron microscopy. Antiserum specificity was demonstrated by immunoblotting and immunoprecipitation methods, and method specificity was shown by the lack of staining produced by appropriate control sera. DARPP-32 immunoreactivity was very strong in neuronal cell bodies and dendrites in those brain regions that are enriched in dopaminergic nerve terminals: caudatoputamen, nucleus accumbens, olfactory tubercle, bed nucleus of the stria terminalis, and portions of the amygdaloid complex. Within the caudatoputamen, immunoreactivity was present in medium size (10-15 µm diameter) neuronal cell bodies and in dendrites, spines and axons. A small number of nerve terminals in the caudatoputamen were also immunoreactive and these typically formed synapses upon neuronal elements that were likewise immunoreactive. In parasagittal sections, immunoreactive fiber bundles were traced from the caudatoputamen to the globus pallidus, entopeduncular nucleus, and pars reticulata of the substantia nigra, and within these regions, the labeled axons gave rise to immunolabeled nerve terminals. In the pallidal areas, and in the pars reticulata of the substantia nigra, indigenous cell bodies and dendrites were not immunolabeled. In the pars compacta of the substantia nigra and in other dopaminergic nuclei, immunoreactivity was never detected in neuronal cell bodies or dendrites.

DARPP-32 immunoreactivity may be an effective marker for neurons (or a subset of neurons) in which the action of dopamine is mediated through cyclic AMP and its associated protein kinase. In addition, DARPP-32 immunoreactivity will enable the identification of both the afferent and efferent neurons with which the DARPP-32-containing neurons interact. (Supported by USPHS Grants MH-17387 and NS-08440.)

25.14 DOPAMINERGIC NEURONES IN RAT DORSAL ROOT GANGLIA. Jack Price*. (SPON:J.P.Brockes) MRC Neuroimmunology Programme, Dept. of Zoology University College London, Gower Street, London WC1E 6BT, UK.

The sensory neurones of the dorsal root ganglia (DRG) can be classified on the basis of a number of criteria. Classically, a distinction has been drawn between 'large light' and 'small dark' cells (see Lawson et al, Cell Tiss. Res. 153:399-413 1974) and more recently sub-populations of the latter have been characterised on the basis of their peptide content (e.g. somatostatin- or substance P-containing) or the presence of fluoride-resistant-acid-phosphatase (FRAP).

We have recently shown a sub-population of small DRG neurones to be dopaminergic in the rat (Price & Mudge, Nature 301:241-243 1983). This conclusion was drawn from immunohistochemical data which demonstrated that these cells stain with an antibody which specifically binds the enzyme tyrosine hydroxylase (TH: Joh et al, Proc. natl. Acad. Sci. USA 76:509-513 1979) but not with another antibody which reacts with dopamine-β-hydroxylase (Geffen & Jarrott Handbk. Physiol. 15:521-571 1978). Further, the glyoxylic acid fluorescence method of De La Torre (J. Neurosci. Meths., 3: 1-5 1980) was used to show that these neurones synthesised and stored catecholamine, hence demonstrating the immunohistochemically-identified TH to be active.

These TH$^+$ sensory neurones have a number of interesting features. Firstly, they are found in DRG L5, L6 and S1 and in no other spinal ganglia. Secondly, they are not found in animals younger than 9 days of age. As rat DRG neurones become post-mitotic between embryonic days 10 and 14, the most likely explanation for this late development of the catecholaminergic phenotype is that the neurones have switched to dopamine from another neurotransmitter.

The question arises as to how these putative dopaminergic neurones fit into classifications of DRG neurones described hereto. Their size (16-20 um diameter) suggests they are of the 'small dark' variety and this is supported by the observation that they do not stain with RT97, a monoclonal anti-neurofilament antibody (Wood & Anderton, Biosci. Rep. 1:263-268 1981) which only stains 'large light" cells. However, they are smaller than the substance P-, somatostatin- or FRAP-containing cell populations, and studies in which alternate serial sections were stained with anti-TH and an anti-substance P serum, or anti-TH and FRAP, showed that most, if not all, TH$^+$ cells contain no substance P or FRAP. Similar studies with other anti-peptide sera are in progress.

(I would like to thank the Medical Research Council for financial support)

25.15 MAPPING OF HISTAMINE-IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF THE RAT. A.H. Mulder* and H.W.M. Steinbusch (SPON: P. Luiten). Dept. Pharmacology, Medical Faculty, Free University, Van der Boechorststr. 7, 1081 BT Amsterdam, The Netherlands.
A variety of neurochemical and neurophysiological data support the candidacy of histamine as a neurotransmitter in the central nervous system. However, thus far only limited evidence for the presence of histaminergic neurons is available, due to the lack of appropriate histochemical techniques. Here, we report on the development of a new immunohistochemical method and its application for the visualization of histamine in the rat brain. Procedures: Antibodies to histamine were raised in rabbits by immunization with a BSA-histamine or Haemocyanine-histamine conjugate. Optimum coupling conditions were established based upon retention studies using brain slices incubated with radiolabelled histamine. Untreated rats or rats pretreated with either colchicine and L-histidine, or 5,7-dihydroxytryptamine (5,7-DHT) or 6-hydroxydopamine (6-OH-DA) were used. Ten /um thick cryostat sections from different brain regions were obtained after a perfusion fixation with 4% paraformaldehyde and subsequently processed for immunofluorescence. Characterization of the antibodies: Immuno-inhibition tests, using conjugate-complexes to the catecholamines and serotonin, revealed no crossreactivity towards any of the monoamines (MA). Staining was not abolished after pretreatment with the neurotoxins 5,7-DHT and 6-OH-DA. A negative staining was observed in nuclei containing MA other than histamine, i.e. the substantia nigra (DA), the locus coeruleus (NA) or the nucleus raphe dorsalis (5-HT). Distribution: A dense innervation of histamine-immunoreactive varicose fibers was observed throughout the median eminence, especially in the lateral parts of the external and internal zone. These fibers run through the infundibulum stalk into the pars nervosa of the pituitary. A fairly large number of histaminergic fibers were seen in the nucleus hypothalamicus posterior and rostrally in the tractus diagonalis of Broca. Scattered fibers were found in the nucleus periventricularis hypothalami and thalami, the nucleus paraventricularis hypothalami, the amygdala, the subfornical region and in the periaquaductal gray. Weakly stained perikarya were demonstrated in the caudoventrolateral part of the hypothalamus. Strongly fluorescent mast cells were detected at the basal surface of the hypothalamus. In summary: the immunohistochemical method developed in this laboratory appears to be specific for histamine and opens the possibility of a complete mapping of histaminergic neurons in various species. It should be emphasized, however, that the fixation procedure needs further improvement for a better retention of histamine in brain regions with low levels of this monoamine.

25.16 Is such classification of nerves as 'cholinopeptidergic' and 'adrenopeptidergic' more appropriate than that of 'cholinergic', 'adrenergic' and 'peptidergic' in the autonomic ganglion?
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The fine structural characteristics of substance P (SP), somatostatin (SRIF), vasoactive intestinal polypeptide (VIP), enkephalin (ENK) and gastrin releasing peptide (GRP) have been revealed by the immunoelectron microscopic analysis with the PAP method in the celiac ganglion of guinea pigs and rats. SP and SRIF nerve fibers are characterized by abundant small clear vesicles mixed with a few large granular vesicles and the immunoreaction product appears in the axoplasm as well as the core of the large granular vesicle. VIP, ENK and GRPnerve fibers, on the other hand, are characterized by considerably numerous large granular vesicles mixed with small clear vesicles and the immunoreaction product is confined to the core of the large vesicles although their axoplasm appears slightly darker than the control. The small clear vesicle is free of the immunoreaction in either of the five peptide-containing nerve fibers. Each of the five peptide-fibers forms axo-dendritic, axo-somatic and some axo-axonic synapses with its own proportions of the three synapses. The immunopositive granular vesicles are usually located away from the synaptic sites in either of the five peptide fibers.

Serial section analysis of conventionally processed blocks of the same ganglia revealed that neuronal profiles containing considerably numerous large granular vesicles are often in cytoplasmic continuity with those containing small clear vesicle predominantly. The latter has conventionally been regarded as cholinergic type in the autonomic nerves.

All these findings suggest that there are two types of nerve fibers in the autonimic ganglion in terms of vesicle population; the one with single varicosities containing small clear vesicles predominantly, and the other with a varicosity containing large granular vesicles predominantly and another containing small clear vesicles predominantly. SP and SRIF fibers belong to the former type and VIP, ENK and GRP fibers belong to the latter type. It is further suggested that both two types of fibers are cholinergic. This infers that neuropeptides are not present in neuronal entity different from classical cholinergic and adrenergic nerves, but that the co-exstence of peptides and classical transmitters is more likely in the autonomic ganglion.

25.17 A NEW TECHNIQUE FOR THE DETERMINATION OF IMMUNOHISTOCHEMICAL SPECIFICITY. Cathrine A. Sasek and Robert P. Elde. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

Determination of immunohistochemical specificity is most commonly attempted using absorption controls, cross-reactivity studies and radioimmunoassay (RIA). These tests are seriously limited in that they characterize the antiserum for its ability to recognize presently characterized antigens. The method outlined below was developed in order to characterize the antigens revealed in a tissue section by immunohistochemical methods. This method has also been designed to account for changes in antigenicity during fixation. Since fixation has been shown to have a major effect on antigenicity, this is an important factor to consider in testing for specificity.

Antiserum raised against synthetic FMRF-NH_2 was used to develop this technique since, in addition to staining cells within pancreatic islets and cells and fibers throughout the nervous system, it has been shown to recognize a whole family of peptides in cross-reactivity studies. These include pancreatic polypeptide (PP), neuropeptide Y (NPY) and peptide YY (PYY). However, as in all immunohistochemical reactions, the molecular nature of the antigens in the tissue section is unknown. Fresh brain and pancreas were homogenized and extracted in 1M acetic acid. Small molecular weight peptides were collected after gel filtration (Ultrogel AcA 54, 1M acetic acid), desalted with XAD-2 resin and grossly separated according to molecular weight by gel filtration (Biogel P6, 3M acetic acid). Fractions were vacuum dried and reconstituted in small volumes of 1M acetic acid and methanol (1:1). Reconstituted fractions as well as synthetic peptides were subjected to thin layer chromatography on cellulose acetate (butanol, 4: acetic acid, 2: H_2O, 2). The chromatogram was fixed with paraformaldehyde vapors (80°C in vacuo, 1 hr.) and processed according to the PAP technique using anti-FMRF-NH_2 as the primary antiserum.

In the chromatograms immunostained peptides of pancreatic extracts, for example, did not show the same migration pattern as synthetic NPY, PYY, avian PP, and FMRF-NH_2. Thus it is likely that the peptide produced by the rodent pancreas and recognized with FMRF-NH_2 antiserum has a different sequence than previously characterized members of this family of peptides. This method should prove to be generally applicable in identifying the physico-chemical nature of low molecular weight antigens recognized by immunohistochemical techniques. It additionally has the advantage of not requiring special equipment and of allowing for the use of different solvents to seperate structurally similar peptides.
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25.18 LOCALIZATION OF SEROTONIN AND PEPTIDE NEUROTRANSMITTERS IN THE VENTRAL MEDULLARY RETICULAR FORMATION IN THE RAT. J.A. Andrezik, S.G. Remington*, and R.H. Ho. Department of Anatomical Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190 and Department of Anatomy, The Ohio State University, Columbus, OH 43210.

The reticular nuclei of the rostral ventral medulla (VMRF) are important areas influencing analgesia, respiration and cardiovascular reflexes. VMRF is composed of nucleus paragigantocellularis lateralis (PGCL), and nucleus gigantocellularis, pars alpha, (GCα). Serotonin, enkephalins, and other peptides have been reported in the VMRF. As a sequel to normal anatomical studies, we reacted the rostral medulla with antisera to serotonin (5-HT), substance P (SP), methionine enkephalin (ME) and neurotensin (NT). The location of the primary antibodies was visualized in frozen sections using the indirect PAP method of Sternberger. Adsorption controls indicated the specificity of staining.

Antiserum to 5-HT labels neurons mainly in GCα throughout its entire extent and a few neurons in PGCL. In both cases some of these are large triangular neurons. Numerous reactive terminals punctuate the PGCL neuropil, more than in GCα.

PGCL contains more neurons that react with SP antiserum than react with 5-HT antiserum. Most of these are medium-sized neurons or spindle shaped neurons. Some large triangular neurons and neurons close to the ventral surface react with anti-SP serum, indicating possible coexistence of SP and 5-HT within the same neuron. Terminals labeled with SP antiserum are found throughout the PGCL and GCα, but the number of labeled terminals is quite striking in the lateral PGCL.

Antiserum to ME labels some neurons in GCα, mainly in the lateral portion at caudal levels. PGCL contains many labeled cells - medium-sized triangular and small round neurons. At the level of the rostral inferior olive, there is a tightly packed group of neurons (15-20 in number) at the ventrolateral border of GCα, which stains intensely with the anti-ME serum. More rostral, at the level of the facial motor nucleus, most of the cellular labeling is found in GCα. PGCL is densely packed with labeled terminals throughout its extent.

Antiserum to NT labels large round and medium-sized triangular neurons only in the caudal PGCL. There is sparse terminal labeling in the midportion of PGCL, but it is not dramatic.

Antisera to 5-HT, SP, ME and NT label, for the most part, different populations of neurons in discrete areas of VMRF. There is, however, some overlap of terminal labeling in VMRF. We propose that there are local interactions between aminergic and peptidergic elements in VMRF and we are currently investigating the anatomy of these systems at the ultrastructural level. Supported by NIH Grants #NS-16868 (JAA) and #NS-10165 (RHH).

25.19 RADIOIMMUNOCYTOCHEMISTRY WITH ^{3}H-BIOTIN

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The high affinity of radiolabelled biotin for the glycoprotein avidin has been exploited in the design of techniques which can be used as a semiquantitative alternative to the peroxidase-anti-peroxidase (PAP) method or as a second marker system in combination with immunoperoxidase techniques. Tissue prepared by standard immunocytochemical protocols is exposed to primary antibody for 48-72 h followed by incubation with biotinylated protein A or antirabbit immunoglobulin and then incubation with a complex of ^{3}H-biotin and avidin. Tissue is subsequently prepared for autoradiography and dipped in liquid emulsion (Ilford K5 or L4) for light or electron microscopy; or apposed to LKB ultrofilm for semiquantitative analysis. Using these techniques it has been possible to visualise a large number of antigens in animal and human nervous tissue including avian pancreatic polypeptide (APP), enkephalin (ENK), substance P (SP), glutamic acid decarboxylase (GAD), tyrosine hydroxylase (TH) and somatostatin (SOM). Large areas of human brain have been scanned and differences in amounts of bound antibody quantified. Electron microscopy indicated that silver grains reflecting bound substance P antibody were largely over axon terminals and double labelling studies using ^{3}H-biotin in combination with immunoperoxidase markers confirmed the co-existence (APP/TH and APP/SOM) or lack of coexistence (SP/SOM, ENK/SOM) of a number of antigens in the peripheral and central nervous system. As a specific test of this semiquantitative technique we examined sections taken from a human spinal cord removed 48 h post-mortem. One leg had been amputated 24 months prior to death. A loss of substance P from the affected side had previously been noted using immunoperoxidase techniques (Hunt S.P. et al., Lancet 1:1082, 1982). Analysis of LKB film exposed to ^{3}H-biotin labelled tissue for 24 h and using computerised microdensitometry indicated a loss of 50% of the immunoreactive substance P on the affected side, suggesting that little recovery of central peptide levels occurs following section of the peripheral process of the sensory neuron. We are currently exploring the potential of the technique for quantifying peptide changes in a variety of degenerative disorders of the human brain including Parkinson's and Alzheimer's disease and Huntington's Chorea.

25.20 COLLOIDAL GOLD AND PEROXIDASE AS ULTRASTRUCTURAL MARKERS FOR SIMULTANEOUS LOCALIZATION OF TWO NEUROTRANSMITTER ANTIGENS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. Anthony N. van den Pol, Section Neurosurgery, Yale Univ. Sch. Med. New Haven, Ct. 06510.

Postembedding immunostaining with colloidal gold adsorbed to immunoglobulins or protein A was combined with peroxidase (PAP or biotin/ avidin) immunostaining to allow simultaneous identification of two putative neurotransmitter antigens in different neurons. Combinations of primary antibodies against peptides, transmitters, and enzymes localized in somata or processes in rat hypothalamic paraventricular nucleus were tried, including neurophysin, glutamic acid decarboxylase, tyrosine hydroxylase, somatostatin, ACTH, Substance P, and serotonin.

After aldehyde fixation thick vibratome sections were stained with the first primary antibody (e.g.,anti-ACTH) and subsequent series of immunoreagents; horseradish peroxidase was used as the final marker with diaminobenzidine as the substrate. After embedding in plastic, thin sections were cut and treated with hydrogen peroxide and then immersed in the second primary (e.g., anti-neurophysin) which was subsequently localized with either goat anti-rabbit IgG or protein A adsorbed to 5,10,or 20 nm colloidal gold particles. These procedures allowed simultaneous viewing with electron microscopy of two different sets of neurons, one labeled with peroxidase and the other with gold particles. Peroxidase labeling was found throughout the axonal or perikaryal cytoplasm of labeled neurons. Colloidal gold particles were found in highest concentrations over neurosecretory vesicles in perikarya, proximal dendrites, axons, and axonal terminals. Gold particles were found in quantities up to 125 times greater over neurosecretory vesicles than over an equal cross-section area of cytoplasm with an anti-neurophysin serum; areas on electron micrographs were determined with a stereological point count methodology. Colloidal gold was also used for pre-embedding immunocytochemistry; with appropriate dilutions gold was an effective marker for light microscopy. Intensification for light microscopy could be achieved with a FITC-conjugated IgG directed against the colloidal gold adsorbed IgG. Grain density of gold with ultrastructural analysis of sections immunostained prior to plastic embedding was 10 times greater in labeled perikaryal cytoplasm than in the nucleus or in adjacent neuropil. Controls with deletion of primary antiserum, adsorbtion with the homologous antigen, or substitution of an unrelated primary IgG were negative. Cross-reactivity of the first set of immunoreagents with the second set was negligible. The combination of colloidal gold and peroxidase immunocytochemistry allows simultaneous identification of both pre- and postsynaptic neuronal elements in a single thin section, or of two antigens in the same cell.(Supported by NIH NS16296 and NS10174.)

CYCLIC NUCLEOTIDES

26.1 PURIFICATION OF THE STIMULATORY AND INHIBITORY GUANINE NUCLEOTIDE REGULATORY UNITS OF BOVINE CEREBRAL CORTICAL ADENYLATE CYCLASE. E.J. Neer, and L.G. Wolf*. Cardiovascular Division, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 USA.

Hormone or neurotransmitter receptors either stimulate or inhibit the adenylate cyclase from bovine brain. Their actions on the enzyme are mediated by two distinct guanine nucleotide binding regulatory proteins, one of which (N_s) mediates stimulation of the adenylate cyclase catalytic unit (C) while the other (N_i) mediates inhibition.

We have purified both these regulatory proteins from bovine cerebral cortex. The activity of N_s is measured by its ability to stimulate a preparation of solubilized C which has been separated from N_s as described by Strittmatter, S. and Neer, E.J. (PNAS(1980)77,6344). The cholate solubilized N_s was purified by a modification of the method of Sternweis et al (J.Biol.Chem (1981)256,11377) for rabbit liver. The final specific activity was 2μmol cAMP/mg/min. By SDS polyacrylamide gel electrophoresis, the purified preparation contains a peptide of 52,000 daltons and, in lesser quantity, one at 45,000. Like N_s purified from rabbit liver, the brain N_s is associated with a 35,000 dalton peptide which does not, itself, have the ability to stimulate C. The 52,000 dalton protein can be phosphorylated by a protein kinase which copurifies with N_s activity.

N_i is identified by the fact that it can be [32P]ADP-ribosylated by pertussis toxin. The labelled polypeptide has a molecular weight of 40,000. Like N_s, it is associated with a 35,000 dalton protein which is not modified by the toxin.

The availability of purified components of adenylate cyclase from cerebral cortex now allows further characterization of their structure and function.

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26.2 SYNAPTIC MEMBRANE ADENYLATE CYCLASE GTP-BINDING PROTEIN SPECIFICALLY INTERACTS WITH RAT BRAIN TUBULIN. C.M. O'Callahan,* R.J. De Lorenzo and M.M. Rasenick (SPON: S.A. Shefner). Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510.

Activation of hormone-responsive adenylate cyclase involves association among several membrane-associated components: the hormone receptor, the Guanyl nucleotide binding subunit (G-Unit) and the catalytic moiety. Recent studies (Nature 294:560, 1981; Neurosci. Abs. 12:344, 1982) suggest that cytoskeletal elements might affect coupling among the rat cerebral cortex adenylate cyclase components. Colchicine or vinblastine augment adenylate cyclase activation by Gpp(NH)p or NaF but not Mn^{++} suggesting that the locus of their effect might be at the G-Unit. Furthermore, if cerebral cortex membranes are incubated with colchicine or vinblastine and subsequently washed, a 42,000 dalton GTP binding protein is specifically eluted.

The above results suggest that tubulin might serve as a possible "anchor" for some adenylate cyclase G-Units. In order to further explore this hypothesis, three cycle polymerized-depolymerized rat brain tubulin was added to synaptic plasma membrane under conditions where tubulin incorporates into artificial membrane (BBA 647:270, 1981). This resulted in a tubulin-concentration dependent inhibition of Gpp(NH)p or NaF-activated adenylate cyclase. Tubulin exerted this effect at a variety of Gpp(NH)p or NaF concentrations while Mn^{++} activated adenylate cyclase was unaffected by tubulin addition. These tubulin effects were noted only in adenylate cyclase from nervous tissue. Treatments which deplete synaptic membrane tubulin both increased the effects of tubulin addition and generally improved Gpp(NH)p and NaF activation of adenylate cyclase.

When G-Unit was covalently labelled with (^{3}H)P3-4-Azidoanilido P1-5' GTP (AAGTP) and passed over a tubulin affinity column, labelled material bound to the column and was eluted with increasing NaCl concentrations. This procedure resulted in an enrichment of M_r 42,000 AAGTP labelled protein. SDS gel electrophoresis of synaptic membrane associated proteins followed by tubulin hybridization indicates that M_r 42,000 AAGTP labelled protein may specifically interact with rat brain tubulin.

These data are consistent with a hypothetical G-Unit - Tubulin association within the neuronal plasma membrane. It is possible that neuronal cytoskeletal dynamics modulate hormone-responsive adenylate cyclase.

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26.3 ACTIVATION AND INHIBITION OF SYNAPTIC MEMBRANE ADENYLATE CYCLASE BY MULTIPLE GTP BINDING PROTEINS. M.M. Rasenick and R.L. Malina*, Dept. of Neurology, Yale Univ. Sch. Med., New Haven, CT 06510.

GTP or hydrolysis-resistant GTP analogues (e.g. guanylylimidiphosphate - Gpp(NH)p) promote stable activation of rat synaptic membrane adenylate cyclase (AC) which persists subsequent to washing of membranes. This stable activation is time, temperature and Gpp(NH)p concentration dependent, and is thought to reflect the coupling of the AC catalytic moiety with the GTP binding regulatory subunit (G-unit).

We find that "stable" AC activation by Gpp(NH)p is subject to further augmentation by subsequent incubation of membranes with Gpp(NH)p. This phenomenon depends upon multiple exposure to Gpp(NH)p and subsequent washing and not upon total Gpp(NH)p incubation time. Furthermore, if low (<1μM) Gpp(NH)p concentrations are present during AC assay, inhibition of "stable" Gpp(NH)p AC activation results. Colchicine, which is thought to promote brain AC coupling (Nature 294: 560, 1981; Science 219: 65, 1983) augments both the increase and the decrease in "stable" AC activation. Binding studies with (^{3}H)Gpp(NH)p reveal that although (^{3}H) Gpp(NH)p binding is relatively stable to washing, about 50% readily exchanges with unlabelled GTP analogues. Treatment of (^{3}H) Gpp(NH)p-labelled membranes with colchicine, which may favor formation of G-unit/catalytic moiety complexes, diminishes (^{3}H) Gpp(NH)p exchange.

The photoaffinity GTP probe (^{3}H)P3-4-azidoanilido P1 5' GTP (AAGTP) was employed to ascertain whether activation and inhibition of AC might be controlled by separate GTP binding proteins and if their GTP binding varied under conditions of Gpp(NH)p or NaF-activation, or Gpp(NH)p-dependent inhibition. Three AAGTP binding proteins, M_r 42,000, M_r 40,000 and M_r 35,000 respectively, were observed. Conditions for competition of AAGTP label (prior to photolysis) were different for the M_r 40,000 and M_r 42,000 proteins. It is hoped that extensions of these studies will provide insight into the mechanisms of activation and inhibition of neuronal adenylate cyclase.

26.4 RECEPTOR-MEDIATED MODULATION OF GTPase ACTIVITY BY NEUROTRANSMITTERS IN RAT STRIATUM. M.C.Olianas and P.Onali. Inst.Pharmacology & Biochem. Pathology and Inst. Pharmacology,University of Cagliari,09100 Cagliari,Italy.

In synaptic plasma membranes of rat striatum neurotransmitters which affect adenylate cyclase activity stimulate a high affinity GTPase. Acetylcholine (Ach) inhibits adenylate cyclase and increases GTP hydrolysis via muscarinic receptors. Chronic acetylcholinesterase inhibition by diisopropylfluorophosphate(DFP) administration results in desensitization of muscarinic inhibition of striatal adenylate cyclase. The maximal decrease of basal enzyme activity elicited by Ach is reduced by 40-50% in membranes of DFP treated rats when compared to controls. The reduction of Ach-inhibition of adenylate cyclase is associated with a proportional decrease of the maximal stimulation of GTPase activity elicited by Ach. The EC_{50} values of Ach in inhibiting adenylate cyclase (3.5 μM) and in stimulating GTPase (1 μM) are slightly increased (two-three fold) by DFP treatment. Occupancy of striatal dopamine (DA) receptors by DA causes activation of adenylate cyclase and GTPase. At maximal concentrations of each agonists (100 μM) the stimulation of GTPase by DA is additive with that elicited by Ach. When the DA-activated adenylate cyclase increases after unilateral lesioning of the nigrostriatal pathway with 6OHDA,the DA-stimulated GTPase activity becomes supersensitive. The supersensitivity is characterized by 80% increase of the maximal GTPase activation by DA with no significant changes of the EC_{50} of DA (1.8μM)These results indicate that alterations of the central neurotransmission in vivo elicit changes in the responses of GTPase to neurotransmitters which parallel those of adenylate cyclase.

26.5 DIFFERENTIAL MODIFICATION OF THE INHIBITORY EFFECTS OF GTP, GPP(NH)P AND GTPγS ON FORSKOLIN-STIMULATED ADENYLATE CYCLASE. M.M. Smith* and T.K. Harden. Dept. of Pharmacology, Univ. North Carolina Sch. Med., Chapel Hill, NC 27514.

Opiate, alpha$_2$, and muscarinic receptor-mediated inhibition of adenylate cyclase (AC) apparently occurs through an inhibitory guanine nucleotide regulatory protein (N_i). We have previously reported that relatively low concentrations of N-ethylmaleimide (NEM) blocks inhibitory coupling of these three receptors to AC in membranes from NG108-15 neuroblastoma x glioma cells, without affecting receptor-mediated stimulation. Experiments have been carried out to determine if the effect of NEM occurs at the level of N_i. Forskolin-stimulated AC activity is inhibited in a concentration-dependent manner by guanine nucleotides, and this effect has been proposed to be an expression of N_i activity (J. Biol. Chem. 257:14723, 1982). In membranes from NG108-15 cells, forskolin-stimulated AC was inhibited by GTP, Gpp(NH)p, and GTPγS by 35%, 50%, and 50%, respectively. The corresponding $K_{0.5}$ values were 300 nM, 10 nM, and 3 nM. Subsequent to NEM treatment, GTP stimulated, rather than inhibited, forskolin-stimulated AC activity. The concentration effect curve for NEM-induced blockade of the inhibitory activity of GTP was the same as for NEM-induced blockade of alpha$_2$, opiate, and muscarinic receptor-mediated inhibition of AC. In contrast to the effect of NEM on GTP-mediated inhibition, the inhibitory effects of Gpp(NH)p and GTPγS were not affected by NEM.

These results have been confirmed in another cell line, the cyc$^-$ mutant of S49 lymphoma cells, which apparently expresses N_i but lacks the stimulatory guanine nucleotide regulatory protein, G/F. GTP, Gpp(NH)p, and GTPγS inhibited basal and forskolin-stimulated AC activity of cyc$^-$ membranes with efficacies and potencies similar to those observed in NG108-15 membranes. NEM pretreatment resulted in increased basal and forskolin-stimulated activities, but had no effect on 10 mM Mn^{++}-stimulated activity. NEM blocked the inhibitory effect of GTP on forskolin-stimulated AC activity with the same concentration effect curve as was seen in NG108-15 membranes, but had no effect on Gpp(NH)p- or GTPγS-mediated inhibition.

These data are consistent with three conclusions: I) NEM blocks inhibitory coupling of receptors to AC by an effect distal to the receptor-N_i interface. II) Gpp(NH)p- and GTPγS-mediated inhibition of forskolin-stimulated AC are not equivalent to GTP-mediated inhibition. III) N_i mediates a tonic inhibition of AC as evidenced by the increase in activity in NEM pretreated cyc$^-$ membranes. Supported by GM29536 and an Established Investigatorship of the American Heart Association.

26.6 CALMODULIN-STIMULATED ADENYLATE CYCLASE ACTIVITY IN BOVINE RETINA. N. Muirhead*, G. Treisman*, P. Simpson*, and M.E. Gnegy. Dept. Pharmacology, Univ. of Michigan Med. Sch., Ann Arbor, MI 48109

Ca^{2+} and calmodulin (CaM)-stimulated adenylate cyclase (AC) has its greatest activity in brain although AC in a few other tissues such as pancreas are slightly responsive to CaM. Retina is a CNS tissue with the advantage of having limited types of neurons and being responsive to varied lighting conditions. Retina also has a relatively pure population of dopamine (DA) D-1 type receptors that stimulate AC activity and that are responsive to changes in lighting. We find a high level of CaM-stimulated AC in retina with activity comparable to that of cerebral cortex. In addition, the ability of CaM to stimulate in bovine retina was responsive to lighting conditions in the same manner as DA stimulation of AC.

CaM-stimulated AC was found in both rat and bovine retina. AC was measured in retinal particulate preparations (27,000 x g) that were washed twice in a buffer containing 1.2 mM EGTA. Maximal stimulation was achieved by 5 μg/0.2 ml (1.4 μM) CaM. Stimulation was dependent upon CaM and was biphasic as a function of Ca^{2+}. Maximal stimulation occurred at an effective Ca^{2+} concentration of 10^{-7} M. AC was stimulated a maximum of 5.5-fold in rat and 7-fold in bovine retinal membranes.

Stimulation of bovine AC by CaM was altered by the lighting conditions under which the retina was dissected. The sensitivity of AC to CaM was greatest when retinas were dissected under a red safety light in a darkroom. The apparent Ka (K_a^{app}) for CaM was 40 nM and the apparent Vmax (V_{max}^{app}) was 184 pmol/min/mg protein. When retina was dissected under fluorescent lights the K_a^{app} for CaM was 80 nm and the V_{max}^{app} was 174 pmol/min/mg protein. In contrast, the lowest activity was found when the retinas were dissected in dim light. The K_a^{app} for CaM-stimulated AC was 141 nM and the V_{max}^{app} was 103 pmol/min/mg protein. These results were duplicated when retinas were dissected under red light and then incubated in an oxygenated Krebs Ringer Buffer for 30 min in red, fluorescent or dim lighting. Lighting effects on DA-stimulated AC paralleled that of CaM-stimulated AC. In contrast, stimulation of AC by guanyl nucleotides was not affected by lighting. Under all of the lighting conditions CaM could increase the V_{max}^{app} of the DA-stimulated AC activity. This work is significant because there is a high activity of CaM-stimulated AC in retina and it can be physiologically regulated by light in a manner similar to that for DA-stimulated AC.

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26.7 CALMODULIN-STIMULATED ADENYLATE CYCLASE ACTIVITY IS INCREASED IN STRIATUM FROM CHRONIC HALOPERIDOL TREATED RATS. G. Treisman*, N. Muirhead* and M.E. Gnegy. Dept. Pharmacology, Univ. MI Med. Sch., Ann Arbor, MI 48109

Chronic dopamine (DA) receptor blockade with haloperidol (HAL) results in behavior supersensitivity to dopaminergic agonists. We have previously shown that chronic HAL treatment results in a biochemical supersensitivity of striatal adenylate cyclase (AC) to DA. This change was accompanied by an increase in the endogenous Ca^{2+}-binding protein, calmodulin (CaM) in HAL membranes. In vitro CaM increased DA sensitivity of striatal AC. CaM directly activates AC in striatal membranes in a Ca^{2+}-dependent manner. We investigated whether factors that are known to affect the striatal AC beyond the DA receptor, CaM and guanyl nucleotides, have altered responses in DA supersensitive animals.

We and others have shown that two components of AC are present in striatal membranes, a CaM-stimulated component and a CaM-insensitive component. We first examined whether the CaM-stimulated AC activity was altered by chronic HAL. Male Sprague-Dawley rats were injected with 1 mg/kg HAL s.c. for 10 days and then withdrawn for 3 days. Control rats were injected s.c. with 0.1 ml vehicle (VEH). CaM-stimulated AC activity was measured in a striatal particulate preparation (27,000 x g) washed twice with buffer containing 1.2 mM EGTA. Membranes from HAL-treated animals were 3-fold more sensitive to CaM than the VEH-treated controls. This was demonstrated by a significant shift in ED_{50} from 300 ng in VEH-membranes to 100 ng in membranes from HAL animals.

Guanyl nucleotides are required for DA-stimulation of AC activity. We found previously that the CaM-stimulated component could be selectively activated by low GppNHp concentrations, but not by GTP. The CaM-sensitive activity is stimulated by high GppNHp or GTP. We therefore examined the effect of guanyl nucleotides on striatal AC in VEH- and HAL-treated rats. We found that membranes from HAL animals showed nearly twice the activation by a low concentration of GppNHp (0.2 µM) than controls; 97% stimulation versus 56% stimulation. This concentration selectively activates the CaM-sensitive AC fraction. Stimulation by GTP and high concentrations of GppNHp, on the other hand, did not differ significantly in membranes from HAL- and VEH-treated animals.

These results demonstrate that chronic blockade of the DA receptor results in an increase in CaM-stimulated AC activity as well as an increase in the DA-stimulated AC activity. This may be due to a selective change in a CaM-sensitive component of striatal AC activity. Supported by MH36044-02.

26.8 CHOLINERGIC RECEPTOR-MEDIATED INHIBITION OF FORSKOLIN STIMULATED ADENYLATE CYCLASE IN THE INTACT HUMAN FIBROBLAST: DIVALENT CATION EFFECTS. R.H. Lenox[1], D.A. Van Riper[1]* and M. Absher[2]*. [1]Neuroscience Research Unit, Dept. Psychiatry and [2]Dept. Medicine, Univ. of Vermont College of Med., Burlington, VT 05405

Regulation of adenylate cyclase (AC) enzyme system involves the interaction of multiple protein components including a catalytic subunit, a guanine nucleotide binding protein and a hormone receptor. Cholinergic inhibition of AC via a muscarinic receptor has been demonstrated in a number of tissues and cell types. Forskolin (FSK), a diterpine, has been shown to stimulate AC activity in both intact cell and broken membrane preparations by an apparent direct interaction with the catalytic subunit. Ions and guanyl nucleotides can modulate AC activity in cell membrane preparations independent of interaction with hormone specific cell-surface receptors. Recently, Hoffman et al. (BBRC, 1981) reported that manganese (Mn++) could preferentially uncouple the α_2 adrenergic receptor mediated inhibition of AC in human platelet membranes. As part of our investigation of receptor regulation of AC activity in intact human cells, we have initiated a series of studies examining divalent cation effects in cultured human diploid fibroblasts which possess muscarinic receptors that inhibit AC activity in response to cholinergic stimulation.

Human fetal lung fibroblasts (IMR-90) were cultured in Minimal Essential Medium with 10% Fetal Bovine Serum. 10^5 cells were seeded into 35 mm culture dishes in 3.0 ml medium and incubated at 37°C in 5% CO_2·95% air. At stationary phase cultures were labeled for one hour with 2 µCi [^{3}H]adenine. Buffers for cation studies were supplemented with varying concentrations of either magnesium (Mg++), calcium (Ca++) or Mn++. The enzyme reaction was initiated by the addition of FSK ± carbachol and terminated after 10 min at 37°C. The [^{3}H]cAMP formed was isolated using a modification of the sequential chromatography method of Salomon et al. (ACNR, 1979).

Incubation of the cells with FSK (0.01-10 µM) resulted in a concentration-dependent increase in cAMP production. Basal cAMP accumulation was unchanged by concentrations of Mg++ or Ca++(0.01-10 mM), but did increase in the presence of 1.0-10 mM Mn++. FSK stimulated cAMP accumulation was enhanced in the presence of higher concentrations of Mg++ and Mn++ but not Ca++. Carbachol (10 µM) inhibited up to 85% of FSK stimulated cAMP production and was unaffected by Mg++ or Ca++. In the presence of Mn++, however, there was a concentration dependent antagonism of the carbachol inhibition, resulting in complete elimination of carbachol activity at 10 mM Mn++. These findings indicate a selective effect of extracellular Mn++ on muscarinic receptor mediated inhibition of AC in the intact human fibroblast. These data will be discussed in light of current models of receptor mediated inhibitory regulation of AC activity.

26.9 ASCORBIC ACID DECREASES [^{3}H]-DOPAMINE BINDING IN STRIATUM WITHOUT INHIBITING DOPAMINE-SENSITIVE ADENYLATE CYCLASE. David W. Schulz* Mark H. Lewis, John Petitto* and Richard B. Mailman (SPON: C. Mitchell). Biological Sciences Research Center and Depts. of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27514

Ascorbate, which occurs in high concentration in cells of neural crest origin (e.g., the CNS and adrenal), is a chemical messenger candidate. One of the first non-biosynthetic biochemical effects ascribed to ascorbate in the CNS was its ability to inhibit dopamine stimulated adenylate cyclase (DA-ACase) in homogenates from striata of Long Evans rats (J. Neurochem. 28:663, 1977). Because of the importance of changes in cAMP concentrations in vivo, we investigated this phenomenon further. An improved adenylate cyclase assay based on reverse phase preparative high performance liquid chromatography (HPLC) (Schulz and Mailman, J. Neurochem. submitted) was used. After the usual incubation, the reaction is stopped by addition of SDS, heating, addition of 4.5% $ZnSO_4$-10% $Ba(OH)_2$, and centrifugation. The supernatant is subjected to automated preparative HPLC using a Waters Z-Module with a 10 µ C_{18} reverse phase cartridge, and 0.15M sodium acetate-20% methanol (pH 5.0) mobile phase. The presence of nonradioactive cAMP in the enzyme incubation mixture allows the use of UV (254nm) monitoring to trigger fraction collection (via Isco FOXY® with Peak Separator) automatically. Using this method, we were unable to detect any inhibition of DA-ACase by ascorbate at concentrations as high as 1mM, although 5.68 mM ascorbate inhibited basal adenylate cyclase activity by ca. 30% while not inhibiting the stimulation by dopamine. This failure to find inhibitory effects of ascorbate on dopamine-stimulated adenylate cyclase occurred when using striatal homogenates from Sprague-Dawley or Long-Evans rats, or N.C. Board of Health mice. However, ascorbate was found to decrease significantly the binding of [^{3}H]-dopamine to striatal membranes. Thus, it appeared that the sites binding [^{3}H]-dopamine that are affected by ascorbate are unlikely to be the same ones coupled to adenylate cyclase. This hypothesis was supported by our finding that the profile of DA-ACase inhibition by cis-flupenthixol is unaltered in the presence of 5.68 mM ascorbate. Interestingly, as has been reported, 5.68mM ascorbate did not affect the binding of [^{3}H]-spiperone to these striatal membranes. Although ascorbate may play a neuromodulatory role, it does not appear that its effects are mediated through effects on cAMP biosynthesis.

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26.10 MODULATION OF CYCLIC AMP STIMULATED SLOW INWARD CURRENT IN A BURSTING NEURON BY A CALMODULIN ANTAGONIST, pH_i, AND Ca^{++}. Rhanor Gillette and Daniel J. Green. Neural and Behavioral Biology Program, Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

Food stimuli induce prolonged and endogenously sustained burst episodes in feeding command neurons, the ventral white cells (VWCs), of the carnivorous marine snail Pleurobranchaea californica. Such burst episodes drive multiple cycles of vigorous feeding motor output. The neuromodulatory action of appetitive chemosensory pathways on the VWCs is mimicked by treatments that elevate intracellular cyclic AMP (J. Neurosci., in press; J. Comp. Physiol. 146:461), which is the presumed natural regulation of VWC excitability. Cyclic AMP potentiates a slow inward current that sustains the burst episode (Green and Gillette, this volume). We previously showed that the action of cyclic AMP is itself mimicked by the effects of treatments that slightly elevate intracellular pH and by the effects of the anti-calmodulin Phenothiazine drugs (J. Neurophysiol. 49:509; Brain Res., in press). These agents may act through Ca^{++}-activated, pH sensitive phosphodiesterase activity (Brain Res., in press). In order to assess the effects of those agents on cyclic AMP action, we measured the slow inward current response to iontophoretically injected cyclic AMP in voltage-clamped cells. Iontophoretic pulses of 0.2 M cyclic AMP (pH 7.4) 5-10 seconds long caused a initial steep rise in inward current which peaked several seconds after the pulse and declined slowly over 30-60 seconds. Iontophoretic currents were adjusted to give a replicable response (1-2 nA peak) far below the cell's maximum. While the rate of rise, time to peak and peak amplitude of the response were stable over long periods, the period of decline was less stable. This last may reflect dephosphorylation processes in cyclic AMP regulation of inward current.

The calmodulin blocker trifluoperazine, and intracellular alkalinization by 15 mM NH_4^+ salines both caused marked increases in rate of rise, time to peak, and peak amplitude of the cyclic AMP current response. Conversely, prolonged depolarization, spike discharge and attendant Ca^{++} influx for 1-2 minutes caused reductions in those parameters of cyclic AMP responsiveness.

These data suggest that Ca^{++} influx contributes to burst termination in part through regulation of cyclic AMP levels. These data are also consistent with the action of a Ca^{++}-calmodulin activated PDE in mediating the effects of phenothiazines, pH and Ca^{++}. Supported by NSF BNS 79-18329 to R. G. and PHS 5T32 GM07143 to D.J.G.

26.11 POSSIBLE ROLE OF CYCLIC GMP AND CYCLIC AMP IN MEDIATION OF MUSCARINIC RESPONSES IN XENOPUS OOCYTES. N. Dascal*, I. Lotan*, Y. Oron* and Y. Lass. Dept. Physiol. Pharmacol., Sackler Sch. Med., Tel Aviv University, Ramat Aviv 69978, Israel.

Acetylcholine (ACh) and other muscarinic agonists evoke a complex response in Xenopus oocyte membrane, which consists of four components: a transient inward current, D1, followed by a slow prolonged inward (D2) or/and outward (H) currents, accompanied by large current fluctuations (F). At high (usually $>1\mu M$) ACh concentrations H is obscured by D2. The response components may be separated pharmacologically and on the basis of a seasonal variation. D1, D2 and F result from changes in Cl^- conductance, H is a K^+ current. D1 and H are partially mimicked by extracellularly applied cyclic GMP (cGMP) and its analogs and inhibited by external cyclic AMP (cAMP) analogs(Kusano K., Miledi R. & Stinnakre J., Nature, 270:739(1977); Kusano K., Miledi R.,& Stinnakre J., J.Physiol.,328:143(1982); Dascal N. & Landau E.M., Life Sci.,27: 1423(1980); Dascal N. & Landau E.M., Proc.Natl.Acad.Sci.USA, 79: 3052; Dascal N., Landau E.M. & Lass Y., in preparation).

cGMP was injected intracellularly, by pressure, through a micropipette, while the oocyte was voltage clamped using other two micropipettes. Injection of 0.6--10 pmoles of cGMP into the cells evoked large outward current accompanied by a conductance increase and having reversal potential of -85 to -96 mV, which is close to the K^+ equilibrium potential. This H-like response was inhibited by external tetraethylammonium (TEA; 40mM) and thus was concluded to represent a K^+ current. Higher doses of cGMP elicited outward currents with less negative reversal potentials (-52 to -75 mV) or inward current superimposed on the plateau of the outward current evoked earlier by a lower cGMP dose.F-like current fluctuations were frequently observed. When the H-like response was abolished by TEA, the inward current was shown to be associated with a conductance increase and to depend on external Cl^- concentration and was thus concluded to represent a Cl^- current. When ACh was applied at the peak of cGMP-induced H-like response, D1 and D2 were enlarged while H was suppressed. Externally applied phosphodiesterase inhibitors reduced D1, D2 and H. Intracellular cAMP level decreased following ACh application by about 30%, with a kinetics which parallelled the time course of D1 and D2. The results are in line with a hypothesis assuming mediation of Cl^- conductance increase through cAMP level decrease, and of K^+ conductance increase through cGMP level increase.

26.12 THE ROLE OF CYCLIC AMP IN ADENOSINE-EVOKED SLOW POTASSIUM CURRENT IN XENOPUS OOCYTES. I. Lotan*, N. Daskal*, Y. Oron*, S. Gelerstein*, S. Cohen* and Y. Lass. (SPON: Z. Wollberg). Dept. Physiol. & Pharmacol., Sackler Sch. Med., Tel-Aviv University, Ramat Aviv 69978, Israel.

The membrane of Xenopus laevis oocytes exhibits a complex response to application of adenosine: i) an early transient inward current (D1) carried by Cl^- ii) a slow, steady outward current (H) carried by K^+ iii) a slow Cl^- inward current (D2), usually masked by H. The adenosine induced H current is selectively inhibited by theophylline. The potency sequence of purinergic agonists in eliciting the H-response is: 5'-N-ethylcarboxamide - adenosine > adenosine $\simeq$ AMP > ADP $\simeq$ ATP (Lotan I., Dascal N., Cohen S. and Lass Y., Nature, 298:572, 1982). The data suggest that the H response is mediated through the activation of a purinergic receptor belonging to R_a, or P_1 type which is supposed to exert its physiological effects through elevation of intracellular cyclic AMP (cAMP) level (Londos C., Cooper D.M.F. and Wolff J., PNAS, 77:2551, 1981).

Intracellular injection of 0.15-5 pmole cAMP (applied iontophoretically or by pressure) caused a dose-dependent outward K^+ current similar to the adenosine induced H-response. Intracellularly injected AMP was almost ineffective even at very high doses. The adenosine induced K^+ current superimposed on a relatively small cAMP induced current (up to 30 nA) was additive; however, when the cAMP response was increased, the additivity was lost. Extracellularly and intracellularly applied phosphodiesterase inhibitors, which by themselves evoked a moderate outward K^+ current, strongly enhanced the cAMP response.Intracellularly applied phosphodiestrase inhibitors (theophylline and IBMX) enhanced the cell response to adenosine. Preliminary measurements of cAMP showed an increase by 20-300% following adenosine application. These results suggest that the adenosine-induced K^+ conductance increase in Xenopus oocytes membrane is mediated by elevation of cAMP level.

26.13 ADRENALECTOMY MODIFIES NEUROTRANSMITTER-STIMULATED CYCLIC AMP ACCUMULATION IN HIPPOCAMPAL SLICES. A.L. Harrelson* and B.S. McEwen, The Rockefeller University, New York, NY 10021, and W. Rostene, INSERM, U.-55, Paris 75571 France (SPON: G.Dohanich).

The hippocampus is the principal CNS target site for glucocorticoid hormones. In order to more closely link steroid hormone action on hippocampus to events involving neurotransmitters, we have investigated the effects of adrenalectomy on the ability of various neuroactive substances to stimulate cyclic AMP (cAMP) accumulation in slices of rat hippocampus. Male rats 8-10 weeks old were bilaterally adrenalectomized under ether anaesthesia. Four to six days later, animals were decapitated, the brains were rapidly removed, and dissected hippocampus cut twice on a McIlwain tissue chopper at 325µm. Slices were pre-incubated in Krebs-Ringer buffer (pre-equilibrated with oxygen) for 60 min. at 37°C in a shaking water bath, then aliquoted into incubation tubes. After 12 minutes of stimulation with the appropriate agonist, the incubation was stopped with 10% TCA. After ether extraction, cAMP was measured using a competitive binding assay. Where indicated, 30µm ZK 62,711 (Schering AG) was included in the incubation medium to inhibit phosphodiesterase activity.

Basal cAMP values were not different between adrenalectomized (Adx) and sham-operated or intact controls. Hippocampal slices from Adx rats showed greater cAMP accumulation than controls with vasoactive intestinal peptide (VIP) at both 10nM and 100nM (levels of 315 pmoles cAMP/mg protein vs. 183 pmoles/mg protein for controls, $p < 0.005$, Student t-test). When ZK 62,711 was added to the medium, similar results were obtained. Cyclic AMP accumulation stimulated by isoproterenol (10µM) was increased in Adx rats (23.5 vs. 16.4, $p < 0.005$). There was no significant difference when slices were stimulated with adenosine, prostaglandin E1, forskolin, and with 30µM norepinephrine + 5µM propranolol (a protocol used to stimulate α-receptors). Histamine-stimulated cAMP accumulation tended to be less in Adx rats. We conclude that adrenalectomy changes the response of hippocampal adenylate cyclase to several putative neurotransmitters; furthermore, the Adx effect is not due to a general increase in adenylate cyclase activity, since other adenylate cyclase agonists show no change after Adx, and the effect of one agonist (histamine) appears to change in the opposite direction. Future studies will examine the effects of steroid replacement in Adx animals, the biochemical mechanism responsible for the increase in cAMP accumulation, and the extent to which these changes are seen in other brain regions which concentrate glucocorticoids to a lesser extent than hippocampus.

26.14 A CORTICOSTERONE SYNTHESIS INHIBITOR (METOPIRONE) RAPIDLY UP REGULATES NORADRENALINE STIMULATED CYCLIC AMP FORMATION IN HIPPOCAMPUS IN VIVO. V.J. Roberts and R.L. Singhal*. Department of Pharmacology, University of Ottawa, Ontario, Canada. K1H 8M5

Beta adrenergic stimulation is known to produce a number of biochemical responses such as gluconeogenesis in liver and lipolysis in adipose tissue. These effects are mediated via adenylate cyclase stimulation and require the «permissive» effect of corticosterone (CORT). Recent evidence suggests that CORT modulates noradrenergic mechanisms in the brain as well. Mobley and Sulser (Nature, 286: 608, 1980) reported an increase in noradrenaline (NA) stimulated cyclic AMP (cAMP) production in rat frontal cortex 2 weeks following bilateral adrenalectomy. We reported (Neurosci. Abs. 93.8, 1982) a similar effect in rat hippocampus. In addition, we demonstrated that treatment with a CORT synthesis inhibitor (Metopirone) resulted in a substantial increase in NA-stimulated cAMP production in rat hippocampus. Tolerance to this effect developed within one week. Metopirone had no significant effect on the cAMP generating system in vitro. In order to characterize this modulation further the time course of the effect was examined and various in vitro manipulations were performed. Male Wistar rats (200 g) received a 50 mg/kg i.p. injection of Metopirone 1, 2 or 4 hours prior to sacrifice. Control animals received an equal volume of the vehicle (40% propylene blycol in water). The hippocampi from 3 rats were pooled and sliced. The slices were pre-incubated for 20 min then incubated for 40 min in the presence of (^{3}H) adenine to lable ATP stores. Adenylate cyclase was stimulated by the addition of 0, 1, 10 or 100µM NA for 10 min. For in vitro experiments agents were added to and remained in the medium during all incubations (Total time = 1 hr). While Metopirone treatment had no significant effect on cAMP production after 1 hour, a significant elevation in NA-stimulated cAMP formation did occur 2 hours following Metopirone treatment. A slight but non-significant elevation remained at 4 hours. This effect parallels the CORT synthesis inhibiting effect of Metopirone as determined by serum CORT levels. The addition of CORT to the incubation medium did not modify cAMP production in hippocampal slices from treated animals. In addition, two in vitro CORT antagonists, 11-Deoxycortisol and progesterone, did not alter cAMP production in hippocampal slices from control animals. A longer incubation may be necessary to observe an effect. While we have hypothesized a direct modulation of CORT on NA-stimulated cAMP production, the in vitro data underscores the possibility that the observed increase in cAMP formation may be due to secondary changes such as increased ACTH levels. (Supported by grants from the OMHF and the MRC).

26.15 THE EFFECTS OF MUSCLE MEMBRANE ACTIVITY AND NERVE-CONDITIONED MEDIA ON cAMP and cGMP LEVELS IN CULTURED MOUSE MYOTUBES, J.A. Powell*, L.J. Standish* and E.S. Swearengen* (SPON: D. Fambrough). Clark Science Center, Smith College, Northampton, MA 01063.

Cyclic nucleotides have been implicated in the mechanism by which both muscle activity and neurotrophic factors modulate the metabolism of acetylcholine receptors in skeletal muscle (Betz, H. and Changeux, J., Nature, 278: 748, 1979; Powell, J.A. and Glenn, C. J. Cell Biol., 95: 357a, 1982). We have investigated both the short and long term effects of muscle activity on cyclic nucleotide levels in cultured mouse myotubes. Short term activity was monitored by initiating contractions in quiescent muscle (12 mM K^+ or TTX treated) by addition of serum-free medium (normal K^+, 5 mM). Cultures were harvested at short intervals up to 120 min. Cyclic nucleotides were extracted from homogenates and measured by a radio immune assay. Long term activity was monitored in active or quiescent (TTX or high K^+ treatment) muscle maintained in complete medium for 1 to 8 days. Long term muscle activity had little effect on cAMP steady-state levels relative to that in quiescent muscle. However, cGMP concentrations were elevated 1.5 to 5 fold in active muscle. Short term muscle activity produced a coordinate surge (up to 14 fold of "quiet" steady-state levels), peaking at 5-30 min following onset of contractions, and returning near to "active" steady-state levels by 120 min.

The short and long term effects of action potential generation on cyclic nucleotide levels in dysgenic (mdg/mdg) muscle (which fires action potentials in the absence of contractions) were similarly assayed. Changes in cyclic nucleotide levels were modulated in the membrane-active dysgenic muscle in an equivalent fashion to that observed in normal, active, contracting muscle.

Neurotrophic effects were assessed by measuring cyclic nucleotides in normal and dysgenic cultures maintained for several days in media conditioned by nerves (14 day spinal cord cells) or mature muscle. No significant differences were found in cAMP levels in muscle treated in these two ways, while nerve-conditioned medium produced a two fold elevation in cGMP levels relative to levels in myotubes maintained in muscle-conditioned medium.

In summary, our findings suggest that 1) the influence of muscle activity on cyclic nucleotide levels is mediated via action potential generation and that both cAMP and cGMP are transiently increased by brief periods of such activity, and 2) cGMP is relatively more affected by, and therefore involved in, the longer term effects of either membrane activity or neurotrophic influence.

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26.16 MUSCARINIC CHOLINERGIC RECEPTOR-MEDIATED REDUCTION IN CYCLIC AMP ACCUMULATION OCCURS BY TWO MECHANISMS IN A SINGLE CELL. L.I. Tanner* and T.K. Harden. (SPON: R.L. Glasser) Neurobiology Program and Dept. Pharmacology, Univ. North Carolina, School of Medicine, Chapel Hill, NC 27514.

Activation of muscarinic cholinergic receptors (MR) results in a decrease in PGE_1-stimulated cyclic AMP accumulation in intact WI-38 fibroblasts (R.W. Butcher, J.Cyclic Nucleotide Res. 4:411, 1978). The purpose of the present study was to further characterize the mechanisms responsible for this MR-mediated decrease in cyclic AMP levels. The MR agonist oxotremorine (OXO) inhibited PGE_1- and isoproterenol-stimulated cyclic AMP accumulation by 60-70% in intact cells. This effect appeared to be due in part to activation of phosphodiesterase (PDE) since in the presence of the PDE inhibitor 3-isobutyl-1-methylxanthine, MR-mediated inhibition was reduced to 40%. Similar results were obtained with another PDE inhibitor, Ro20-1724. Direct evidence for MR-mediated activation of PDE was obtained. That is, in the presence of OXO the rate of degradation of cyclic AMP was increased from 0.24 min^{-1} to 0.50 min^{-1}. This effect of OXO was blocked by a PDE inhibitor.

In addition to PDE activation, OXO also decreased cyclic AMP accumulation by inhibition of adenylate cyclase (AC). The evidence for this effect was two-fold. First, the effect of MR activation in intact cells was only partially attenuated by maximally effective concentrations of a PDE inhibitor. Second, MR-activation inhibited PGE_1- and isoproterenol-stimulated AC activity by 30-35% in a washed membrane preparation. Thus, activation of MR of WI-38 fibroblasts reduces cyclic AMP accumulation by an activation of PDE as we have demonstrated previously in 132IN1 human astrocytoma cells (Mol. Pharmacol. 22:310, 1982) and by inhibition of AC as has been demonstrated in a variety of tissues. Whether these two mechanisms are mediated through a common population of MR or through separate MR subtypes remains to be determined. Supported by GM-29536 and an Established Investigatorship of the American Heart Association.

26.17 INVOLVEMENT OF CYCLIC NUCLEOTIDES IN AMINE AND PEPTIDE MODULATION OF THE LIMULUS HEART. J.R. Groome* and W.H. Watson, III (SPON: S. Reingold). Dept. of Zoology, UNH, Durham, NH 03824.

The neurogenic heart of the horseshoe crab (Limulus polyphemus) is a simple system modulated by several amines and the pentapeptide proctolin (Watson, W.H., Augustine, G.J., Peptides, 3:485, 1982). Dopamine has positive inotropic and chronotropic effects on the heart (Augustine, et al., J. Neurobiol., 13:61, 1982); proctolin only increases the strength of heart contractions (Watson, et al., J. Exp. Biol., 103:55, 1983). The extraordinary in vitro viability of the Limulus heart coupled with the presence of large identifiable neurons in the cardiac ganglion have facilitated determination of the site of action of these compounds. However, the molecular mechanisms underlying their action remains uncertain. The slow onset and long duration of the effects produced by dopamine and proctolin suggests that cyclic nucleotides play a role in this process. Several experiments utilizing radioimmunological, pharmacological, and electrophysiological techniques have provided direct evidence for this hypothesis.

Levels of cGMP increase transiently in cardiac muscle following application of 10^{-6}M proctolin (10-fold increase) or 10^{-5}M dopamine (5-fold increase); while ganglion cGMP levels are not markedly affected. Dopamine also appears to increase levels of cAMP in the ganglion as well as in the muscle.

Pharmacological elevation of intracellular cyclic nucleotide levels elicits a change in the cardiac rhythm that mimics the effect of dopamine application. Forskolin, an activator of adenylate cyclase (Seamon et. al., PNAS, 78(6):3363, 1981) increases the rate and amplitude of heart contractions in a dose-dependent manner (threshold, saturation). Forskolin (10^{-5}M)) also increases the burst frequency of the isolated ganglion 80%, probably as a result of its action on the pacemaker cells. IBMX (3-isobutyl 1-methyl xanthine), an inhibitor of phosphodiesterase, also increases contraction amplitude and rate. It is approximately two log units less potent than Forskolin.

These findings indicate that cyclic nucleotides are involved in amine and peptide modulation of the Limulus heart and confirm previous reports that dopamines act directly on the cardiac ganglion neurons while proctolin exerts its effects on the cardiac muscle.

26.18 A NEW PHOTOACTIVATED cAMP ANALOGUE AND ITS EFFECT ON THE SLOW INWARD CURRENT IN HEART. J. Nerbonne, J. Nargeot*, and H. A. Lester. Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Previously, we reported that the photolabile analogue of cAMP, o-nitrobenzyl cAMP, could be utilized to produce step changes in intracellular cAMP in heart (Nargeot et al., Proc. Natl. Acad. Sci. 80, 2395-2399 (1983)). In voltage-clamped, TTX-treated atrial trabeculae from bullfrog, this compound is physiologically inert at concentrations up to 100 μM. Flash-induced concentration jumps of cAMP increase the slow inward current (carried principally by calcium ions). The amplitude of the effect is dependent on the absolute magnitude of the cAMP concentration jump: at 30 μM o-nitrobenzyl cAMP, a single light flash produces a cAMP jump of 0.75 μM, increasing the slow inward current by 50%. The increase is linear with time and the effect reaches a maximum in 30-40 sec. In very stable preparations, a 2% increase in the slow inward current can be detected within 150 msec after the light flash.

In order to extend the useful concentration range of intracellular cAMP jumps accessible with this methodology, we have designed and synthesized 3,4-dimethoxy-6-nitrobenzyl cAMP. We find that this new photolabile analogue, under the same experimental conditions used for o-nitrobenzyl cAMP, releases cAMP with twice the efficiency of the parent compound. The chemical reactions leading to photorelease for this derivative are complete within at most 5 msec. In voltage-clamped bullfrog atria in the absence of light, this derivative causes no change in the amplitude or the waveform of the slow inward current at concentrations up to 120 μM. A single light flash in the presence of 50 μM 3,4-dimethoxy-6-nitrobenzyl cAMP, however, increases the amplitude of the current to three times its pre-flash value (with little or no change in kinetics). The increase in the amplitude of the slow inward current is linear with time for the first 10 sec and the response reaches a maximum in 20-25 sec. Simultaneous measurements of the phasic contraction show that a light flash results in a threefold increase in the phasic tension over 20-25 sec (agreeing with the time course and the magnitude of the effect on the slow inward current).

The response latency following single light flashes as well as experiments employing the 3,4-dimethoxy-6-nitrobenzyl ester of cGMP will be discussed.

26.19 ELECTROPHYSIOLOGICAL AND BIOCHEMICAL CORRELATES OF DEVELOPMENTAL LEAD TOXICITY IN RAT RETINA. D.A. Fox and D.B. Farber. College of Optometry, Univ. Houston, Houston, TX and Jules Stein Eye Instit., UCLA Sch. Med., Los Angeles, CA.

Low-level developmental lead (Pb) exposure produces toxic amblyopia in adult rats with greater spatial vision deficits observed under scotopic (rod-mediated) than photopic (cone-mediated) luminance conditions (Fox and Wright, Neurosci. Abs. 8:81,1982). In vitro electrophysiological studies, in isolated bullfrog retinas, reveal that low-level Pb depresses the amplitude of the rod, but not cone, photoreceptor potential (Fox and Sillman, Science 206: 78,1979). To determine if rod and/or cone photoreceptors were preferentially altered in adult rats following developmental Pb exposure, electroretinographic (ERG) and retinal cyclic nucleotide metabolism studies were conducted. Female Long-Evans hooded rats were exposed to Pb (or no Pb) from parturition to weaning (days 0-21) via the milk of dams drinking 0.2% Pb acetate solution (or water). ERGs were recorded in anesthetized rats at 4 levels of luminance, scotopic to photopic, and the V-log I relationships were determined. The a-wave amplitude, which represents the photoreceptor response, was decreased 35% under photopic conditions and greater than 50% under scotopic conditions. The b-wave was decreased 20% under photopic conditions and over 35% under scotopic conditions. Thus, the ERG data reveals a preferential rod-mediated effect of Pb. Next we investigated, in litter-mates, whether there were changes in the retinal cGMP system (mainly associated with rod photoreceptors) and/or the cAMP system (mainly associated with cone photoreceptors) related to the ERG effects. In vivo, Pb caused cGMP levels in dark-adapted and light-adapted retinas to increase 40% and 25%, respectively, above controls whereas cAMP levels remained unchanged. Light-activated cGMP-phosphodiesterase (PDE) showed similar kinetics in control and Pb exposed tissue, but the V_{max} of the latter was decreased. At 10^{-4}M substrate, cGMP-PDE was inhibited 40%. In Pb-retinas, guanylate cyclase was activated by 20%. To verify the effects of Pb on cyclic nucleotide enzymes we performed in vitro retinal studies with adult control retinas incubated with different concentrations of Pb. In vitro, a dose-response inhibition (10-40%) of cGMP-PDE occurred with 10^{-6} to 10^{-4}M Pb. Guanylate cyclase was activated 120-250% by 1 to 5 X 10^{-4}M Pb. Thus, the in vivo effects were confirmed in vitro. The effects of Pb on the retinal cGMP system show that developmental Pb exposure selectively disrupts rat rod photoreceptors. This result is consistent with the preferential rod-mediated deficits we observed in this study with the ERG and in previous electrophysiological and psychophysical studies in adult rats following developmental Pb exposure. Supported by ES 03183 (DAF), EY 02651 and RCDA EY 00144 (DBF).

26.PO DIFFERENT EFFECTS OF ADENOSINE ON CYCLIC AMP REGULATION IN RETINA AND BRAIN. K.M. Campau*, D.A. Kinscherf*, and J.A. Ferrendelli (SPON: D.B. McDougal, Jr.). Dept. of Pharmacology, Washington Univ. Med. Schl., St. Louis, MO 63110.

Adenosine is a well known regulator of cyclic AMP levels in mammalian brain. This nucleoside increases cyclic AMP levels several fold and also greatly enhances the stimulatory effect of norepinephrine on cyclic AMP concentrations in incubated slices of brain. Indirect evidence suggests that adenosine is a major factor regulating levels of cyclic AMP in brain, in vivo. This study was carried out to determine whether adenosine has similar effects on cyclic AMP in mammalian retina.

Normal retinas were obtained from young adult C57BL/6J mice. Animals were either light- or dark-adapted for one hour, killed by decapitation, and their retinas removed in the light or under dim red light illumination. The isolated retinas were then preincubated in buffer containing 120 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 26 mM $NaHCO_3$, 0.9 mM NaH_2PO_4 and 10 mM glucose saturated with 95% O_2 - 5% CO_2 at 37° for 30 min. Following this the tissue was exposed to various agents or other conditions, as indicated below; then the buffer was removed and the tissue was treated with trichloroacetic acid and analyzed for cyclic AMP concentration.

Isobutylmethylxanthine (IBMX) (1 mM), dopamine (100 µM), and norepinephrine (100 µM) all increased levels of cyclic AMP 1 - 2-fold in normal light-adapted retina, but adenosine (100 µM) had no effect. The combination of adenosine with norepinephrine with or without IBMX also failed to demonstrate a stimulatory effect of adenosine on cyclic AMP levels. Adenosine, however, did slightly augment dopamine-induced elevations of cyclic AMP levels in the presence of IBMX in light-adapted retinas. In dark-adapted retinas IBMX increased cyclic AMP levels more than those in light-adapted retinas, and dopamine caused an additional elevation. Adenosine did not affect any of these changes. Dopamine in combination with IBMX increased levels of cyclic AMP almost 40-fold in dystrophic (receptorless) retinas, but adenosine had no effect in this tissue either. In contrast to the findings in retina, adenosine (100 µM) increased levels of cyclic AMP 6 - 10-fold in slices of striatum from C57BL/6J mice. This effect was inhibited by IBMX and augmented by norepinephrine and dopamine.

These results demonstrate that adenosine has little or no effect on cyclic AMP levels in incubated mouse retina and suggest that adenosine-sensitive adenylate cyclase is absent in this tissue. The results indicate that regulation of cyclic AMP levels in brain is under different control than that in retina.

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26.PO MORPHINE: A NEW SITE OF ACTION IN PURINE NUCLEOSIDE PATHWAYS. M.L. Cohn, M. Cohn, J. Larrinaga*, D. J. Wooten*, J. Samora* and G. Fernandez*. Dept of Anesthesiology Research, C.R. Drew Med Sch, Los Angeles, CA 90059.

In 1977, we reported the potent central analgetic properties of the dibutyryl analog of guanosine 3':5' monophosphate (dbcGMP). Although morphine has been shown to increase cGMP concentrations in rat brain structures and rat brain slices, the relationship between the pharmacologic actions of morphine and brain levels of cGMP is not well understood. Based on our analytical evidence which increasingly suggested that in addition to cGMP, one or several of its metabolic products may be involved in the biochemical processes regulating analgesia, we investigated here the effects of morphine on cGMP metabolism. Sprague-Dawley male rats were divided into three groups. Rats of control and second group were injected IP with saline 0.9% and morphine (1.7mg/kg) respectively and sacrificed 2 hrs later. Rats of third group were addicted to morphine (100mg/kg--in slow releasing preparation of Collier et al., 1972) injected SC once a day for 2 days and sacrificed on 3rd day. Brain slices were incubated in tonometer at 37°C with Krebs-Ringer bicarbonate/glucose buffer, pH 7.35, a constant flow of O_2/CO_2 (20:5) and 2.90 x 10^{-1} mM of standard cGMP. Sequentially withdrawn aliquots of incubation mixture were analyzed by high performance liquid chromatography (HPLC). In control rats, primary metabolic products were GMP, guanosine (GUS), guanine, xanthine, and inosine. After the first 10 min of incubation, cGMP catabolism resulted in accumulation of GUS, suggesting that GUS nucleoside phosphorylase may be a regulatory enzyme and that, like adenosine, GUS may be active in physiological processes. Appearance of inosine on chromatograms agreed with our earlier reports of GMP reductase activity in rat brain slices. In IP morphine treated rats, concentrations of GUS were significantly increased over control values at the 30 and 60 min of incubation. Most striking, however, were GUS concentrations in rats addicted to morphine with accumulations 2 to 3 fold higher than control values at 30 and 60 min of incubation. In both groups of rats treated with morphine cGMP metabolism resulted in accumulation of inosine. Morphine (1.46 x 10^{-2} mM) added to incubation of brain slices of untreated rats, with cGMP as substrate, produced GUS and inosine accumulations similar to those observed in rats treated IP with morphine. Noticeable also are our findings that morphine (1.46 x 10^{-2} mM) added to incubation of standard GUS with GUS nucleoside phosphorylase resulted in accumulations of GUS which were significantly increased over control values. Therefore, the inhibition of GUS nucleoside phosphorylase by morphine in rat brain slices was reproduced in a GUS incubation mixture containing the enzyme preparation.
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26.PO A SUPPRESSION OF DARK IBMX-ENHANCED CYCLIC AMP LEVELS OF INCUBATED MOUSE RETINAS BY 5-METHOXYTRYPTAMINE. A.I. Cohen. Dept. of Ophthalmology, Washington Univ. Med. Sch., St. Louis, MO 63110.

When incubated C57BL/6 mouse retinas were exposed to 1 mM IBMX, cyclic AMP levels rose 6.4 fold over those of control retinas in IBMX-free medium for dark-adapted, dark-incubated retinas but only 4.5 fold for light-adapted, light-incubated retinas. However, if the medium included 10 µM - 100 µM 5-methoxytryptamine (5 MTA), dark cyclic AMP levels were held to values identical to those obtained with light-adapted, light-incubated retinas in IBMX media with or without 5 MTA. No effect on dark cyclic AMP levels in IBMX medium was obtained with 100 µM serotonin, melatonin, or 5-methoxytryptaphol. In addition, when dark-incubated retinas which lack rod photoreceptors were used from dark-adapted 3 mo C3H/HeJ mice homozygous for rd/rd, 5 MTA had no influence on the ability of IBMX to achieve a 7-fold increase in cyclic AMP in this retina. The data suggest that rod photoreceptors in mice may possess receptors for a molecule resembling 5 MTA or that rd/rd retinas behave like light-adapted retinas despite their well-known more vigorous production of cyclic AMP.

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27.1 SOME STEROID HORMONE CONCENTRATING CELLS IN THE MEDIAL BASAL HYPOTHALAMUS (MBH) AND ANTERIOR PITUITARY CONTAIN β-ENDORPHIN OR DYNORPHIN. J. I. Morrell, J. McGinty[1] and D. W. Pfaff. The Rockefeller University, New York, NY 10021. [1]East Carolina University, School of Medicine, Greenville, NC.

Steroid hormone receptors located in particular brain and pituitary cells may be involved in the regulation of the genome of these cells, ultimately resulting in altered protein production. Steroid autoradiography has revealed the number and distribution of steroid hormone concentrating cells; immunocytochemistry has revealed the number and distribution of cells containing endogenous opiates. These distributions overlap. Levels of endogenous opiates in brain and pituitary vary over the estrus cycle, and in response to steroid hormones, including estradiol (E_2) and dexamethasone (dex). We applied the combined steroid autoradiographic-immunocytochemical method (Morrell & Pfaff, Methods in Enzymology, 1983) to ask the question: Do E_2 or dex concentrating cells contain endogenous opiates?

Eighteen adult, (9 colchicine pretreated, 50μg/2μl, I.C.V.) ovariectomized, adrenalectomized female rats were given either .8μg/250g of ^{3}H-E_2 (S.A. 100Ci/mM) or 10μg/100gm ^{3}H-dex (S.A. 38Ci/mM) I.P.; 1-2 hrs later perfused with saline, then 4% paraformaldehyde. After a 24 h 30% sucrose soak, the tissue was frozen in freon chilled by nitrogen, and steroid autoradiograms were prepared. After exposure times of 2 to 12 months the autoradiograms were photodeveloped, fixed and immunocytochemistry was carried out on the autoradiograms using primary antibodies to β-endorphin or dynorphin (gifts of R. Benoit, Salk Inst.; L. Terenius, Uppsala, Sweden) and either the PAP or ABC method with DAB for visualization. As expected, using the combined method we found many E_2 concentrating cells in the MBH; many β-endorphin and many dynorphin containing neurons were also found in the MBH, including within the arcuate and the region lateral to it, but ventral to the ventromedial nucleus. Of the β-endorphin containing neurons, about 4% concentrated E_2; about 6% of the dynorphin containing neurons concentrated E_2. In the anterior pituitary, a few cells that contained β-endorphin concentrated E_2. This is in contrast to the results from the anterior pituitary of animals administered ^{3}H-dex; many β-endorphin containing anterior pituitary cells concentrated dex.

These data are consistent with the hypothesis of a genomic effect of E_2 on a particular subset of MBH neurons that produce endogenous opiates. In the anterior pituitary dex decreases the levels of pro-opiomelanocortin (POMC) m-RNA (Schachter et al., Endo., 110: 1442-4). This regulation could result from the steroid acting thru autoradiographically localizable nuclear receptors in POMC-producing pituitary cells.

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27.2 INTERMEDIATE FILAMENTS OF THE NEONATAL AND ADULT HUMAN PITUITARY: AN IMMUNOHISTOCHEMICAL STUDY WITH ANTI-NEUROFILAMENT AND ANTI-GLIAL FILAMENT MONOCLONAL ANTIBODIES. J.Q. Trojanowski, D. Gordon* M.A. Obrocka* and V.M.-Y. Lee*. Div. of Neuropathology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19147.

Normal pituitary glands from adults and neonates were obtained at autopsy and immersion fixed in either Bouin's fixative or in 4% mercuric chloride/8% formaldehyde. After paraffin embedding, serial 6 u thick sections were prepared from each pituitary and affixed to glass slides. Sections were deparaffinized, endogenous peroxidase activity was quenched with methanol/hydrogen peroxide and monoclonal antibodies which recognize either neurofilament (NF) or glial filament (GF) proteins were applied to each section. Immunoreactive NF or GF were then localized using the peroxidase anti-peroxidase immunohistochemical method. Peroxidase was visualized with diaminobenzidine as the chromogen in medium containing imidazole and hydrogen peroxide. Sections lightly counterstained with hematoxylin were dehydrated, cover slipped and examined. Controls included sections incubated with supernatant from unfused parent mouse myeloma cultures and similarly prepared sections of normal rat cerebellum treated with the same monoclonal antibodies or mouse myeloma supernatant. Immunoreactive NF was present as linear or punctate profiles in the neurohypophysis, consistent with an intra-axonal localization and was more prominent in adults compared with neonates. Only rarely were similar profiles observed in the adenohypophysis and there was no unequivocal immunoreactive NF in perikarya of the neuro- or adenohypophysis. In adult, but not in neonatal pituitaries, large extracellular "blobs" of immunoreactive NF were seen in the neurohypophysis. Immunoreactive GF was present in the cell bodies and processes of only a small number of neurohypophyseal cells, in some cells lining cysts of the residual pars intermedia and only occasionally in astrocyte-like profiles of the adenohypophysis of adult pituitaries. In neonatal pituitaries, immunoreactive GF was localized to the cells of the neurohypophysis and only rarely was it present in cells lining cysts of the residual pars intermedia. It was never seen in cell bodies of the the adenohypophysis. GF containing "blobs" were less common in the adult neurohypophysis than NF containing "blobs" and none were seen in the neonatal pituitaries. We conclude that the adult and neonatal pituitary contains both NF and GF and that they are differentially distributed. The "blobs" containing immunoreactive NF or GF in the posterior pituitary of normal glands may reflect a limited capacity to degrade externalized GF and NF by elements of the neurohypophysis.

27.3 PROTEIN CARBOXYL METHYLATION IN POSTERIOR PITUITARY LOBE OF SALT TREATED, PITUITARY STALK SECTIONED AND BRATTLEBORO RATS. J.M. Saavedra and Y. Kloog*. Section on Pharmacology, Lab. of Clinical Science, NIMH, Bethesda, Md. 20205, USA.

Enzymatic methylation of free carboxyl groups of proteins is catalyzed by protein carboxyl-O-methyl transferase (E.C.2.1.1.24)

Enzyme activity and levels of endogenous methyl acceptor proteins are very high in the posterior pituitary gland. Protein carboxyl methylase was postulated to play a role in the neurosecretory process, which involves the exocytotic release of neurohypophyseal peptides and their corresponding neurophysins. Although neurophysins are good substrates for protein carboxyl methylase in vitro, the actual methylation of proteins in posterior pituitary has not been demonstrated.

The Brattleboro rat is derived from the Long Evans strain, and presents genetic deficits in the formation of vasopressin and neurophysin-associated vasopressin. Homozygous Brattleboro rats are unable to synthesize these peptides and show a chronically increased stimulation of the hypothalamo-neurohypophyseal axis. Alterations in protein carboxyl methylation are selectively present in the posterior lobe of homozygous Brattleboro rats. Posterior lobe homogenates from male homozygous Brattleboro rats incubated in the presence of the natural methyl donor, S-adenosyl-L-methionine, show higher protein carboxyl methylase activity (+40%) and lower endogenous methyl acceptor protein capacity (-80%) than control heterozygous Brattleboro or Long Evans rats. This latter change is correlated with decreased methylation of proteins of a molecular weight of approximately 11,000, similar to that of neurophysins, as determined by polyacrylamide gel electrophoresis.

Whole posterior pituitary lobes from control rats can enzymatically form protein carboxyl methyl esters using methionine as a precursor. At least six proteins are methylated in the intact posterior lobe. Among these is neurophysin, identified by its characteristic properties: molecular weight, [^{35}S]-cysteine labelling, disappearance after salt loading or pituitary stalk section and low levels in homozygous Brattleboro rats. In addition to neurophysin, four other still unidentified proteins are methylated in the hypothalamo-hypophyseal nerve endings, as indicated by their disappearance after pituitary stalk section.

These results indicate that genetically (Brattleboro model) or experimentally induced (salt loading) stimulation of the neurohypophyseal tract result in specific alterations in posterior pituitary protein carboxyl methylation. Our results support the hypothesis of a role for protein carboxyl methylation in the neurosecretory activity of the posterior pituitary, probably related to a modulation of the exocytotic release process in this organ.

27.4 PRODUCTION OF MONOCLONAL ANTIBODIES USING SUPRAOPTIC, PARAVENTRICULAR AND NEUROHYPOPHYSIAL MEMBRANES AS IMMUNOGENS. C.M. Paden and S.J. Hapner*. Dept. of Biology, Montana State Univ., Bozeman, MT 59717

Monoclonal antibodies which recognize cell-type-specific antigens have great promise as probes of neuronal development. We have employed membranes prepared from rat neurohypophysis and supraoptic (SON) and paraventricular (PVN) nuclei as immunogens in an attempt to develop monoclonal antibodies for use as markers of cell surface antigens of hypothalamic neurosecretory cells.

The neurohypophysis (and intermediate lobe) were dissected from unperfused, decapitated adult Holtzman albino rats. SON and PVN tissues were removed from 200 μ cryostat sections of unfixed brain using a 400 μ Palkovits punch. All tissues were homogenized in a hypotonic Tris buffer and a crude membrane pellet obtained. In various experiments, BALB/c mice were immunized with 100 to 200 μg of membrane protein suspended in Freund's Complete Adjuvant ip one or more times. After a period of one to 3 months, mice received either (a) a final ip boost injection, (b) an iv boost, or (c) spleens were removed and splenocytes activated in vitro with whole membrane or detergent extracts (R.A. Luben et al., Science 218, 887, 1982). Four days later splenocytes were fused with NS1 myeloma cells and plated into 48 culture wells (G. Galfre and C. Milstein, Methods in Enzymology 73, 1981). Immunocytochemical screening for antibody production was done on rat pituitary and hypothalamic sections (Zamboni's fixed) with FITC conjugated sheep anti-mouse immunoglobulins sera.

A total of 23 positive wells have been obtained from 5 fusions. Three monoclonal hybridoma lines have been isolated from these, producing antibodies which stain (1) tissue throughout the neurohypophysis and median eminence, (2) myelin and (3) cytoplasm of scattered neurons throughout the brain. Among the hybridomas being cloned, some secrete antibodies which appear to stain the neurohypophysis and cytoplasm of magnocellular neurons in the SON, or cytoplasm in both the SON and PVN. While further immunocytochemical characterization is required to determine the specificity of these antibodies, two conclusions are possible. First, membrane preparations from tissue enriched in rat neurosecretory cells or their processes are immunogenic in mice and have the potential to stimulate production of cell-type-specific antibodies. However, the majority of our antibodies recognize cytoplasmic antigens. We are trying different membrane extraction procedures to increase the production of antibodies to cell-surface molecules. Second, activation in vitro is much more effective than in vivo boosts with our immunogens. Two fusions using in vitro stimulated splenocytes have produced 20 of the 23 positive wells. This technique offers great advantages when immunizing with small amounts of protein containing many antigenic substances.

27.5 ULTRASTRUCTURE OF VENTROMEDIAL HYPOTHALAMIC NUCLEOLI AND NUCLEI REVEALED BY ENZYME DIGESTIONS IN DE-EMBEDDED THIN SECTIONS. S. K. Chung*, R. S. Cohen and D. W. Pfaff. Dept. of Anatomy, Univ. of Illinois Health Sci. Center, Chicago, IL and Rockefeller Univ., NY.

Estrogen affects ventromedial hypothalamic (VMH) nerve cell nucleoli in ovariectomized rats, causing an increase in the number of protuberances associated with nucleoli (Cohen & Pfaff, Soc. Neurosci. Abst., 2:531, 1982). Ultrastructural examination of these protuberances revealed dense aggregations of material, separated from the nucleolus by a narrow gap, penetrated by strands of electron-dense material that seem to be connected to the main portion of the nucleolus. Previous ultrastructural studies indicated increased synthesis of a secretory product in estrogen-activated neurons (Cohen & Pfaff, Cell Tiss. Res., 217:451, 1981), possibly associated with greater rRNA synthesis in the nucleolus. Here we describe the morphological and chemical nature of the nucleolar protuberances and explore the nuclear matrix in VMH nerve cells of ovariectomized, estrogen-treated rats. Based on sodium tungstate staining (Takeuchi, J. Electron Microsc., 30:150, 1981), which differentiates between RNA- and DNA-containing structures in epon-embedded thin sections, the dense aggregated material was shown to contain DNA. Since epon interferes with enzyme digestion, as well as obscuring the visualization of fine filaments, other hypothalamic tissue was embedded in polyethylene glycol (PEG) (Wolosewick, J. Cell Biol., 86:675, 1980), a water-misible wax, thin sectioned, and de-embedded before enzyme digestion. Pepsin did not alter significantly the morphology of the nucleolus nor its associated structures. However, treatment with RNAse and pepsin reduced the density of the nucleolus, while that of the nucleolus-associated structure and other chromatin-like structures remained unchanged. Treatment with DNAse and pepsin reduced the density of the nucleolus-associated structures and other chromatin structures, but did not affect the density of the nucleolus. Stereo viewing of the de-embedded sections shows that the nucleolus, its associated structures, and other chromatin-like structures, are associated with fine filaments that may comprise the nuclear matrix. The nucleolus-associated structure containing DNA may direct RNA synthesis at an increased rate in estrogen-treated hypothalamic cells.

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27.6 AN INTERACTION BETWEEN HYPOTHALAMIC SLICES INCUBATED IN VITRO. P. Cobbett* & G. I. Hatton (SPON: ENA). Neuroscience Program and Psychology Dept., Michigan State University, E. Lansing, MI 48824.

Dye coupling of central mammalian neurons in vitro has been demonstrated in various regions of the CNS. In some regions the incidence of dye coupling may vary according to the condition of the animal from which the slices were prepared and the conditions of incubation. The paraventricular nucleus of the rat hypothalamus is one such area (see Hatton & Cobbett, this meeting). In this study we have investigated whether the presence of slices from one treatment group may affect the incidence of dye coupling in slices of another group incubated in the same chamber. Slices were prepared from male and female animals aged 60-90 days. Rats were given water (Control) or 2% NaCl (Dehydrate) for 8 days before sacrifice. In each experiment equal numbers of Dehydrate and Control slices were incubated together in an oxygenated medium of 310 mOsmol./kg. Slices of different sex were incubated separately. Using appropriate precautions to prevent artefactual dye filling of cells and incorrect counting of dye coupling, neurons in the magnocellular region of the PVN in Dehydrate slices were injected with Lucifer Yellow CH. Subsequently fixed, cleared slices were examined using epifluorescence microscopy to determine the number of filled neurons. For each group the Dye Coupling Index (DCI) was calculated as the ratio of the number of coupled dye filled neurons to the total number of dye filled neurons.

The DCI of Dehydrate males incubated with Control males was 0.475 (n=30 injections) which is significantly (χ^2 =10.487, p<0.01) greater than that recorded previously for Dehydrate males incubated alone (DCI=0.121, n=31). In contrast the DCI of Dehydrate females incubated with Control females (0.263, n=33) was not significantly different to that of Dehydrate female slices incubated alone (0.229, n=30).

These data indicate that dye coupling of magnocellular neurons in a slice can be dependent on its "incubation partner" slice type and this interaction is sex dependent. The underlying cause of this phenomenon is not clear but may in part be the differential levels of 1) sex steroid hormones in the brain of males and females, and 2) other steroid hormones in the brain of normal and dehydrate animals.

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27.7 DYE COUPLING IN THE IN VITRO HYPOTHALAMUS: DEPENDENCE ON SEX AND HYDRATION STATE. G. I. Hatton & P. Cobbett* Neuroscience Program and Psychology Dept., Michigan State Univ., E. Lansing, MI 48824.

To achieve sufficient release of hormone in conditions of high demand, homotypic magnocellular neurons of the hypothalamus must be coordinated. Such coordination might be achieved by electrotonic coupling which is indicated by dye coupling. We have investigated the incidence of dye coupling of magnocellular neurons in vitro in the paraventricular nucleus of male and female rats to determine whether the incidence of coupling is sex dependent and is affected by dehydration (one stimulus of hormone secretion). Slices were prepared from 30-90 day old animals given water (Control) or 2% NaCl (Dehydrate) for 8 days prior to sacrifice. The slices were incubated in an oxygenated medium of 310 mOsmol./kg. (310 medium) or 340 mOsmol./kg. (340 medium): these are similar to the plasma osmolalities of normal and dehydrate animals respectively. Male and female slices were incubated separately, as were Control and Dehydrate slices. Neurons in the magnocellular region of the PVN were injected with Lucifer Yellow CH using appropriate precautions to prevent artefactual dye-filling of neurons and incorrect counts of dye coupling. Fixed, cleared slices were examined microscopically using epifluorescence to determine the number of filled neurons. In each group the Dye Coupling Index (DCI) was calculated as the ratio of the number of dye filled coupled neurons to the total number of dye filled neurons.

In 310 medium, Control males had a DCI of 0.333 (n=35 injections) whereas the DCI of Control females was 45% lower (0.182, n=30) indicating a different basal incidence of coupling between males and females. The DCI of Dehydrate females was 0.229 (n=30) in 310 medium and 0.367 (n=24) in 340 medium: these are increases of 25% and 102% respectively compared to the basal (Control) coupling index. Thus dehydration in vivo probably increases coupling but this increase is more completely maintained in vitro if the "dehydration" is also maintained. In contrast the incidence of dye coupling in males was decreased following in vivo dehydration. The DCIs were 0.121 (n=31) and 0.261 (n=20) in Dehydrates incubated in 310 and 340 media respectively. Thus even if dehydration increases coupling in vivo, it is not sustained in vitro under the conditions tested.

These data indicate that the incidence of dye coupling is dependent on sex and in vivo and in vitro "hydration states". The underlying causes of these differences are not known but may be related to steroid hormone concentrations. In addition the argument that dye coupling is a real phenomenon and not an artefact of incubation is supported by these data.

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27.8 CYSTEAMINE DEPLETES PROLACTIN, BUT DOES NOT ALTER THE STRUCTURE OF PROLACTIN-CONTAINING GRANULES. L.A. Weinstein* and D.M.D. Landis (SPON: J.J. Halperin) Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Systemic administration of cysteamine (mercaptoethyl amine) causes a profound, dose-dependent depletion of prolactin in the rat anterior pituitary as assessed by radioimmunoassay (RIA) and by bioassay. Similar reversible depletion occurs in cells of dispersed pituitary cell cultures, and assay of the culture medium shows that the depletion is not caused simply by release of cell content. Cysteamine has no effect on purified prolactin in solution, and so its effect appears to occur within cells.

To assess the mechanism of cysteamine action, we have examined the ultrastructure of rat anterior pituitary 1, 2, and 4 hours after systemic administration of cysteamine (300mg/kg), an interval during which prolactin levels are very low, and again at 24 hours, when levels have climbed to the normal range. Lactotropes initially were identified by comparison with observations made by previous investigators. Qualitative and quantitative studies showed no significant change in the appearance and number of secretory granules or lysosomes during the interval of prolactin depletion.

Lactotropes were also identified by light and electron microscopic immunocytochemical techniques (ICC), using a primary antiserum supplied by the National Hormone and Pituitary Program. No change in the pattern of prolactin-like immunoreactivity was detected at 1, 5, and 24 hours after systemic injection, in contrast with the depletion detectable by RIA. At low dilutions (1:400) most immunoreactivity was present over large secretory granules, but with more highly diluted antisera (1:2000-4000) the rough endoplasmic reticulum stained as well. Usually, staining of granules was of variable intensity in a single cell. Omission of primary antiserum or preabsorption with prolactin eliminated staining.

We suggest that cysteamine alters prolactin structure only in the microenvironment of secretory granules, probably by interacting with the disulfide bonds of prolactin. The altered peptide is inactive in bioassay, and is not bound by the highly diluted antiserum in the RIA, though it is detected by ICC after aldehyde fixation. The presence of cysteamine-altered prolactin in secretory granules does not seem to trigger degradation of granules by the lysosomal system.

27.9 NEURONS OF THE CAUDAL NEUROSECRETORY COMPLEX RECONSTRUCTED ULTRASTRUCTURALLY. R.M. Kriebel*, S.M. Gerttula* and R.L. Parsons. Dept. of Anatomy & Neurobiology, Univ. of Vermont Col. of Medicine, Burlington, VT 05405.

It has been shown that the activity of neurosecretory cells is influenced by synaptic input, however, the integrative mechanisms of this synaptic influence are yet to be understood. The caudal neurosecretory complex (CNC) of fishes, specifically P. latipinna (green molly), is well suited for studies to this end. We have started a multidisciplinary program which is aimed at studying the synaptic control of neurosecretory cells using the CNC model. One phase of these studies is concerned with the distribution of synaptic terminals on these neuroendocrine cells. The size and linear orientation of the CNC makes this neuronal nucleus convenient for examining three-dimensional characteristics of the synaptic input to individual nerve cells. The CNC, located within the terminal five vertebrae, was fixed in vivo with 2.0% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer and processed routinely for plastic embedding. One micron sections were used to locate an area of CNC suitable for serial sectioning, i.e. numerous soma and adjacent neuropil. The block face was trimmed to remove excess tissue and plastic, however, the size of this structure permitted thin sectioning of the entire spinal cord cross-sectional area. Serial thin sections were mounted on single slot formvar coated grids; three sections were placed on each grid. Meticulous records were kept during the process including section interference coloration as well as loss of sections. Multiple section loss resulted in the initiation of a new series of serially collected sections. The sections were stained with uranyl acetate and lead citrate. Randomly selected neurons were serially photographed to include their somal area, processes, and the synaptic contacts on these cells. The three-dimensional reconstructions were completed with computer assistance. Ultrastructurally the neurons of the molly caudal system appear a homogeneous cell type, but reconstructions showed that some cells had smooth cellular profiles while others had a more varied cellular contour. The microenvironment of the smooth cells consists almost exclusively of other apposing neurosecretory cells or glial processes. The synaptic profiles on the smooth neurons were found at the axonal pole of the cell. The other neuroendocrine cells were more isolated and surrounded by neuropil. These cells received more synaptic terminals than the smooth neurons and the terminals were distributed randomly over the somal membrane. These studies suggest that some of the caudal system neurosecretory neurons receive more direct synaptic influence than others.

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27.10 ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDIES OF CRYO-PREPARED (CP) NEURAL TISSUES. J.G. Linner* and J.M. Krolak* (SPON: J.C. Waymire). Dept. Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

Immunocytochemical (ICC) localization of soluble chemical moieties in the cytosol has been difficult with previous tissue preparation techniques. This study describes a cryo-preparation (CP) approach which renders these moieties present and visible via ICC staining (post-embedding avidin-biotin complex technique).

Small biopses of fresh supraoptic nucleus, median eminence and neural lobe were 'quick-frozen' with a bounce-free mechanical device[1] and subsequently freeze-dried in an apparatus developed in this laboratory. The tissues were either treated with osmium tetroxide vapors or left unosmicated. In either case, the tissues were infiltrated directly with pure resin under vacuum. Ultrathin (silver-gold) sections were collected on Delrin staining blocks coated with a Butvar-88 support film. These sections were incubated for 2 h at room temperature in solutions containing antibodies to either vasopression (from Dr. G. Nilaver), calmodulin (from Dr. J. Dedman) or tyrosine hydroxylase (from Dr. J. Haycock) at dilutions of 1:500,000-1:1,000,000.

Ultrastructural morphology was well preserved when compared to tissues fixed with low % glutaraldehyde or commercially available freeze-drying apparatuses. Cytosolic antigens were readily present and stainable, in contrast to aldehyde fixation, and the apparent antibody avidity and spatial stain resolution were enhanced with the present CP technique. Osmication was found to have little or no effect upon the avidity of these antibodies. Extracellular compartments contained localizable material, and cisternae of intracellular membranous organelles were readily stained with no overriding concern relative to the diffusion artifact ubiquitous with pre-embedding ICC techniques.

The procedural artifacts necessarily introduced by preparative methodologies are always constraints to the interpretation of ICC data. As alternative procedures become viable, these artifactual constraints are altered. The present CP technique overcomes cytoplasmic reticulation (and concomitant loss of ultrastructure) during freeze-drying--previously a major disadvantage. Thus CP tissues prepared by this method can be intepreted with less artifactual constraint which should allow heretofore unobtainable information to be gathered. For example, the present CP technique will allow us to investigate labile, soluble cell constituents (such as sugars, metallic ions and membrane lipids) with powerful techniques including cytochemistry, X-ray STEM analysis, autoradiography and serial reconstructions of cryo-prepared tissues.

[1]J. Neurosci. Meth. 1, 353 (1979)

AGING AND BEHAVIOR I

28.1 EFFECT OF AGE ON ESTRADIOL CYTOPLASMIC RECEPTOR (E_2R_C) LEVELS IN MICRODISSECTED BRAIN NUCLEI: CORRELATIONS WITH CHANGES IN STEROID-INDUCED SEXUAL BEHAVIOR. P.M. Wise, B. Parsons, T.C. Rainbow and B.S. McEwen. Dept. of Physiology, U. of Maryland, Baltimore, MD 21201 and the Rockefeller University, NY, NY 10021.

We have reported that the maximal number of E_2 nuclear receptors (E_2R_n) is lower in the preoptic area of middle-aged compared to young cycling rats while no change was detectable in the medial basal hypothalamus (MBH) (Camp, P. and Wise, P.M., Endocrine Soc. Abs #478, 1981). The purpose of this study was to answer the following questions. (1)Are changes in E_2R_n due to changes in maximal E_2R_C levels? (2)At what age are changes in E_2R_C first observed? (3) Are changes in E_2R_C evident in discrete brain nuclei which may be masked in grossly dissected brain areas? (4)Are changes in E_2Rc levels accompanied by changes in E_2-mediated functions? To assess the effect of age on maximal E_2R_C levels, we used 3-4, 7-8 and 10-11 mo old virgin female Sprague-Dawley rats ovariectomized 1 wk prior to use. The following nuclei were microdissected: bed nucleus of the stria terminalis (BNST), suprachiasmatic preoptic area (SCPOA), medial preoptic nucleus (MPN), periventricular preoptic nucleus (PVPN), periventricular anterior hypothalamic area (PVAHA), paraventricular nucleus (PVN), dorsomedial nucleus (DMN), ventromedial nucleus (VMN), arcuate-median eminence (ANME), medial and cortical amygdala and pituitary gland. Cytosolic fractions were prepared and E_2R_C quantitated according to the method of Rainbow et al (J Neurosci 2:1439, 1983). There was no difference in maximal E_2R_C in any brain area in 7-8 compared to 3-4 mo old rats. By 10-11 mo of age there was a decrease in the number of E_2R_C in the SCPOA and MPN with a similar trend in the BNST and PVPN, areas included in the grossly dissected preoptic area used in our previous study. We observed a decrease in the VMN, but no change in the PVAHA, PVN, DMN or ANME, areas included in the grossly dissected MBH. Since the VMN is involved in reproductive behavior, we examined if the decrease in E_2R_C in this area is correlated with behavioral changes. Young (3-4 mo) and middle-aged (10-11 mo) rats were ovariectomized and 2 wk later, they received Silastic capsules which produced physiological levels of plasma E_2. Two days later, 4h before testing, rats received sc injections of progesterone. Proceptive behaviors, lordosis quotient and lordosis quality score were assessed. Middle-aged rats showed deficits in all aspects of mating behavior. The data demonstrate that by 10-11 mo, rats exhibit decreased E_2R_C levels in components of the preoptic area which parallel previously observed changes in E_2R_n. The use of microdissection methods allowed us to uncover changes in a particular nucleus of the MBH, the VMN. This change was correlated with deficits in E_2-induced behavior. The data suggest that changes in estradiol receptors may affect the ability of aging rats to respond to E_2 and may contribute to the age-related transition to acyclicity and infertiltity.

28.2 INCREASED PHYSICAL ACTIVITY IS NOT ASSOCIATED WITH IMPROVED COLD STRESS RESPONSE IN SENESCENT MICE RECEIVING HYPOTHALAMIC SELF-STIMULATION. M. Talan*, J. Whitaker*, D. K. Ingram*, B. T. Engel* (SPON: E. Bresnahan). Gerontology Research Center, National Institute on Aging, Baltimore, MD 21224.

Previous research in our laboratory (Talan, M., Neurosci., Abs. 8, 1982) indicated that electrical self-stimulation of the hypothalamus can retard an age-related loss in thermoregulatory ability to withstand cold stress in senescent male C57BL/6J mice. The objective of the present study was to determine whether this effect was due to increased physical activity which this paradigm also induced. In one experiment senescent mice (29 mo) were surgically implanted with hypothalamic electrodes and then were administered a cold stress test (3 hr exposure to 10° C with physical restraint). After matching for cold tolerance, an experimental group obtained hypothalamic stimulation of 0.5 sec train of 80-100 imp/sec square waves 0.5 msec each at a current of 10-40 μa for each bar press during 30 min daily sessions, while a yoked-control group received the same degree of electrical stimulation without bar pressing. Three weeks later there was no significant difference in response to cold stress. Neither group showed a decline in cold tolerance commonly seen in age-matched, control animals tested at 3-week intervals. A second experiment examined whether increasing the level of general activity produced effects in thermoregulation similar to those observed with self-stimulation. One group of 26-mo old mice was housed singly in standard cages; another group also was housed singly in standard cages but received forced treadmill exercise (daily 60 min sessions at 5 m/min); and a third group was housed singly in activity-wheel cages and voluntarily exercised at a daily rate of 1.5 m/min. After being matched initially on their cold-stress responses, the groups were retested 3 weeks later. No significant differences in responses were found in spite of the different exercise regimens. Thus, the retardation of cold-stress response observed in senescent mice receiving hypothalamic self-stimulation is not due to increased physical activity but rather results from the effect of hypothalamic stimulation.

28.3 HYPOTHALAMIC MONOAMINES IN C57BL/6J FEMALE MICE: EFFECTS OF AGE AND ESTRADIOL N.Telford*, C.V.Mobbs*, H.H.Osterburg*, and C.E.Finch (Spon: R.E.McCaman). Andrus Gerontology Ctr. and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles,CA 90089-0191.

This study evaluates the effects of age and estradiol (E_2) on hypothalamic monoamines. Three groups of C57BL/6J female mice (young, Y, 6 mo; middle-aged, M, 12mo; and old, O, 17 mo) were ovariectomized (OVXed) for 7 d, then implanted subcutaneously with either an empty silastic capsule (sham), or a 6mm (1x, = ca. 6 pg E_2/ml plasma) or 18mm (3x, = ca. 10 pg E_2/ml plasma) E_2-containing silatic capsule, or an E_2-containing glass capsule (>3x, = ca. 15-20 pg E_2/ml plasma). Three weeks after implantation, mice were sacrificed at 1400h (12:12 L:D; lights on 0600h). Monoamines and their catabolites were measured in the whole hypothalamus using an HPLC equipped with an electrochemical detector. Levels of all compounds are expressed as ng/mg protein. All levels and ratios are given as mean±S.E.M.

Serotonin (5-HT) levels declined about 10% with age (all E_2 groups combined): Y=30.7±0.7 (n=24); M=28.5±0.8 (n=26); O=27.4±1.0 (n=22) ($p<0.05$, ANOVA). The ratio of 5-hydroxyindole acetic acid (5-HIAA) to 5-HT, an indication of the relative rates of 5-HT degradation and synthesis, increased with age: Y=0.308±0.011 (n=24), M=0.313±0.007 (n=26), O=0.343±0.014 (n=22) ($p<0.05$, ANOVA). The increase in this ratio was primarily due to the decrease in 5-HT levels, as 5-HIAA levels did not increase due to age alone. Both 5-HIAA/5-HT and 5-HIAA levels were positively correlated with E_2 implant size, but were more strongly correlated with uterine weight, an indicator of E_2 effect. 5-HIAA/5-HT for the four implant groups (all ages combined) were: sham=0.305±0.011 (n=18), 1x=0.318±0.013 (n=19), 3x=0.315±0.010 (n=17), >3x=0.345±0.016 (n=18) ($p<0.05$ vs implant size, <0.01 vs uterine weight, analysis of regression). 5-HIAA levels for the four implant groups were: sham=8.52±0.41 (n=18), 1x=9.13±0.39 (n=19), 3x=9.40±0.39 (n=17), >3x=9.76±0.35 (n=18) ($p<0.05$ vs implant size, <0.01 vs uterine weight, analysis of regression). There was a significant interaction between age and E_2 implant size in increasing 5-HIAA levels ($p<0.05$, ANOVA).

Norepinephrine (NE) levels decreased with age: Y=18.3±0.6 (n=24), M=17.5±0.5 (n=26), O=16.0±0.6 (n=22) ($p<0.05$, ANOVA). Other compounds measured were 3-methoxy- 4-hydroxyphenyl glycol (MOPEG), dopamine (DA), 3,4-dihydroxyphenyl acetic acid (DOPAC), and homovanillic acid (HVA).

28.4 PENTOXIFYLLINE IMPROVES AGE RELATED DEFICITS IN MEMORY. L. de Toledo-Morrell,* F. Morrell, S. Fleming* and M.M. Cohen, Departments of Neurological Sciences and Psychology, Rush Medical College, Chicago, Ill. 60612.

We recently demonstrated a striking relationship between age related deficits in spatial memory and in hippocampal plasticity measured in the same subjects. We now report that pentoxifylline, a phosphodiesterase inhibitor which has been shown to increase intracellular ATP and to augment oxygen consumption in tissue slices, produces a remarkable reversal of the memory deficit in aged rats.

The behavioral task we used was the 8-arm spatial maze. In this task, every arm of the maze is baited with food. Since, once consumed, food is not replaced, the optimal strategy for an experimental animal is not to go back to an arm which has already been visited.

Different groups of young (3 mo. old) and aged (26 mo. old) Fischer 344 strain male rats were treated daily, 30-40 min. prior to testing in the 8-arm maze, with IP injections of a) saline, b) 20 mg./kg. of pentoxifylline, c) 20 mg./kg. of pentoxifylline plus 100 mg./kg. of choline chloride and d) 100 mg./kg. of choline chloride. The pentoxifylline plus choline group tested whether any effect shown by pentoxifylline might be mediated through facilitated use of neurotransmitter substrate.

A comparison of the performance of 'young-saline' and 'old-saline' groups indicated that choice accuracy was markedly impaired (as shown by repeated entries into already chosen arms) in aged animals after about the third or fourth response. Pentoxifylline treatment, however, markedly improved choice accuracy among the aged animals. This effect was not due to improved substrate utilization since the performance of the 'pentoxifylline plus choline' group did not differ significantly from that of pentoxifylline alone group. Although choline did not improve performance in old animals, young rats treated with choline reached the criterion of three consecutive trials with no errors significantly faster than young saline control rats.

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28.5 EPINEPHRINE FACILITATION OF MEMORY PERFORMANCE IN AGED MICE. D. B. Sternberg, P. E. Gold, J. L. Martinez, Jr., and J. L. McGaugh. Department of Psychobiology, University of California, Irvine, CA 92717 and Department of Psychology, University of Virginia, Charlottesville, VA 22901.

Considerable evidence indicates that with aging there are learning and retention deficits in both mice and rats. While there is evidence that changes in cholinergic systems in the central nervous system may underlie some deficits in memory, recent evidence suggests that changes in catecholamines may also be important.

It is known that, in comparison with young animals, old animals show a diminished release of epinephrine with acute stress, such as a training-related footshock. If this diminished release is important to age-related memory deficits, then replacement therapy should be possible. When administered shortly after training, epinephrine (and other stress-related hormones) can enhance or impair retention performance in young animals. The present study investigated the memory modulatory role of epinephrine in aged mice.

Male CFW mice (4 mo and 24 mo old) were trained in a one-trial inhibitory (passive) avoidance task using a 600 μA, 2 sec footshock. Immediately posttraining, the animals received subcutaneous injections of saline or epinephrine (0.01, 0.1 or 1.0 mg/kg). Twenty-four hrs later the animals were tested for retention. All doses of epinephrine used caused significant facilitation in both young and old mice. The age-related differences were attenuated with the epinephrine injection. Thus, it appears that the epinephrine injections may replace normal function.

Comparable findings were obtained in a study using ARS Sprague-Dawley rats, tested one week following training. Once again, facilitation was seen. The old animals administered epinephrine had retention scores which were comparable to those of young animals.

These findings support the view that adrenergic mechanisms play an important role in memory modulation in aged as well as young animals.

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28.6 THE EFFECT OF AGE ON DIAZEPAM PHARMACOKINETICS AND DIAZEPAM-INDUCED HYPNOSIS. P. Hicks, C. Rolsten*, C. Harrington*, C. Davis*, T. Samorajski, and J. Schoolar. Texas Research Institute of Mental Sciences, 1300 Moursund, Houston, Texas 77030.

We have previously reported that marked tolerance develops to the hypnotic effect of diazepam in mice (Hicks, et al., Soc. Neurosci. Abstr. 8:443, 1982). The rate of tolerance development is much slower in old mice than in younger mice, and the older mice respond with a shorter latency. The mechanism of the age-associated difference in response latency and tolerance development is unknown. We have measured plasma concentrations of diazepam and its two biologically active metabolites, desmethyldiazepam and oxazepam, to assess the role of pharmacokinetic differences in the age-associated behavioral changes.

Male 12-, 18- and 28-month old C57BL/6J mice were used that had been purchased as retired breeders from Jackson Laboratories (Bar Harbour) and aged in our animal facilities. Diazepam was administered intraperitoneally at weekly intervals at a dose of 45 mg/kg. Sleeping time was assessed weekly for the first six weeks. On the seventh week mice were sacrificed at various times between 1/3 to 16 hours. Whole body, liver and abdominal fat weights were recorded. The plasma was frozen at -70°C until analysis. Diazepam, desmethyldiazepam and oxazepam were measured in plasma by high performance thin-layer chromatography with flurazepam as an internal standard.

At the time of sacrifice the average body weight of the oldest mouse group was approximately 10% lower than either younger group ($\bar{X}$= 33.0 g, $\bar{X}$= 33.2 g, $\bar{X}$= 30.7 g for the 12-, 18- and 28-month old mice, respectively). Liver weight in the oldest mice was greater than for either younger group ($\bar{X}$= 1.46 g, $\bar{X}$= 1.57 g, $\bar{X}$= 1.89 g for the 12-, 18- and 28-month old mice, respectively). A significant proportion of the whole body weight loss in the oldest mice could be accounted for by a marked decrease in abdominal fat ($\bar{X}$= 1.47 g, $\bar{X}$= 1.46 g, $\bar{X}$=0.52 g for the 12-, 18- and 28-month old mice, respectively). Consistent with the shorter response latency, the older mice had a markedly higher and faster plasma accumulation of diazepam and both of its biologically active metabolites.

The benzodiazepines are very lipid soluble drugs. Their distribution in plasma is markedly influenced by the amount of body fat present. In post-mature mice there is loss of fat stores. This loss of adipose tissue seems to be a major factor in the higher accumulation of diazepam and its metabolites in plasma in older mice. The higher plasma levels clearly are responsible for the shorter response latency seen in older mice following hypnotic doses of diazepam. Further evaluation is indicated to determine the role these pharmacokinetic differences have in the age-associated decreased tolerance to the hypnotic effect of diazepam.

28.7 ANOMIA IN ALZHEIMER'S DISEASE: ASSOCIATED COGNITIVE DEFICITS
F. Jacob Huff*, Suzanne Corkin, and John H. Growdon* (SPON: W.H. Sweet). Dept. Psychol., Mass. Inst. Tech., Cambridge, MA 02139

A study of the cognitive deficits underlying the anomia of Alzheimer's disease will be reported. Patients with this clinical diagnosis were grouped by severity of anomia on the basis of ability to name pictures on the Boston Naming Test (BNT). Additional tests were administered in order to assess visual form perception, verbal fluency (listing members of natural categories, such as vegetables), recognition of the names of objects, and recognition of the category membership of objects and of the words that name the objects. Results were obtained for 12 patients with Alzheimer's disease and 11 healthy, age-matched control subjects.

Patients with Alzheimer's disease were divided into 3 groups on the basis of their scores on the BNT. Non-anomic patients (N=3) were defined as those who obtained scores of 68 or higher, mildly to moderately anomic patients (N=5) as those with scores from 32 to 67, and severely anomic patients (N=4) as those with scores less than 32. In addition, ratings of the overall severity of dementia were made on the basis of the patient's independence and competence in activities of daily living. All 3 non-anomic patients were mildly demented. Among the mildly to moderately anomic patients, dementia was mild in 3 and moderate in 2 patients. In the severely anomic group, dementia was mild in 1, moderate in 2, and severe in 1 patient.

On a test of verbal fluency, deficits were observed in 2 of the 3 non-anomic patients and in all of the anomic patients. Name-recognition tests revealed no impairment in non-anomic patients; varying degrees of deficit were found in 3 of the 5 mildly to moderately anomic patients and in all of those who were severely anomic. Poor performance was characterized by a tendency to accept incorrect names belonging to the same category as the correct name, for example, accepting the word "hat" as the name for a picture of a coat. Recognition of the category membership of words and pictured objects was relatively preserved in all groups. A test of visual form perception revealed no deficit in non-anomic and mildly to moderately anomic patients, but impaired performance was observed in 2 of the 4 severely anomic patients.

The results suggest that two cognitive deficits may interact to produce anomia: a word retrieval deficit resulting in impaired verbal fluency, and a semantic deficit resulting in impaired name recognition. The word retrieval deficit may be present in patients who are unimpaired in naming pictures on the BNT. The semantic deficit occurs independently of a deficit in visual form perception, which is present only in some severe cases.

Experience with additional patients will be incorporated into these results for presentation and discussion.

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28.8 AGE-RELATED INCREASED SUSCEPTIBILITY TO ANISOMYCIN-INDUCED AMNESIA IN MICE: EFFECTS OF PROLONGED TREATMENT WITH DIETARY CHOLINE CHLORIDE.
S. J. Y. Mizumori*, T. A. Patterson, M. R. Rosenzweig and E. L. Bennett. Dept. of Psychology and Melvin Calvin Lab., Univ. of Calif., Berkeley, CA 94720.

Aged mice (14-15 mo) are susceptible to anisomycin (ANI)-induced amnesia for longer periods of time after avoidance training than young controls (2-3 mo) (Davis et al., Exp. Aging Res., 7:33, 1981). The purposes of this experiment were: 1) to investigate in more detail the characteristics of the extended retrograde amnesia (RA) gradient and 2) to assess whether the extended RA gradient could be ameliorated by dietary choline chloride.

Male CD-1 mice were trained in a step-through passive avoidance task. ANI (120 mg/kg) was injected either 10, 15, 20, 30 or 45 min after training. Following a 7-day retention interval, it was found that while 2-3 mo old mice were susceptible to the effects of ANI administered up to 10 min post training, memory of 14-16 mo old mice was susceptible to disruption out to 20 min post training. Retention of 17-20 mo old mice was significantly impaired when ANI was injected as late as 30 min post training. Saline injected animals of all ages showed good retention. These data support the hypothesis that with advanced age, protein synthesis necessary for long-term memory formation progressively slows down. Based on evidence of a possible interaction between the cholinergic system and protein synthesis and findings of a decline in function of the cholinergic system with increased age, naive mice were placed on a choline enriched diet for 5.5 mo. Choline chloride (1.5 mg/ml) was added to the drinking water; standard lab chow was provided ad libitum. At 14.5 mo of age, ANI (120 mg/kg) was injected either 15 or 20 min after passive avoidance training. ANI induced amnesia in choline treated mice only when injected 15 min post training, not when injected 20 min post training. In contrast, control mice maintained on a standard diet were amnesic when ANI was injected either 15 or 20 min post training. These results suggest that dietary choline treatment can render new long-term memories less susceptible to disruption after training. In addition to assessment of overall retention, one may measure age-related changes in memory function more sensitively by examining specific characteristics of RA gradients.

To assess the extent to which this dietary manipulation specifically affected the cholinergic system, the brains were removed for regional weight measures, ChAT assay and cell counts. Brain weights did not differentiate choline treated mice from age-matched controls. However, cerebral hemispheres of both groups of aged mice weighed significantly less than that of young mice. Results of ChAT assay and cell counts will be presented.

28.9 AN IMPORTANT STRAIN DIFFERENCE BETWEEN THE BEHAVIORS OF CLOSELY-RELATED AGING C57Bl MICE.
Kimberly A. Caris*, Susan B. Beall*, Donald R. Meyer, and Ronald F. Mervis#. Departments of Psychology and Pathology#, The Ohio State University, Columbus, Ohio 43210.

The use of C57Bl/6NNIA mice obtained from the National Institutes of Aging (NIA) in various studies is attractive because such subjects can be purchased at older ages than a closely-related strain from the Jackson Laboratories (C57Bl/6J). As part of a continuing series of investigations evaluating the effects of different dietary factors on behavior (and other parameters) in aging mice, we have found in studies using the Bartus passive-avoidance paradigm, that NIA mice are better performers than JAX mice. The male NIA and JAX mice were 13 months-old when tested, having been obtained when 8 months-old and assigned to various dietary groups for 5 months. The dietary treatments included variations in amounts of choline, phosphatidylcholine, and commercial lecithin (Central Soya Centrolex®). Of 114 JAX mice, 33 (or 29%) passed the test (avoided for 300 sec.) and 81 failed it. Of 101 NIA mice, 63 (or 62%) passed the test and 36 failed it. There were no striking interactions between the strain difference and dietary treatments.

28.10 PARKINSON-LIKE MOVEMENT DISTURBANCES OF THE AGED RODENT: RELATIONSHIP TO NEOSTRIATAL HIGH-AFFINITY ^{3}H-DOPAMINE UPTAKE.
J.F. Marshall and C.A. Altar. Dept. of Psychobiology, University of California, Irvine, CA 92717.

Aged rodents show movement disturbances that resemble some of the impairments of Parkinson's disease. These movement disorders, such as impaired swimming, are temporarily reversed by administration of the dopamine (DA) agonist, apomorphine or the DA precursor, l-dopa.

Several known abnormalities in central DA transmission may contribute to the decline of dopamine-dependent sensorimotor skills in advanced age: (1) a loss of dopaminergic nerve terminals in the neostriatum, (2) a decreased number of postsynaptic dopamine receptors (D-1 and/or D-2), and (3) a reduced rate of DA synthesis or an enhanced rate of its enzymatic degradation in this structure. The present study examines a fourth possible contributor, alterations in the inactivation of dopamine via its reuptake into nerve terminals.

We determined the concentrations of neostriatal catecholamines as well as the uptake of ^{3}H-DA into neostriatal synaptosomes prepared from young adult (3 mo) or aged (26-28 mo) Fischer 344 rats. The Na^+-dependent uptake was examined using a range (5 nM to 1 μM) of concentrations of exogenous DA.

Relative to young adults, the aged rodents showed significant decreases in the DA concentration of the neostriatum (5.9 ± 0.4 vs 4.7 ± 0.3 ng/mg tissue) and olfactory tubercle (3.8 ± 0.3 vs 2.9 ± 0.3 ng/mg tissue) without changes in the norepinephrine content of these structures. In contrast, the kinetics of dopamine uptake into neostriatal synaptosomes showed no age change (K_m for young adult, 0.12 μM; for aged, 0.11 μM; and V_{max} for young adult, 98 pmol/mg tissue/ 8 min; for aged, 96 pmol/mg tissue/8 min). The uptake of 0.5 μM DA into the olfactory tubercle also did not vary with age.

These data indicate that, in aged rats, the ratio of available dopamine uptake sites to dopamine content is elevated in both neostriatum and olfactory tubercle. This increased ratio in aged animals could favor the inactivation of released DA by reuptake, particularly when extracellular DA concentrations are high. If so, then administration of DA uptake blockers should facilitate DA transmission and lead to improvements of motor function. The DA reuptake blocker bupropion HCl (Burroughs-Wellcome) facilitated the vigor and success of aged rats' swimming at 25-50 mg/kg i.p.

These experiments draw attention to the importance of considering age-dependent changes in the inactivation of neostriatal dopamine by reuptake and indicate the possible behavioral consequences of these alterations.

28.11 THE NIGROSTRIATAL SYSTEM AND AGING. T.H. McNeill, L.L. Koek*and J.W. Haycock. Department of Neurology, University of Rochester, Rochester, New York 14642 and Department of Neurobiology and Anatomy, University of Texas, Houston, Texas 77025.

Alterations in neurotransmitter systems of the basal ganglia have been postulated to contribute to the disruption of motor function and balance associated with aging. This study examined nigrostriatal (A9) and mesolimbic (A10) dopamine neurons for qualitative age-correlated changes using fluorescence histochemistry for catecholamines and immunocytochemical techniques for the catecholamine synthesizing enzyme tyrosine hydroxylase.

A total of 30 C57BL/6NNia mice 3, 6, 10, 20, 25, and 30 months-of-age (5/group) were used for these studies. Mice were anesthetized with sodium pentobarbital, exsanguinated with saline, and perfused with 4% paraformaldehyde for peroxidase-anti-peroxidase immunocytochemistry or with 4% paraformaldehyde-.5% gluteraldehyde for histofluorescence of catecholamines. Coronal sections were cut at 25 um in thickness on an Oxford vibratome and tissue sections for immunocytochemistry were floated freely in a staining dish following the technique of Grzanna et al. (1978).

Results from this study suggest that age-correlated morphological changes in A9 but not all A10 neurons in the midbrain are present in the mature adult (10 mo.) C57BL/6NNia mouse and show progressive increase in severity until at least 30 months-of-age. These changes are characterized by progressive accumulation of lipofuscin in dopamine-containing perikarya, markedly reduced dopamine content per cell as determined visually by histofluorescence, and an increase in the number of large, fluorescent axonal dilations in dopamine-containing fibers of the mesolimbic nigrostriatal pathways. These data suggest that heterogeneous morphological aging patterns exist within dopamine-containing neurons of the midbrain and that based upon their terminal projection sites various regions of the striatum and cortex may be differentially affected in the aged brain. In addition, these findings support the belief that age-related changes in neural structure are not generalized to an entire brain nucleus or cell type but are selective for individual cells within an affected area.

This work was supported by PHS Grant AG 03254.

28.12 ACCELERATION OF AGE-RELATED FUNCTIONAL DECLINE IN MICE FOLLOWING PRIOR CHRONIC ETHANOL CONSUMPTION. S.F. ZORNETZER, DEPT. PHARMACOLOGY, U.C. IRVINE, IRVINE, CA 92717

The degree to which chronic alcoholism and aging independently produce a shared set of deleterious biological changes is unknown. This study investigated some neurobiological domains sensitive to the potential interactions of prior chronic alcohol consumption and aging. The experiments were designed to determine whether chronic alcohol consumption during a defined period of life-span is capable of altering the normal consequences of aging upon both behavior and brain morphology.

The experiments included behavioral and quantitative neuroanatomical studies performed on male C57BL/6J mice. Three major age groups (starting age: 2, 12 or 24 mo.) were tested. Each was subdivided into three subgroups (minimum number per subgroups = 12). One subgroup received the alcohol-containing liquid diet as the only source of fluid and calories for a 4-month period (Group A). A second group of age-matched mice was a liquid diet nutritional control and received the sucrose-containing liquid diet (Group S). Mice in Group S were pair-fed with mice in Group A, on a cage-by-cage basis. The third basic group of mice received lab chow and water ad libitum. Alchohol was administered to one third of the mice via an ethanol-containing liquid diet in which ethanol provided 35%-42% (8.1% - 10.3% ethanol v/v) of the total caloric content. Blood ethanol concentrations (BEC) were sampled from mice chosen randomly from each cage receiving alcohol containing liquid diets.

Behavioral tests began either at the end of the 30 day "dry out" period for half the mice in Group A (or the equivalent period for mice in Groups S or Lab Chow) or at 28 months of age (typically, life-span for these mice is 32 months of age) for the remaining mice. The behavioral tests for the mouse were selected as relatively robust probes for functional damage in either the hippocampal formation (spontaneous alternation) or the cerebellum (swimming and balancing behavior).

The neuroanatomical experiments evaluated quantitatively a number of morphological changes in the mouse brain produced by (1) normal aging and (2) the possible interaction of aging and chronic alcoholism. Cell count studies, using the correction procedures described by Abercrombie (1946), were caried out.

The results indicate that prior chronic ethanol consumption produces significantly greater behavioral and morphological deterioration in mice at all ages tested. These data support the hypothesis that the deleterious effects of chronic ethanol consumption are compounded with the ongoing processes of age-related behavioral and CNS deterioration.

28.13 PLATELET MAO AND RESPONSE TO MAO INHIBITION IN ELDERLY DEPRESSIVES. R. C. Young, G. S. Alexopoulos*, K. Lieberman*, E. Kent*, C. A. Shamoian. Dept. of Psychiatry, Cornell Univ. Med. College and N. Y. Hosp.-Cornell Med. Ctr., Westchester Div., White Plains, N.Y. 10605.

Major depressive illnesses are common in the elderly. The pathophysiology of these disorders has been hypothesized to involve amine neurotransmitter dysfunction. The activity of monoamine oxidase (MAO), an enzyme that degrades amine neurotransmitters, increases in brain and in platelets with aging; it is further increased in patients with degenerative dementia. Platelet MAO activity has been used as a possible index of brain MAO activity and as a marker of adequacy of MAO inhibitor therapy in young adults. While it has been speculated that MAO inhibitors would be especially useful in depressed patients with high MAO activity, little data is available concerning such therapy in the elderly.

Inpatients $>$ 55 years of age with major depression by RDC were evaluated after one week of psychotropic washout. Baseline platelet MAO activity was measured using benzylamine as substrate. Clinical history and symptoms were documented using the Schedule for Affective Disorders and Schizophrenia. Symptom severity was assessed using the Hamilton Depression Rating Scale (HDRS). Cognitive function was monitored using the Minimental State Scale (MMS). Resting pulse and sitting and standing blood pressure were monitored and serial electrocardiograms were obtained. Subjective side effects were rated using the Asberg Scale. Symptom ratings and platelet MAO activity were recorded weekly for at least four weeks of treatment with phenelzine at a fixed final dose of 30-60 mg/day (median 60 mg/day).

Of the first thirteen patients studied, twelve (mean age 69.4) have completed the four week period. Their HDRS scores (median 26.5; range 17-35) decreased during treatment (median 13.0; range 0-29; $p < .001$). Changes in MMS scores and side effects scores were not statistically significant. Platelet MAO activity was inhibited during treatment (mean 80.9%; S.D. 16.6%; $p < .001$) and inhibition was apparent within the first week. Decreases ($p < .01$) in sitting and standing systolic and diastolic blood pressure occurred during treatment. Neither heart rate nor electrocardiographic conduction times changed during treatment.

Phenelzine can be therapeutically effective in elderly depressives. It is tolerated adequately. Hypotensive effects may limit its use in some patients.

Supported by Dept. of Psychiatry, C.U.M.C.

29.1 PLATELET MAO ACTIVITY IN GERIATRIC DEPRESSION. G.S. Alexopoulos,* K.W. Lieberman,* R.C. Young, C.A. Shamoian. Dept. of Psychiatry. New York Hospital-Cornell Medical Ctr., White Plains, N.Y. 10605

Monoamine oxidase (MAO) is an enzyme responsible for the oxidative deamination of biogenic amines and participates in the intraneuronal regulation of brain monoamine neurotransmitters. Platelet MAO activity has been studied in various psychiatric disorders. There have been contradictory findings concerning platelet MAO activity in unipolar depression. Platelet MAO activity is partly determined by heredity but also increases with age. Studies of elderly depressives may reveal a differential effect of aging on the MAO system in some clinical subgroups.

Platelet MAO activity was assayed in 38 geriatric hospitalized women with primary major depressive disorder and in 16 elderly women with no personal or family history of psychiatric disorders. None of the subjects was on psychotropic medication or other drugs known to influence platelet MAO activity. Diagnosis was made according to Research Diagnostic Criteria. Symptomatology was rated with quantitative scales and family history was systematically obtained and classified according to Family History-Research Diagnostic Criteria. Platelet MAO activity was assayed using benzylamine as substrate and was expressed in nmol/mg protein/hour. Data were analyzed with one way ANOVA and Scheffé's post hoc comparison of means. Significance levels are two-tailed.

The platelet MAO activity of elderly depressed women as a group (N=38, MAO mean: 51.7, SD:21.0) was comparable to that of elderly normal controls (N=16, MAO mean: 66.0, SD=18.5). However, elderly depressed women with early age ($\leq$50 years) of illness onset (N=19, MAO mean: 41.6, SD: 16.9) had significantly lower platelet activity than depressed women with late age ($>$60 years) of illness onset (N=19, MAO mean: 61.9, SD: 20.2) and elderly normal controls. ($F_{(2,51)}=8.91$, $P<.005$). Differences in the clinical picture and family history of the two groups of elderly depressives did not appear to account for the lower platelet MAO activity of early age of onset depressives.

The data suggests that aging reveals biological heterogeneity amongst unipolar depressed women.

(Supported by award from Department of Psychiatry, New York Hospital-Cornell Medical Center)

29.2 AROMATIC L-AMINO ACID DECARBOXYLASE ACTIVITY IN MICROVESSELS AND PARENCHYMA FROM BRAIN REGIONS OF AGING RATS. Isaac F. Roubein, Larry J. Embree and David W. Jackson*. VA Med. Ctr., and Dept. of Neurology, LSUMC-Shreveport, LA 71130.

The presence of aromatic L-amino acid decarboxylase (AAD) in the cerebral microvessels (CMV) constitutes an enzymatic barrier to biogenic amine precursors at the microvascular level. Therefore, alterations in AAD activity with aging may adversely affect normal brain function.

We have determined the activity of AAD in CMV and parenchyma utilizing the carbidopa model. When carbidopa is given at a dose of 100mg/kg it inhibits AAD activity in CMV leaving the enzyme in brain parenchyma (P) essentially unaffected.

Groups of aging male rats (24-27 months) and young adults (3-4 months) were pretreated with reserpine (10 mg/kg i.p.) and 4 hr later with pargyline (75 mg/kg i.p.), followed after 30 minutes by carbidopa (100 mg/kg i.p.). The animals were sacrificed by decapitation 1 hr after the last injection. A second group of animals of identical ages received the same drug treatment except for carbidopa. The following regions were dissected: striatum (S), hypothalamus (HT), midbrain (MB), and cerebral cortex (CX), and the activity of AAD was determined in the brain regions of both groups of animals. The amount of dopamine synthesized was determined using HPLC with amperometric detection. Protein was determined by the Lowry method.

Age (Months)		(n)	A: Total (CMV+P) Activity*	B: Parenchymal Activity*	A-B=CMV Activity*
3-4	S	(9)	10.70±0.79	8.89±0.69	1.81
	HT	(5)	6.49±0.61	4.70±0.70	1.79
	MB	(9)	3.85±0.39	3.11±0.34	0.74
	CX	(5)	1.03±0.18	1.00±0.14	0.03
24-27	S	(9)	10.53±0.59	8.13±0.52	2.40
	HT	(5)	5.95±0.56	4.59±0.26	1.36
	MB	(9)	3.22±0.24	2.39±0.27	0.83
	CX	(5)	1.09±0.13	0.93±0.09	0.16

*AAD activity is expressed as the amount of dopamine synthesized (μg formed/mg protein/30min).

We conclude that aging has no effect on AAD activity in the brain regions examined. Therefore, the action of cerebrovascular AAD as an enzymatic barrier regulating the entrance of monoamine precursors into the brain parenchyma is essentially unaffected with aging. Research supported by Veterans Administration Medical Center and Louisiana State University Medical Center, Shreveport, LA.

29.3 SPECIFIC BEHAVIORAL IMPAIRMENTS IN ASSOCIATIONAL TASKS IN MICE WITH AN AUTOIMMUNE DISORDER. D.G. Spencer, Jr. and H. Lal. Dept. of Pharmacology, Texas Coll. of Osteopathic Med., Fort Worth, TX 76107.

Antibodies are selective for neuronal antigens (brain-reactive antibodies - BRA) increase in blood serum concentrations as C57BL/6 mice age (Nandy, K., in The Aging Brain and Senile Dementia, K. Nandy and I. Sherwin (Eds.). Plenum: New York, 1977). NZB mice have elevated titers of autoantibodies, including BRA, at all ages. In addition, NZB mice acquire the active avoidance task at a slow rate, relative to C57BL/6 mice (Nandy, K., Lal, H., Bennett, D., and Bennett, M., unpublished data). Since BRAs in humans have been hypothesized to produce the neuromorphological abnormalities observed following pre-senile and senile dementia (Ingram, C.R., Phegan, K.J., and Blumenthal, H.J., J. Gerontol., 29: 20, 1974), we undertook to characterize the NZB mouse behaviorally in order to determine whether their apparent cognitive deficits are specific or due to a more general sensorimotor impairment. NZB and CFW mice, ranging in age from 7 to 9 months, were studied in performance on a number of procedures designed to evaluate sensorimotor and stimulus-response associational learning and memory tasks. NZB subjects were no different from CFW mice in basic reflexes or startle thresholds, but the magnitude of the NZB shock startle response was less. NZB mice were also quicker to fall off a rotating rod than CFWs. Conversely, NZBs flicked their tail away from a radiant heat source faster than CFWs did. Although NZBs displayed the same locomotor activity as CFWs after four minutes in the open field, NZBs did not display the same burst of exploratory activity in the first few minutes. Finally, although NZBs displayed large deficits in active and passive avoidance relative to CFWs, no strain difference was observed on the taste aversion task. From this pattern of information, we conclude that there is good evidence for a specific deficit in the formation or retention of stimulus-response associations in NZB mice. Further corroborative neurochemical and neuropathological studies should provide additional information on the appropriateness of the NZB autoimmune disorder as a model for the neuronal and behavioral changes occurring in pre-senile and senile dementias. (Supported by NIA grant #1R03AG03623-01).

29.4 AGE AND NEUROCHEMICAL CORRELATES OF BEHAVIORAL RIGIDITY IN RATS. A. M. Lowy* and D. S. Olton, Dept. Psychology, The Johns Hopkins University, Baltimore MD 21218. D. K. Ingram*, S. B. Waller, and M. S. Reynolds. Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224. E. D. London. Addiction Research Center, National Institute of Drug Abuse, Baltimore MD 21224. (Spon: H. B. Popolow)

Previous experiments have demonstrated that rats have age-related impairments in the performance of complex mazes, and have attributed these impairments to perseverative errors, indicating the presence of increased behavioral rigidity. The present study investigated further the effects of aging on learning and memory, specifically with regard to the phenomenon of behavioral rigidity. Two experiments, similar in procedure, were performed. Two groups of ACI rats, aged 9 and 22 months, respectively, were food deprived and tested five days a week. The design used two different T-mazes, each of which had two different components. The first component was a position discrimination on the stem of the T-maze. The other component was a discrete trial rewarded alternation discrimination on the arms of the same T-maze. Performance in each of the components was first tested simultaneously on one maze. Then the position discrimination in the stem was reversed using the second maze. Consequently, the procedure assessed rigidity using two different procedures simultaneously. Following training, the brains were removed and dissections were made to remove the hippocampus and the following areas of the cortex: cingulate, sensory-motor, occipital, frontal, auditory, and pyriform-perirhinal. The activities of L-glutamic acid decarboxylase and choline acetyltransferase were assayed in these regions, as was muscarinic binding with (^{3}H)Quinuclidinyl benzilate as the ligand. Although both groups of rats were able to learn the task, the rate of learning was more rapid in the younger group. Furthermore, the older rats were impaired in both components of the task. Additional experimentation suggested that the impaired performance of the aged rats was not due to visual defects because there was no age difference in a simple visual discrimination task. The results of the experiments support the hypothesis that behavioral rigidity increases with age in rats. Neurochemical correlates of performance are also presented.

29.5 SERUM DOPAMINE-β-HYDROXYLASE ACTIVITY IN ALZHEIMER TYPE DEMENTIA. M.A. Oleshansky,* B. Reisberg* and S.H. Ferris* (SPON: L.N. Neckers). Millhauser Labs, Dept. of Psychiatry, New York University Medical Center, New York, N.Y. 10016.

Current research in Alzheimer Type Dementia (ATD) is focused on the relationship between CNS pathology and cognitive deterioration. The degree of dementia appears to correlate with both the extent of senile plaques and the reduction of cortical choline acetyltransferase activity in post mortem brain tissue. Similarily, a reduction of noradrenergic cell bodies in nucleus locus coeruleus has been associated with significantly higher dementia scores and younger age at death. As these neuropathological studies require invasive procedures or post mortem examination, they are of limited value in the evaluation of a patient with dementia. We have assayed serum dopamine-β-hydroxylase (DBH) in patients with a clinical diagnosis of ATD in an attempt to develop a laboratory marker for this disorder. Serum DBH has been proposed to be an index of peripheral sympathetic nervous system (SNS) activity. We reasoned that peripheral SNS activity should reflect in some way the CNS pathology that has been reported in ATD.

Blood was obtained from twenty four cases of ATD age 60 - 79 years (mean age 71), twenty four age-matched control subjects age 60 - 79 years (mean age 70) and fifteen young controls age 20 - 40 years (mean age 30). The older subjects were all ambulatory and were attending an outpatient clinic as patients or spouses. A diagnosis of ATD was made by two investigators (B.R. and S.F.) and patients with any other medical illness particularily cardiovascular disease were excluded. A battery of cognitive tests was given to the older subjects and a Global Deterioration Score (GDS) was assigned (Reisberg et al. Am. J. Psychiatry, 139:1136, 1982). DBH activity was measured by photometric assay in 20 µl of serum (Nagatsu and Udenfriend Clinical Chemistry, 18:980, 1972).

Mean serum DBH activity in the group of older subjects as a whole was nearly identical to that for the younger controls. Patients with moderate to severe ATD (GDS 4-6) had serum DBH activity that was not different from their age matched controls (GDS 0-2). ATD subjects and the age matched controls were divided into two groups on the basis of median age (below 70 and above 70). There was a significant reduction ($P < 0.05$) in DBH activity in the younger ATD cases as compared to the older subjects with ATD. No significant correlation between DBH activity and severity of dementia within the range GDS 4-6 was found for either age group. Among the younger ATD cases, women appeared to have the lowest serum DBH activities.

Our data confirm and extend to an older population the finding that serum DBH activity does not change significantly as a function of age in a healthy adult population (Freedman et al. Nature, 236:310, 1972). It further demonstrates that serum DBH activity appears to be altered in ATD in an age-dependent manner. Serum DBH activity may be useful in the prospective evaluation of patients with ATD.

29.6 HIPPOCAMPAL NEUROPEPTIDES AND NEUROFIBRILLARY TANGLES IN AGING AND ALZHEIMER'S DISEASE. H. K. Kulmala and M. J. Ball*. Department of Pathology, University of Western Ontario, London, Canada, N6A 5C1.

Hippocampal neurons are afflicted by neurofibrillary tangles in normal aging and in Alzheimer's Disease (AD). Since a number of neuropeptides are putative hippocampal neurotransmitters, it is possible that peptidergic perikarya contain neurofibrillary tangles (NFT). We report the development of a dual staining procedure used to determine whether NFT are contained in peptidergic neurons.

A block containing hippocampus was obtained from 10 persons dying with AD and 6 control subjects; ages 30 - 95 yrs. (postmortem delay 3 - 16 hrs.). Tissues fixed by immersion in 4% paraformaldehyde for 48 - 72 hrs. were stored 24 hrs. in 5% sucrose in phosphate buffer, then sectioned on a CO_2 freezing microtome at 40 µm. Sections were stained using the PAP procedure with antisera against somatostatin (SOM), cholecystokinin (CCK), vasoactive intestinal peptide (VIP) (Immuno Nuclear) and Leu-enkephalin (ENK) (Dr. R. J. Miller, Univ. of Chicago), along with appropriate controls. Mounted sections were stained with Congo red-gallocyanin.

Postmortem delay (PMD) was found to affect the immunohistochemical localization of these peptides. Best results were obtained after the shortest PMD, while perikarya were not stained after 8 - 10 hrs. ENK neurons were seen in several subregions, similar to their distribution in rat hippocampus. Many VIP neurons, but few SOM or CCK neurons, were stained. However, less than 10% of VIP and ENK neurons in the subiculum and H_1 of aged or AD brains contained NFT. Such immunoreactive cells thus appear relatively unaffected in aging or AD. Several of the tangle-bearing peptidergic neurons displayed large tangles, indicating that neurotransmitter synthesis may not have ceased in such cells. Most tangle-bearing neurons did not stain for any of the peptides; some of these may contain one of the neuropeptides at concentrations below the sensitivity of the present method.

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29.7 CORTICAL CHOLINERGIC AND BEHAVIORAL IMPAIRMENT FOLLOWING KAINIC ACID LESIONS OF RAT BASAL FOREBRAIN. B.Lerer, J.Warner*, M.Zolcinski*, E.Gamzu and E.Friedman*. Depts. of Psychiatry and Pharmacology, New York University Medical Center, New York, NY 10016 and Pharmacology I, Hoffman-LaRoche Inc., Nutley, NJ 07110.

The magnocellular nuclei of the basal forebrain (MNBF) provide extensive cholinergic innervation to fronto-parietal cortex and are analogous to the nucleus basalis, implicated in the cognitive dysfunction in Alzheimer's disease. Bilateral MNBF neurotoxic lesions depleted cortical choline acetyltransferase (CAT) in frontal and parietal cortex but not in striatum or hippocampus.

Behavioral testing began 3 wk post-operative, after MNBF-lesioned rats recovered from the aphagia, adipsia and locomotor abnormalities resulting from the lesion. Rats were tested only if they weighed 20 g more than pre-operative baseline weight. The MNBF rats were compared to unoperated controls, sham-operated controls and control rats injected with kainic acid in the cortical area directly above the MNBF. Cortically-lesioned controls displayed the hyperactivity characteristic of MNBF rats for 7-10 hr after surgery, but were not aphagic, adipsic or behaviorally impaired.

MNBF-lesioned rats were impaired in 24 hr retention of a passive avoidance task with escapable footshock; however, their acquisition performance during the initial training trial was not different from that of the three control groups. There were no differences between the four groups in mean number of daily avoidances on a bar-press active avoidance task; however, the data suggested a slower rate of avoidance learning in the MNBF rats. Mean rates of motor activity were not different between groups and, therefore, do not account for the results obtained in these two tasks. Finally, all rats were tested in a task involving an exploratory response to one of two recessed feeding devices (see Gamzu et al. this meeting). The procedure required serial reversals of this spatial discrimination. MNBF-lesioned rats showed evidence of acquiring the task, as indicated by a decrease in the mean number of daily errors; however, their performance was significantly poorer than that of the three control groups.

A cortical cholinergic deficit may underlie the memory impairment and reduced cognitive functioning of Alzheimer's disease. We believe our rodent model is useful for studying the role of the cholinergic system in memory dysfunction and for developing treatment strategies to alleviate the deficits of Alzheimer's disease.

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29.8 COUNTS OF THE MAGNOCELLULAR CHOLINESTERASE-POSITIVE NEURONS IN THE BASAL FOREBRAIN OF YOUNG AND AGED MICE. J.C. Hornberger* and S.J. Buell* (SPON: R.J. Joynt). Dept. Neurology, Univ. Rochester Sch. Med. & Dent., Rochester, NY 14642.

Severe depletion of cholinergic markers and relative sparing of markers for other transmitter systems have been reliable findings of studies of dementia of the Alzheimer type (DAT). Evidence for loss of up to 80% of cells of the nucleus basalis of Meynert, the presumed cells of origin of cholinergic input to cortex has also been reported in DAT. Depletion of cortical cholinergic markers has also been reported in normal human and rodent aging. We determined quantitatively the number of magnocellular acetylcholinesterase-staining (AChE) neurons in the basal forebrain of the C57Bl/6NNia mouse. Four 15-month and five 53-month-old mice were injected with diisopropylphosphorofluoridate (2.0 mg/kg). Four hours later they were perfused transcardially with 2% paraformaldehyde, 2% glutaraldehyde fixative. The brains were then removed, placed in the fixative overnight, and Vibratome-sectioned in the coronal plane at 20µm thickness. Every third section was stained for AChE. This method, in conjunction with in vivo treatment with DFP, is selective for soma of cholinergic neurons, staining them with substantially greater intensity than acetylcholinoceptive neurons and leaving unstained nonspecific esterases. We collected data from the level of the rostral pole of the globus pallidus to the level of the posterior commissure in the region shown by AChE staining to contain intensely positive neurons. Cytological criteria included size (25-35µm diameter), shape, and intensity of staining. Neurons of the nucleus basalis magnocellularis, globus pallidus, nucleus interstitialis ansa lenticularis, and the ventral aspect of the internal capsule were included. AChE-containing neurons of the paraventricular nucleus, the supraoptic nucleus, and the posterior hypothalamic nucleus were not included. There was less than a 5% difference in total AChE-positive neuron number between age groups. There were no significant differences in cell numbers in any of the above specified anatomical loci. Additional cell count and size studies of the medial septum, tractus of the diagonal band of Broca, and the lateral preoptic nucleus will be presented. These findings of maintenance in aged animals of the numbers of cholinergic neurons which innervate the neocortex must be considered when interpreting data indicating depletion of cholinergic markers in aging rodent neocortex. Supported by NIH Grant AG 02680.

29.9 NORADRENERGIC CHANGES IN SENESCENT MEMORY LOSS. F.M. Leslie, S.E. Loughlin, J.L. McGaugh, D.B. Sternberg and S.F. Zornetzer. Departments of Pharmacology and Psychobiology, University of California, Irvine, CA 92717.

A decline in memory is commonly observed in aged animals. Although it has been proposed that cholinergic dysfunction may underlie this process, recent behavioral and biochemical data suggest that other neurotransmitter systems may be involved. We have utilized morphological and biochemical techniques to examine the role of central noradrenergic systems in age-related memory decline. Male mice (CFW) were trained in a step-through inhibitory avoidance task, and tested 24 hours later for retention of the response. Animals were then sacrificed and their brains removed. One group of brains were formalin-fixed, embedded in paraffin and sectioned serially through the nucleus locus coeruleus (LC). The sections were nissl-stained and analyzed to determine the number of nucleoli within the boundaries of LC. Brains from a separate experimental group were dissected into three areas - cortex, forebrain and cerebellum/brainstem -and analyzed by radioligand binding assay for changes in α_2-receptor number. Regional receptor density was determined by Scatchard analysis of the binding of [^{3}H]rauwolscine, a selective α_2-antagonist, to well-washed brain membranes. Tissue from 4-5 animals was pooled for each analysis.

In general, aged mice (24-27 months) showed significantly lower retention of the shock avoidance response than did young controls. There was, however, considerable variability in the performance of individual aged animals. Analysis of the data suggested the presence of two sub-groups within the aged population - memory-impaired and non-impaired. No significant differences were observed in the density of α_2-receptors in the brains of aged and young mice. It may be, however, that our method of analysis obscured individual differences in memory-impaired animals. We are, therefore, currently using autoradiographic techniques to examine this question in further detail. In the morphological study, a significant decrease in LC cell count was observed in the aged population. Furthermore, there was a highly significant correlation between cell number and the degree of memory impairment. These data suggest that age-related degeneration of the noradrenergic locus coeruleus may have an important role in senescent memory loss.

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29.10 LESIONS OF THE NUCLEUS BASALIS OF MEYNERT IMPAIR MEMORY IN SPRAGUE-DAWLEY RATS. R. F. Berman, R. D. Crosland, D. J. Jenden, and H. J. Altman. Department of Psychology, Wayne State University, Detroit, MI 48202, Department of Pharmacology, UCLA Los Angeles, CA 90024, and Lafayette Clinic, Detroit, MI 48207.

Converging lines of evidence indicate an important role for the basal forebrain cholinergic system in memory processes. The principal origin for this cholinergic projection appears to be the magnocellular neurons in the region of the nucleus basalis of Meynert (NbM) (Fibiger, H.C., Brain Res. Rev., Vol. 4, 327-388, 1982). In Alzheimer's disease there appears to be a loss of basal forebrain cholinergic neurons (Whitehouse, et.al. Science, 215: 1237-1239, 1982). This suggests that the loss may underlie cognitive deficits associated with Alzheimer's disease, and that destruction of this region in experimental animals may impair memory. To date, there have been few experimental studies addressing this possibility. In a series of experiments we examined the effects of neurotoxin lesions (i.e., Ibotenic acid) of the NbM in rats on retention of shock avoidance training. Sixty male, Sprague-Dawley rats 3 months of age were used. Twenty rats were stereotaxically injected, bilaterally, with 0.5µl of Ibotenic acid (15 µg/µl) into the region of the NbM and 20 rats were injected with vehicle only. Animals were allowed a 2 week post-operative recovery. The remaining 20 rats served as non-injected controls. All rats were then placed in the lighted chamber of a two-chambered shuttle box, allowed to enter the larger darkened chamber, and then given a 1.0 mamp, 3 sec inescapable footshock. Retention of footshock training was then tested 30 minutes or 24 hours later by measuring the animals latency to again enter the darkened chamber. Animals were decapitated after retention testing and cortical choline acetyltransferase (CAT) and acetylcholinesterase (AChE) assayed. Rats with lesions of the NbM showed significantly impaired shock avoidance performance compared to non-operated controls at both 30 minutes and 24 hours after training. Lesioned animals showed a significant decrease (-28%$\pm$ 14.4 and -29% $\pm$ 14.2, respectively) in cortical CAT and AChE activities compared to non-operated controls. There was also a siginificant correlation between the impairment in retention performance and changes in cortical CAT and AChE activities, as well as a large and significant correlation between the changes in CAT and AChE activities. The results demonstrate the usefulness of NbM lesions as a model for studying the role of the basal forebrain cholinergic system in memory processes. Supported by the Neuroscience Small Grants Program, Wayne State University, School of Medicine.

29.11 PERFORMANCE OF YOUNG AND AGED MICE IN AN EIGHT-ARM RADIAL MAZE AND NEUROCHEMICAL CORRELATES. D. E. Bernstein* and D. S. Olton. Dept. Psychology, The Johns Hopkins University, Baltimore MD 21218. D. K. Ingram*, S. B. Waller, and M. S. Reynolds. Gerontology Research Center, National Institute on Aging, Baltimore City Hospitals, Baltimore, MD 21224. E. D. London. Addiction Research Center, National Institute of Drug Abuse, Baltimore, MD 21224. (Spon: B. Gordon).

Past studies have demonstrated that the performance of aged rats is impaired relative to that of young rats in complex learning tasks. The present experiment examined whether the age related decrement of rats previously observed in radial maze performance extended to another species, the C57BL/6J mouse. This study consisted of two experiments similar in procedure. Old and young mice, aged 28 and 8 months, respectively, were water deprived for five days each week, and their performance was assessed on a radial arm maze with isotonic saline as the reward. At the beginning of each test session, some isotonic saline was placed at the end of each arm so that the mice were rewarded for their first visit to each arm. Therefore, the optimal strategy was to visit each arm once during a trial. The number of choices made and the number of drinks taken in the first eight choices were recorded. Following training, the the rats were sacrificed and the brains were removed. The hippocampus and six cortical regions (cingulate, sensorimotor, occipital, frontal, auditory, and pyriform-perirhinal) were dissected. The activities of L-glutamic acid decarboxylase and choline acetyltransferase were assayed in each of these regions, as was muscarinic binding with (^{3}H)Quinuclidinyl benzilate as the ligand. Behavioral results indicated that the mice clearly learned to choose accurately on the maze. Both young and old mice began the task at chance levels and showed considerable improvement during the testing period. However, no significant age-related differences in performance were found. Neurochemical correlates of this performance are also presented.

29.12 EFFECTS OF DIETARY RESTRICTION ON NEUROTRANSMITTER SYNTHETIC ENZYMES AND MUSCARINIC BINDING IN AGING RAT BRAIN. E.D. London, D.K. Ingram* and S.B. Waller. Gerontol. Res. Ctr., Natl. Inst. Aging; Addict. Res. Ctr. Natl. Inst. Drug Abuse, Balto., MD 21224.

Dietary restriction increases the lifespan of rats, and retards age-related decrements in striatal dopamine receptor concentrations (Levin et al., Science 214:561, Goodrick et al., Gerontol. 28:233). The present investigation was intended to assess effects of dietary restriction on other neurochemical markers in the striatum and three other brain regions.

Male Wistar rats were given a diet of 24% protein ad lib (AL) or every other day (EOD) from shortly after weaning. Rats were killed at 6 or 24 mo. The striatum (24 mo only), cerebral cortex, hippocampus and cerebellum were assayed. Muscarinic binding site densities (Bmax) and affinities for [H-3]quinuclidinyl benzilate were determined. Specific activities of choline acetyltransferase (CAT), L-glutamic acid decarboxylase (GAD) and tyrosine hydroxylase (TH) were determined radiometrically. All differences indicated below were significant($p \leq 0.05$).

Muscarinic binding studies revealed few differences between AL and EOD rats. At 24 mo, Bmax was 92% higher in the EOD striatum. At 6 mo, K_D differences were seen in the hippocampus and cerebellum where the values were 53% lower and 83% higher, respectively, in EOD than in AL rats. The hippocampus showed age-related declines in Bmax and K_D in both groups. In the cerebellum, no age differences in Bmax were noted; however, K_D increased between 6 and 24 mo in both groups.

CAT was lower at 24 mo than at 6 mo in the cortex and cerebellum of EOD rats. Although EOD feeding did not affect cortical CAT, activities in EOD rats were higher than in AL rats in the striatum at 24 mo, the hippocampus at 6 and 24 mo, and the cerebellum at 24 mo.

The only age difference in GAD was in the cerebral cortex of EOD rats, where values at 24 mo were lower than at 6 mo. The only diet effect was in the cortex at 6 mo, where EOD rats had greater activity than AL rats.

Cortical TH was higher at 24 mo than at 6 mo in both groups. Hippocampal TH was lower at 24 mo than at 6 mo in AL rats. In contrast, EOD rats showed no age differences in hippocampal TH between 6 and 24 mo. The only dietary effect on TH at any age was a lower cortical activity in EOD rats at 6 mo.

Subjecting AL rats to the EOD regimen for 2 weeks at 24 mo did not influence the neurochemical parameters discussed above.

These findings demonstrate that EOD feeding from weaning selectively affects neurotransmitter systems in different brain regions. Effects on cholinergic parameters are greater than those on adrenergic or GABAergic markers, and represent chronic rather than acute effects.

29.13 SPATIAL VISION IN ALZHEIMER'S DISEASE. Mary Jo Nissen* Suzanne Corkin and John H. Growdon (Spon: Edith V. Sullivan) Department of Psychology, Massachusetts Institute of Technology, Cambridge, MA 02139.

Alzheimer's disease is a degenerative brain disease that produces marked impairments in domains of performance that are mildly affected in normal aging. Although memory impairment is the most common and often the earliest symptom of Alzheimer's disease, deficits in language function and, occasionally, visual perception can be prominent even in the early stages. Human aging causes changes in spatial contrast sensitivity; Alzheimer's disease may produce an exaggeration of these effects. In addition, patients with Alzheimer's disease who present with visual complaints may have disorders of spatial vision that could be characterized more specifically by the patients' contrast sensitivity functions.

In order to investigate these possibilities, spatial contrast sensitivity was measured in 17 patients with Alzheimer's disease, one of whom had severe perceptual deficits, and 8 healthy, age-matched control subjects. The stimuli were stationary 700-msec vertical sinusoidal gratings of 0.5, 1, 2, 4, and 8 cpd. The mean luminance of the gratings, which were generated on an oscilloscope, was 5 cd/m^2.

The results from 16 of the patients indicated threshold elevation, relative to control subjects, of about 0.37 log unit at all frequencies tested. Although the possibility that the results reflect a criterion difference between groups cannot be ruled out, the similarity of their false alarm rates makes this interpretation unlikely. Rather, the neural degeneration of Alzheimer's disease appears to produce a true sensitivity loss.

The remaining patient was unique. This 63-year-old woman had an impairment in object and face recognition so severe that she could not recognize her husband visually, despite good visual acuity. She had a right inferior homonymous quadrantanopia but an otherwise normal ocular examination. A CT scan showed enlargement of the cortical sulci over the lateral convexities posteriorly. An occasional sulcus in the posterior parietal region was capacious, especially on the left. This patient's contrast sensitivity to low and intermediate spatial frequencies was dramatically reduced relative to other Alzheimer patients. Her sensitivity loss was 0.84 and 0.68 log unit at 0.5 and 1 cpd, respectively, whereas her sensitivity at 8 cpd was equal to that of the other patients. This dramatic sensitivity loss to low spatial frequencies in a patient with a severe impairment in object and face recognition supports the view that low spatial frequency information is essential for those functions.

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29.14 BIOCHEMICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF CHOLINE ACETYLTRANSFERASE IN AGING AND SENILE DEMENTIA. P. L. McGeer, E. G. McGeer, J. H. Peng* and J. Suzuki*. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, University of British Columbia, Vancouver, B. C., Canada, V6T 1W5.

The primary cholinergic innervation of the neocortex is believed to be the substantia innominata area of the basal forebrain. We have prepared a detailed map of cholinergic neurons in the human medial and basal forebrain by immunohistochemistry using a monoclonal antibody to human neostriatal choline acetyltransferase (ChAT) developed in a mouse hybridoma. By cutting thin sections of paraformaldehyde-glutaraldehyde fixed tissue and staining first for ChAT and then for cresyl violet, it has been possible to establish that all giant neurons greater than 30 microns in diameter in these areas are cholinergic. The numbers of cholinergic cells in the substantia innominata in normal and senile dementia cases have been determined as a function of age. We have also correlated such changes with ChAT levels measured biochemically in various cortical areas on fresh post-mortem tissue. Both cholinergic cell counts in the medial basal forebrain and ChAT levels in the frontal cortex decline substantially with age. Senile dementia cases have medial basal forebrain cell counts and biochemical levels of cortical ChAT reduced to less than half those of controls of similar age. These data support the cholinergic hypothesis of memory and suggest that cholinergic deficiency dementia may be an appropriate clinical description for dementia cases that may or may not show extensive development of neurofibrillary tangles.
(Supported by the M.R.C. of Canada)

29.15 BEHAVIORAL AND FINE STRUCTURAL ASSESSMENT OF 13 MONTH OLD C57BL/6j MICE ON DIFFERENTIAL CHOLINE DIETS. K. A. Doyle*, L. D. Trombetta* and H. P. Davis. Dept. Psychol. and Dept. Pharmaceutical Sciences, St. John's Univ., Jamaica, NY 11439.

Retired breeder C57BL/6j male mice at 8-9 months of age were placed on either a choline enriched diet (≈50 mg choline/gm diet), choline control diet (≈1-2 mg choline/gm diet), or choline deficient diet (<1 mg choline/gm diet). At 13 months of age mice were assessed for 7-day retention of one-trial passive avoidance training. The median step-through-latencies at test for enriched, control, and deficient animals were 364 sec, 101.5 sec, and 15 sec, respectively (N=19-22 per group), and application of the Mann—Whitney U-test indicated that mice on the deficient diet had significantly poorer retention than control or enriched animals ($p<0.05$). Enriched and control mice were not significantly different ($p>0.20$).

Light microscopy and electron microscopic ultrastructural analysis of the hippocampus revealed early degenerative changes in the astrocytes of the animals on the choline deficient diet. These effects included cytoplasmic clearing, with loss of cytoplasmic integrity, increases in smooth surfaced vesicles, and mitochondrial enlargement. Observations of neurons showed no significant changes in synaptic regions or in the appearance of neurofibrillary structures. Occasionally large abnormally shaped mitochondria were seen in the axoplasm. No degenerative changes were observed in the control or enriched choline groups.

29.16 BIOCHEMICAL STUDIES IN RATS AFTER PROLONGED PHYSICAL CONDITIONING. Lewis L. Truex, Norman R. Mason and Michael J. Schmidt, The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

Physical conditioning decreases fat stores, increases cardiac capacity and increases oxidative metabolism in muscle. Physical conditioning is also purported to affect brain function and has been used in therapy programs for affective disorders. The present studies were undertaken to determine if physical conditioning resulted in measureable neurochemical changes in rodents, and to determine if there was a difference in the overall adaptive physical capabilities of young (6-mo) and old (24-mo) rats.

Animals were exercised by treadmill running on a graduated intensity scale that continued for 13 weeks. An initial endurance test was used to establish baselines for the young and old animals. Following 8 weeks of conditioning, endurance was tested again, and biochemical determinations were made at the end of the 13-week conditioning period. The most striking observation was the effect chronic exercise had on the endurance capacity of young and old rats. Young animals were initially able to run a distance of 1072 ± 60 meters, while old animals could only run 420 ± 27 meters. After conditioning, both groups demonstrated improvement: young, 74 percent increase and old rats, 113 percent increase. Other changes noted were a 37 percent decrease in the epididymal fat pads of young rats and a 13 percent body weight reduction. Exercise did not reduce the fat pads of aged rats although a 7 percent reduction in body weight was observed. Changes in heart weight were not observed in either age group.

Although evidence of conditioning was apparent in the young and the old rats, no biochemical changes in muscle or brain were detected: brain and gastrocnemius muscle mitochondrial cytochrome oxidase activity; mitochondrial P/O ratios and respiratory rates; the concentration of mitochondrial protein; or the binding of ^{3}H-serotonin or ^{3}H-diazepam to cortical membranes. There also were no age-related differences in these parameters. The post-decapitation induced accumulation of cyclic AMP was less in the cerebellum of old rats compared to young rats; but conditioning did not change these values.

In summary, prolonged physical training, which was adequate enough to produce marked physical improvements in both young and old rats was not sufficient to result in detectable biochemical differences in the muscle or brains of animals after a 13-week training period. However, the experiments do demonstrate that aged rats have the capability of enhancing their endurance thresholds through a physical training regimen.

29.17 INFLUENCES OF AGEING AND PHOTOPERIODS ON ANALGESIA IN MICE. M. Hirst* and M. Kavaliers* (SPON: A. Hudson). Depts. of Pharmacology-Toxicology and Zoology, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Old mammals display altered behavioral responses to narcotic analgesics. Recent studies by us have shown that there are major age-related differences in the daily rhythms of aversive thresholds and analgesia induced by morphine. To pursue these findings we investigated the effects of various light-dark conditions on analgesia in mice of different age classes. Times of reactions to a hot-plate (55+/-0.5^{0}) were assessed in young (1-3 month), mature (8-12 month) and senescent (24-30 month) male mice exposed to particular light-dark cycles (L:D 12:12; 16:8, 8:16; 12:12) after administrations of morphine sulphate (10 mg/ml/kg of body weight), or saline. The dark phase tests were conducted under low intensity (photosafe light - 0.005 uW/cm^{2}) lighting conditions (which is necessary to observe diel changes in this period as normal laboratory lighting has a disruptive influence on dark phase responses). Composites were prepared of aversive threshold and analgesia measures so that 24-hour patterns accumulated from independent tests, with 2-hour intervals. Individual animals were not used for at least 4 days between two testing sessions. Significant day-night rhythms of responsiveness were observed in the treated mice. The diel rhythms were characterised by increases in aversive thresholds during the course of the light phase, with a significant dark phase enhancement and a precipitate fall in threshold with the dark-light transition. The timing of the nocturnal increase in analgesia, as well as the extent of the elevation, was dependent on the duration of the dark period. Other features of the response also varied with the L:D conditions. In all cases, however, there was a significant age-dependency in response in the dark phase; the old animals demonstrated a markedly lower level of analgesia at this time, but no consistent differences were evident in the light phases. The observed effects suggest that photoperiods and age interact to influence endogenous opiate systems. It remains to be seen if there are alterations in opiate levels, receptor sensitivities, or perhaps pineal-related endocrinal changes that occur in parallel.

29.18 AGING-RELATED CHANGES IN THE CORRELATIONS BETWEEN SNIFFING AND HIPPOCAMPAL RHYTHMIC SLOW-WAVE ACTIVITY IN THE RAT. Wm. B. Forbes and F. Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

During odor discrimination training, sniffing exhibits a preferred latency relationship with rhythmic slow-wave activity (RSA) in the hippocampal formation; this relationship is strongest when animals are evaluating the behavioral relevance of odors (Macrides, Eichenbaum, and Forbes, J. Neurosci., 2:1705-1717, 1982). The present study analyzed sniffing (monitored with a nasal thermocouple) and hippocampal RSA (bipolar recording in dorsal field CA1) during open field behaviors in Fisher-344 rats aged 3, 18, 30, and 36 months. Analytic procedures were based on the fast-Fourier transform. There was a monotonic decrease with age in the incidence of higher frequency (7-10 Hz) sniffing and hippocampal RSA during free behavior. However, high frequency (8 Hz) hippocampal RSA could be elicited in animals of all ages by inducing them to struggle, suggesting that the reduced incidence of high frequency hippocampal RSA in the older animals might be due to a decrease in the vigor of spontaneous movements in the open field. In the youngest animals, (1) the dominant frequency of sniffing matched that of hippocampal RSA (frequency entrainment) more often than would be predicted on the basis of chance; (2) preferred phase differences between sniffing and hippocampal RSA were reliably observed in the 5-9 Hz range; and (3) these preferred phase differences varied linearly as a function of frequency, implying an underlying latency relationship. These phenomena changed progressively with age as follows: (1) the incidence of frequency entrainments decreased; (2) the frequency range within which preferred phase differences were observed became increasingly narrow; and (3) the incidence of preferred phase differences decreased. In the oldest animals, preferred phase differences between sniffing and hippocampal RSA were reliably observed only at 5 Hz. The preferred phase difference at that frequency was similar, nonetheless, to that seen in younger animals. This suggests that though the robustness of the preferred temporal relationship between sniffing and hippocampal RSA declined with age, the underlying nature of the phenomenon was similar in young and old animals. These observations may reflect aging-related changes in either the predisposition or the ability of rats to evaluate odor relevance in the open field.

This research was supported by NIH grants AG00779 and NS12344.

29.19 PROLONGED NEUROLEPTIC EXPOSURE AND DOPAMINERGIC SUPERSENSITIZATION IN AGING MICE. P.K. Randall*, J.S. Randall* and J.A. Severson (Spon. F.N. Pitts, Jr.). Depts. of Physiology & Biophysics and Psychiatry, USC Sch. of Med. and Andrus Gerontology Ctr., L.A., CA 90089

Reports on supersensitization of striatal dopamine (DA) receptors in the aging rodent have been inconsistent. We previously reported that aging C57BL/6J mice show diminished supersensitization to moderate chronic haloperidol regimes by both neurochemical (3H-spiperone binding) and behavioral (apomorphine-induced stereotypic behavior) criteria. In order to determine whether this phenomenon is dependent upon severity of chronic drug treatment, we examined 6 1/2, 13, and 27-30 month-old C57BL/6J mice after 0, 30, 60, or 90 days of treatment with 2.5 mg/kg haloperidol administered in the drinking water. After a 7-day withdrawal period mice were tested for apomorphine-induced stereotypic behavior (1.0 mg/kg, IP.) On the following day mice were sacrificed and striata dissected for analysis of 3H-spiperone binding.

Results were similar for binding and behavioral tests. Although receptor number (Bmax) and behavioral response to apomorphine varied significantly with age and duration of haloperidol treatment duration, the Age X Duration interaction did not approach significance, indicating a similar supersensitization in all ages. Haloperidol treatment produced a highly linear increase in both binding and behavioral response to apomorphine over the 90 days of treatment. Bmax for 3H-spiperone binding decreased significantly with age, while stereotypic behavior increased between 6 1/2 and 13 months and then declined slightly in the oldest group. Analysis of the time-course of stereotypic behavior following apomorphine injection revealed that the elevation of total stereotype scores in the older groups was the result of extended duration of the response rather than increases in peak response. No alterations in Kd for spiperone binding were observed.

In addition, a highly regular linear relationship was obtained between group means of 3H-spiperone Bmax and stereotypic behavior scores (r=.97). Correlations of binding and behavior between individual animals within groups, however, were poor.

These data suggest that diminished neuroleptic-induced DA-supersensitization in aging mice is probably confined to relatively short treatment durations.

29.20 LESION-INDUCED DA SUPERSENSITIVITY IN AGING MICE. J.S. Randall* and P.K. Randall* (SPON: J.K. Engelhardt) Dept. of Physiology & Biophysics, USC Sch. of Med., Andrus Gerontology Ctr., L.A., CA 90089.

Regulatory adjustments of receptor number may be critical in the appearance of therapeutic or motoric side-effects of neuroleptic drugs. We have found that old mice are less likely than young to develop DA receptor-supersensitivity when given a moderate chronic regime (21-day, 1.2 mg/kg injected or 2.0 mg/kg in drinking water). Joseph et al. (Life Sciences, 1982), however, using a unilateral nigro-striatal lesion model in Wistar rats did not detect a difference in supersensitivity with age. Other results have as well been contradictory. In order to examine whether these differences resulted from species variation or from the technique employed for inducing supersensitivity, we investigated the contralateral rotational behavior to dopamine agonists in different ages of mice following unilateral 6-OHDA lesions of the nigro-striatal pathway.

Four-, 10-, or 24-month-old C57BL/6J mice received unilateral striatal (0.5 mm anterior, 2.4 mm lateral, and 3.1 mm ventral from bregma) injections of 4 ul of 4ug/ul 6-OHDA hydrobromide. Five, 10, and 20 days following this injection mice were tested for contralateral rotation to 2.0 mg/kg apomorphine. All mice, regardless of age, showed a regular increase in net contralateral rotations over this time period. There was no significant age effect either in percent of mice showing reliable contralateral rotation or in net contralateral turns of those which exhibited the behavior. In addition, the spontaneous contralateral asymmetry apparent following the lesion disappeared in a regular fashion- highly correlated with the appearance of contralateral rotation to apomorphine in all age groups.

These data suggest that the age related deficits in DA supersensitization which we have observed to chronic neuroleptic treatment are probably specific to that less severe stimulus.

30.1 **WHAT IS THE RELATIONSHIP BETWEEN EMBRYONIC AND ADULT GROWTH PATTERNS IN THE TELEOST VISUAL SYSTEM.** **L.C. SHELTON* and R.D. FERNALD, Inst. of Neuroscience, University of Oregon, Eugene, Or. 97403.**

In the African cichlid Haplochromis burtoni, as in many teleosts, growth continues throughout its lifetime. As the body grows, so do both the retina and tectum maintaining a congruent visual map as they do so.

It has been shown in adult H. burtoni, using ^{3}H-thymidine to locate newly generated neurons, that the retina enlarges symmetrically, partially (≈60%) by stretching the existing tissue and partially (≈40%) by the addition of new neurons at the margin of the eye. The tectum, in contrast, grows asymmetrically, adding new tissue only over about half its extent, primarily at the caudal pole and more medially than laterally.

Since a congruent visual map of the retina on the tectum exists throughout adult life, the distinctly different growth patterns of these two structures suggests the need for rearrangement of the fiber projections during growth. To account for this difference, it has been hypothesized that the sites of retinal ganglion cell termination are continuously re-located during adult growth. We have examined embryonic development in this species to understand whether this hypothesis is consistant with the growth pattern during embryogenesis. For example, compensatory differential growth in the embryo may hold the key to understanding the adult growth process.

By examining ^{3}H-thymidine labelled cells in embryos and young fry, following a variety of survival times, we can show that the retina of the embryo is growing quite asymmetrically. The tectum, on the other hand, is growing in a pattern very similiar to that of the adult. Whether or not this differential growth pattern between retina and tectum in the embryo is sufficient to compensate for the observed adult growth patterns is currently under investigation.

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30.2 TRANSPLANTATION OF EMBRYONIC NEURAL TISSUE INTO THE HEMISECTED SPINAL CORD. B. H. Hallas. Department of Anatomy, New York College of Osteopathic Medicine, Old Westbury, NY 11568.

Previous investigations have demonstrated that embryonic spinal cord or neocortical tissue could be successfully transplanted into the mammalian spinal cord. Such transplants survived, grew, and established connections with the host brain. The nature of these connections, however, was unknown. The present research provides evidence that it is possible to re-establish afferent and efferent connections of the severed spinal cord by the placement of embryonic transplants into the lesioned area.

Hemisections were made in adult rat spinal cord between vertebrae C5 and C6. Immediately following the hemisections either 14-day embryonic spinal cord or 15-day embryonic neocortex was transplanted into the lesioned area. The host animals were then allowed to survive for 90 days at which time either HRP was injected directly into the transplants to determine the afferents to the transplants or electrolytic lesions were made in the transplants for the determination of the efferents. Additional electrolytic lesions were made in areas of the host brain that normally provide afferents to the intact cervical spinal cord.

The afferents to the transplants were provided by neural structures that normally have axons that course through the cervical spinal cord. Labeled neurons were located in the motor cortex, deep cerebellar nuclei, cervical and thoracic spinal cord. The efferents of the transplants terminated in areas of the spinal cord that were immediately surrounding the transplant. Large areas of terminal field degeneration were observed in the cervical spinal cord (C1-C3), in the upper thoracic spinal cord, and in the lower brain stem.

Therefore, the results of these experiments indicate that an embryonic neural transplant is not only capable of completely filling the lesioned area of an adult spinal cord but receives afferents from the host's central nervous system that normally has axons coursing through the hemisected area and also provides efferents to the intact spinal cord. In addition, the transplants prevented a neural glial scar from forming along the entire interface between transplant and host spinal cord. The transplants then become incorporated into the spinal cord and function as a "bridge" between the severed ends of the spinal cord.

30.3 A METHOD FOR LOW MAGNIFICATION HIGH RESOLUTION PHOTOGRAPHY IN THE FLUORESCENCE MICROSCOPE R. Mårtensson, Medical Photographer, Department of Histology, University of Lund, Lund, Sweden.

In order to observe high-resolution histofluorescence one must use a 10x or higher objective depending on the construction of the dark field condenser, otherwise the illumination is not sufficient to photograph evenly throughout the preparation and in sufficient detail. When using these higher powers of magnification, montages are often constructed to represent the entire field of interest. Several technical problems arise in using this procedure: a) variability in background tone between photos within a montage; b) after exposing a tissue to fluorescent light for the time necessary to obtain the montage, the fluorescence can have faded to the point where it is no longer useful; c) cost of making a large montage can be prohibitive; d) the time necessary to photograph, copy, print and assemble the montage can be very long. With these problems in mind a method was developed to use a low power magnification which allows for a large field to be photographed in a single frame, and yet retain brightness, sensitivity and high resolution of detail within the photographed image. A Zeiss fluorescence microscope using transmitted light, standard dark field condenser and a 2.5x objective is used in this work. With this method one is able to: 1) increase the consistency of tone of photographs from experiments requiring large field representations, 2) save preparations from fading due to overexposure 3) save time, and 4) save money. Specifically a montage consisting of 30 separate photos, which exposes the specimen to 30 minutes of constant fluorescent illumination, and which takes 50 hours to develop and copy can now be represented more clearly on one single negative which is exposed to 3 minutes of fluorescent illumination. Examples are presented using catecholamine histofluorescence and serotonin immunohistochemistry. This procedure is also applicable for other tissue stains which require transmitted light using low power magnification.

30.4 INTRACEREBRAL AND INTRASPINAL IMPLANTS OF LOCUS COERULEUS CELL SUSPENSIONS: DELETERIOUS EFFECT OF TRYPSIN IN THE SUSPENSION MEDIUM, A. Bjorklund, H. Nornes, S. B. Dunnett, F. H. Gage and U. Stenevi*. Department of Histology, University of Lund, Sweden, Department of Anatomy, Colorado State University, Fort Collins, Co. and Department of Experimental Psychology, University of Cambridge UK.

Dissociation of embryonic CNS tissue was devised as a technique for implantation of developing neurones into the depth of the brain in adult rats (Bjorklund et al. Cell Tiss. Res. 212:39, 1980; Schmidt et al. Brain Res. 218:347, 1981). This procedure, which involves incubation of the tissue in trypsin followed by mechanical dissociation, has been successfully applied to grafting of a variety of neuronal cell types, such as dopaminergic, serotonergic and cholinergic neurones. Initial attempts to graft suspended noradrenergic (NA) neurones taken from the developing locus coeruleus (LC) region were however unsuccessful. This prompted us to investigate which factors might hamper the survival of LC neurones in the dissociation-implantation procedure.

In the first series of experiments, the age of the donor foetuses was varied between 12 and 17 days of gestation (CRL of 7, 9, 11, 13, 15 and 20 mm were tested). The tissue was collected in room-tempered sterile glucose-saline, incubated in 0.1% crude trypsin (Sigma, Type II), washed and ruptured with a fire-polished Pasteur pipette, according to our standard procedures. 3-5µl aliquots of the resulting cell suspension were injected stereotaxically into the dorsal hippocampal formation denervated by 6-OHDA. After survival times of 4-8 weeks, the rats were processed for monoamine histofluorescence by the ALFA method. Although surviving grafts of good size, rich in non-monoaminergic neurones, were found in most animals, the grafted tissue from all donor ages contained at most only some single, scattered NA-containing neurones, and there was very little NA fibre outgrowth from the grafts.

In the next series of experiments, the LC region taken from 11 mm foetuses was dissociated as above, but the trypsin incubation step was omitted. 3µl aliquots were injected into the dorsal hippocampus and the thoraco-lumbar spinal cord in 6-OHDA denervated rats. All these grafts contained abundant NA-containing neurones. They had formed an extensive plexus of NA-containing fibres throughout the host hippocampus and the gray matter of the spinal cord, similar to what has previously been described with solid LC grafts (Bjorklund et al. Brain Res. 170:409, 1979; Nornes et al. Cell Tiss. Res. in press). These results suggest that some neuronal types may be relatively sensitive to trypsin in the dissociation procedure.

30.5 REINNERVATION OF DENERVATED HIPPOCAMPAL FORMATION BY EMBRYONIC RAPHE CELL IMPLANTS. AN IMMUNOHISTOCHEMICAL STUDY IN THE RAT. H.W.M. Steinbusch, F.H. Gage, A. Björklund* and U. Stenevi*. Dept. Pharmacology, Free University, Amsterdam, The Netherlands and *Dept. Histology, University of Lund, Lund, Sweden.

Previous studies from one of our laboratories have clearly demonstrated that intracerebral implants of embryonic dopamine, noradrenaline, and acetylcholine neuronal cell structures exhibit the capacity to reinnervate specific brain areas in the adult host recipients. In addition, preliminary results on the survival and outgrowth of transplanted embryonic serotonin containing raphe nuclei using fluorescence histochemistry has been presented. In the present communication we present more complete evidence of cell survival and fiber outgrowth using an immunofluorescent method, in conjunction with the cell suspension implantation methodology.

Embryonic raphe cells were dissected from the region of the pontine mesencephalic flexure from embryonic rats with crown-rump length of 15 mm. 2 µl volumes of dissociated cell suspensions were injected into the dorsal anterior and posterior hippocampal formation unilaterally. The rats were pretreated with 5,7-DHT and DMI to induce a complete biiateral serotoninergic denervation. After a survival time of 3 months the rats were perfused and processed for immunofluorescence, using a specific and well characterized antibody to serotonin.

The results demonstrate that long term survival of raphe cells in suspension is effective and moreover, a limited number of 5-HT cells in suspension can induce an extensive density of reinnervation of the host hippocampus. The serotoninergic neurons in the suspensions implanted in the denervated rats give rise to a restored innervation of hippocampus on the side of the transplants. The contralateral side lacks any serotonin immunoreactivity. The ingrowth of newly formed fibers was not restricted to the dorsal and ventral parts of the hippocampus. The pattern of re-innervation of the host hippocampus by the transplanted raphe cells was found to be quite specific and resembles the distribution seen in the control brains. A dense plexus of serotoninergic varicose fibers was found in addition in the area of the medial forebrain bundle mostly in its caudal extent, however only on its ipsilateral side. Studies are currently in progress to investigate not only the ingrowth of serotoninergic fibers into the hippocampus but also the ingrowth of serotonin-containing raphe cell suspensions into the spinal cord after spinalectomy. Functional correlates of these denervating and reinnervating procedures are being tested.

30.7 EFFECTS OF REPETITIVE INDUCTION OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS OF THE RAT. M.C. de Jonge* and R.J. Racine. Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

High frequency trains of pulses applied to the perforant path can produce a long-term potentiation (LTP) of the perforant path to dentate granule cell synaptic response. Most LTP studies have utilized multiple trains to produce LTP and in some cases several sessions of trains have been applied to maximize the LTP effect. Even in these cases, however, LTP is far from permanent. The experiment reported here was designed to test the effect of inter-session interval on levels and duration of LTP and to determine if residual changes in responsitivity of the system could be detected after the response amplitude had decayed back to baseline. For example, although no residual potentiation is evident in terms of response amplitude after decay to baseline, subsequent LTP effects may be larger, last longer or occur at lower threshold.

Three groups of animals each received 5 sessions of 70 trains. The train intensity was increased in 10 steps during each train session in order to determine threshold and peak LTP effects. In group one, the 5 train sessions were 24 hours apart, followed by a nine day decay period during which evoked responses were measured each day. In group two, the amplitude of the population spike was allowed to decay to halfway between the peak potentiated response and baseline, before the application of the next series of trains. After the 5th session, decay was followed for a nine day period. The third group was allowed to decay back to baseline amplitudes before each subsequent train session. A final nine day decay curve was determined for this group as well.

The animals administered a train session once every 24 hours showed daily increments in LTP of both population spike and population EPSP. The animals receiving train sessions after partial (50%) decay showed increases in LTP of the population EPSP but not population spike. The animals allowed to decay to baseline between train sessions showed no increase in peak LTP after the first session. None of the groups showed any changes, over sessions, in LTP threshold or decay rates. There does not then, appear to be any residual changes in responsitivity in the system once the response amplitude has returned to baseline.

30.8 LONG-TERM POTENTIATION OF THE PERFORANT PATH-GRANULE CELL SYNAPSE IN THE CHRONIC RAT PREPARATION: EFFECTS OF CATECHOLAMINE DEPLETION. G.B. Robinson* and R.J. Racine. (SPON: N.W. Milgram). Dept. of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

Previous investigators (Bliss, T.V.P., Goddard, G.W. & Riives, M., J. Physiol., 334: 475, 1983) have shown that catecholamine depletion, in the anesthetized rat, reduces the level of long-term potentiation (LTP) of the perforant path-granule cell (PP-GC) synaptic response but not of the population spike. It is possible, however, that catecholamine depletion may result in a more depolarized GC population. Any further increase in excitatory input might then be expected to produce a smaller increment in the EPSP while increasing the probability of cell discharge. We have investigated this hypothesis in the chronic animal. This allowed us to test the effect of catecholamine depletion both on baseline responses and on that component for LTP which lasts for days or weeks.

Rats were implanted with stimulating and recording electrodes in the PP and hilus of the fascia dentata, respectively, then allowed at least two weeks recovery. The effects of reserpine (same regimen as Bliss et al., 1983) on baseline PP-GC field responses was determined by comparison of I/O curves obtained immediately before and 24 hrs after injection. During potentiation trials, test responses were sampled once every 20 sec for 15 min, to establish baseline amplitude, and for 15 min after each of 8 trains presented in an ascending intensity series. Non-depleted controls received identical treatment. When the LTP had decayed to baseline the animals were tested under the opposite treatment condition. Thus, each animal served as its own control.

Catecholamine depletion itself resulted in a significant increase in the PP-GC population spike amplitude without affecting threshold. This was accompanied by a significant decrease in the EPSP amplitude. These effects on baseline responses are consistent with the proposed disinhibition hypothesis.

During potentiation trials depleted animals consistently exhibited greater levels of LTP (proportional to the current baseline) of the EPSP and population spike than was observed in the control condition. The potentiated EPSP, however, was still smaller in amplitude than before drug treatment. The level of EPSP and spike LTP in the depleted condition remained above that observed in the control condition for several days. Subtraction of drug effects, however, indicated that catecholamine depletion had no effect on that component of LTP which can persist for days or weeks.

30.9 INTRACELLULAR TESTS OF POSSIBLE LTP MECHANISMS. J. S. Taube* and P. A. Schwartzkroin (SPON: H.D. Patton). Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

A description of the mechanisms underlying long term potentiation in hippocampus has been long in coming. Several investigations suggest that changes may be localized to the synapses, whereas other experimental evidence implicate alterations in the dendritic tree or even in interneuron-mediated inhibition. Since most LTP experiments have utilized the technique of extracellular recording of field potentials, it has not been possible to determine what changes might be occurring at the level of the postsynaptic membrane of a single cell. We have monitored the intracellularly-recorded response to glutamate in order to test the hypothesis that glutamate sensitivity is altered, and have recorded from interneurons to examine their role in LTP.

400 µm thick guinea pig hippocampal slices were cut and maintained in vitro at 35°C. Intracellular recordings were made from the soma of CA1 pyramidal cells and extracellular field potentials were monitored simultaneously with another electrode located in nearby stratum pyramidale. Orthodromic input was elicited with stimulation in stratum radiatum. Glutamate (1 mM) was micropressure-injected from pipettes introduced in the CA1 apical dendrites (30 psi, 2 µm tip diameter, 10 msec pulse). The drug electrode was positioned to achieve a short latency, fast-rising depolarization, and the pulse duration adjusted so the response was subthreshold for action potential initiation. Afferent fibers were then tetanized (20 Hz for 5 sec, or 50 Hz for 1 sec) and the response to glutamate monitored for 30 min afterwards.

Following tetanization the pyramidal cell was often hyperpolarized (5-10 mV). Consequently, for subsequent testing, we adjusted the membrane potential to the pretetanized level by injecting depolarizing current into the cell. It was difficult to potentiate the intracellularly recorded EPSP, even though our extracellular field potential showed potentiation. The response to glutamate following tetanization was inconsistent, but usually showed either no change or a decreased amplitude. The amplitude of the glutamate response was extremely sensitive to the position of the electrode; when care was taken to control for this factor, no change could be found in the response to glutamate. It seemed unlikely that LTP was due to a decrease in inhibition since: 1) the EPSP/IPSP sequence (produced by Schaffer collateral stimulation) sometimes included a potentiated IPSP; this occurred independently of whether or not the EPSP was potentiated; 2) recordings from interneurons located in stratum pyramidale indicated that interneuron activity was not decreased following tetanization; 3) intracellularly-recorded EPSPs in the interneurons were not decreased.

(Supported by NS 00413, NS 17111, GM 07108)

30.11 VOLTAGE-CLAMP ANALYSIS OF LONG-TERM SYNAPTIC POTENTIATION. German Barrionuevo, Stephen Kelso and Thomas H. Brown. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Long-term synaptic potentiation (LTP) is an extremely persistent enhanced synaptic efficacy that can be induced by repetitive synaptic stimulation for periods of a few seconds or less. Extracellular recordings performed in vivo on the hippocampal formation, suggest that LTP may last for hours, days, or weeks. LTP has been proposed to play a role in information storage in the nervous system.

As part of an effort to understand more about the mechanisms underlying LTP, we have recently developed the capability of applying the technique of voltage-clamp analysis to synapses in the hippocampal slice (Brown and Johnston, J. Neurophysiol. in press; Johnston and Brown, In: Brain Slices, 1983). Here we report preliminary observations on our voltage-clamp studies of LTP. Intracellular recordings were made, using a timeshare single microelectrode current- and voltage-clamp device, from pyramidal neurons of the CA1 and CA3 regions. The slices were bathed in 10 μM picrotoxin to block the recurrent or feedforward inhibition that normally accompanies the monosynaptic excitatory synaptic inputs to these cells. LTP was induced by stimulating the afferents at 100 Hz for 1 second. This tetanic stimulation was presented 2-4 times at 5 second intervals. The input resistance, synaptic potential amplitude and synaptic current amplitude were measured before and 15 minutes to 1 hour after the tetanus.

LTP was observed, under current- and voltage-clamp conditions, in six pyramidal neurons. As might be expected (Carnevale and Johnston, J. Neurophysiol. 47, 606, 1982), the synaptic currents increased more than the corresponding synaptic potentials. There was no increase in the input resistance after LTP. Mean (± SE) values for the synaptic current amplitude, the synaptic potential amplitude and the input resistance, before and after the induction of LTP, are given in the table below.

Measurement	Control	After LTP	Percent of Control
Synaptic potential amplitude (mV)	9.8 ± 2.4	15.3 ± 4.4	156
Synaptic current amplitude (nA)	0.5 ± 0.1	1.2 ± 0.4	240
Somatic input resistance (MΩ)	53 ± 12	52 ± 11	98

We are presently attempting to determine whether the increased synaptic currents result from an increase in the synaptic conductance and/or a change in the reversal potential. (NIH Grants NS 18861, NS18295 and a McKnight Foundation Scholar's Award).

30.12 ASSOCIATIVE LONG-TERM SYNAPTIC POTENTIATION IN HIPPOCAMPAL SLICES. Thomas H. Brown and German Barrionuevo. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Associative long-term synaptic potentiation (LTP) has been suggested as a candidate mechanism for aspects of learning and memory in the vertebrate central nervous system. The essential phenomenon was first well-characterized in a series of in vivo studies performed on the interactions between two separate sets of synaptic inputs to the dentate gyrus (Levy and Steward, Brain Res. 175, 233, 1979; Levy, et al., Neurosci. in press; Levy and Steward, Neurosci. in press). One input was arranged to generate a weak and the other a strong extracellularly recorded synaptic response. Concurrent tetanic stimulation of both inputs produced a subsequent long-term enhanced synaptic efficacy in the previously weak input under conditions in which tetanic stimulation of either pathway alone failed to have this effect.

One major breakthrough, in the effort to understand such use-dependent forms of cortical synaptic plasticity, was the development of the in vitro hippocampal slice preparation. The advantage of the hippocampal slice preparation is that the circuitry is well-defined and relatively simple and it is possible to apply to the slice powerful neurophysiological techniques that are impracticable in vitro (Brown and Johnston, J. Neurophysiol., in press; Johnston and Brown, In: Brain Slices, 1983; Barrionuevo, et al., Neurosci. Abst. 1983). We were therefore interested in knowing whether associative LTP could be studied in the hippocampal slice.

Intracellular recordings were made from 16 pyramidale neurons of the CA1 region of the slice. One synaptic input to these cells was made to generate a weak (2-5 mV) excitatory postsynaptic potential (EPSP) and the other input was made to produce a strong EPSP that was 2-5 times larger. Seventy five percent of the cells showed associative LTP; that is, simultaneous tetanic stimulation of both input pathways resulted in a subsequent long-term enhanced synaptic efficacy in the weak input under conditions in which the same tetanic stimulation of either input alone failed to have this effect. The average increase in EPSP amplitudes was 81%. Associative LTP was shown in some cases to last hours without decrement. The plastic changes were localized within the CA1 region and appear to reside in the pre- or postsynaptic elements of the monosynaptic excitatory input to the pyramidal neurons. The increased synaptic efficacy could not be accounted for by any of several measured postsynaptic passive membrane properties.

These results demonstrate that associative LTP occurs in vitro and is not unique to the dentate gyrus. The technical advantages of the hippocampal slice should facilitate more detailed analysis of the mechanisms underlying this interesting form of long-term synaptic plasticity. (Supported by NIH grant NS18861 and a McKnight Foundation Scholar's Award).

30.13 QUANTAL ANALYSIS OF LONG-TERM SYNAPTIC POTENTIATION. Douglas Baxter and Thomas H. Brown. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

Long-term synaptic potentiation (LTP) is an extremely persistent form of neuronal plasticity that can be induced by brief repetitive synaptic stimulation. Although LTP was originally thought to be unique to the hippocampal formation, it now appears to be a rather general feature of many central and peripheral synapses (Brown and McAfee, Science 211,1411, 1982; Swanson, et al., Neurosci. Res. Prog. Bull. 20:, 1982).

We were interested in knowing whether the mechanism responsible for LTP is pre- or postsynaptic. In principle, LTP could arise either from an increase in the average number of quanta released (termed the mean quantal content, m), and/or from an increase in the average amplitude of the postsynaptic response associated with the release of each quantum (termed the mean quantal size, $\bar{q}$). An increase in m implies a presynaptic mechanism while an increase in $\bar{q}$ could arise from either pre- or postsynaptic factors.

Three methods of quantal analysis were used to determine whether LTP is due to an increase in m or $\bar{q}$. For the first quantal analysis of LTP, we selected the crayfish opener neuromuscular junction because of its numerous advantages for such studies. Conventional intracellular recordings were made from small muscle fibers in young crayfish. Several hundred evoked excitatory junctional potentials (EJPs) were collected before and 15 minutes to 2 hours after brief repetitive stimulation of the excitatory axon that innervates these fibers.

LTP was observed in 7 fibers. The EJP amplitudes increased on the average to 179% of the pretetanic control values. The increase in EJP amplitude could be entirely accounted for by a corresponding increase in m. In no cases was there a significant change in $\bar{q}$. The table below summarizes the range of average measurements or calculated parameters, before and after LTP, as well as the average percent change. The parameter estimates (m and $\bar{q}$) made by the three methods of quantal analysis were in excellent agreement.

Measurement or Calculated Parameters	Range During Control Period	Range After LTP	Mean % Change
EJP amplitude(uV)	63 - 260	93 - 430	79
m estimate	0.2 - 2.5	0.3 - 4.2	83
$\bar{q}$ estimate (μV)	107 - 320	109 - 328	-2

These findings suggest that LTP results from presynaptic changes that increase the mean number of quanta released. The generality of this conclusion is currently being tested by performing similar quantal studies of LTP in the hippocampus. (NS18861, NS 07190 and a McKnight Foundation Scholar's Award).

30.14 INTRACELLULAR INJECTIONS OF EGTA BLOCK HIPPOCAMPAL LONG-TERM POTENTIATION. J. R. Larson*, G. Barrionuevo, S. R. Kelso, F. Schottler*, and G. Lynch. Department of Psychobiology, University of California, Irvine, CA 92717.

It has been suggested that the long-term potentiation (LTP) of synaptic potentials seen in hippocampus after high frequency stimulation of afferents is due to a calcium-dependent change in the post-synaptic neuron. To test this, we have injected the calcium chelator EGTA into hippocampal CA1 pyramidal cells and measured synaptic potentials before and after high frequency stimulation of the Schaffer-commissural pathway.

Hippocampal slices were prepared from rats and maintained in vitro. An extracellular recording electrode was positioned in field CA1 (s. radiatum or s. pyramidale) and a bipolar electrode placed in s. radiatum to activate the Schaffer-commissural fibers. Only slices in which high frequency stimulation of these fibers (5 trains of 10 or 30 pulses at 300 or 100 Hz) produced stable LTP of the dendritic field potential (at least 10% increase in slope and amplitude for 15 min) were used. We obtained intracellular recordings from 33 cells impaled with control electrodes (filled with 4 M potassium acetate) and 26 cells impaled with EGTA electrodes (4 M potassium acetate + .2-.5 M EGTA). All cells had resting membrane potentials greater than 55 mV, action potential amplitudes greater than 60 mV, and input impedances greater than 15 megaohms.

Single pulse stimulation of Schaffer-commissural fibers evoked stable EPSP's in all cells reported here. EGTA injection (200 ms long .5 nA hyperpolarizing current pulses passed through the electrode at 2 Hz via a bridge circuit for 5 minutes) had no detectable effect on membrane potential, input impedance, and paired-pulse facilitation of EPSP amplitude. Twenty-eight of 33 cells recorded with control electrodes showed LTP of the evoked EPSP (at least 10% increase in EPSP amplitude lasting a minimum of 10 minutes after high frequency stimulation; overall mean of 30 ± 4%), however only 6 of 26 cells injected with EGTA exhibited LTP (by the same criterion; overall mean of 1 ± 3%). The amount of LTP induced in the field potentials of the two groups did not differ.

These results indicate that selective manipulation of the target neuron can prevent the occurrence of LTP. Given the discrete effects of EGTA found here and reported by others for CA1 pyramidal cells, it is reasonable to conclude that the drug's effects on LTP were mediated by calcium buffering.

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31.1 INPUT FROM ERGORECEPTORS AND OTHER SENSORY MODALITIES ONTO LUMBAR SPINORETICULAR NEURONS. Roger Thies. Dept. of Physiology & Biophysics, Univ. of Okla. Hlth. Sci. Ctr., Okla. City, OK 73190.

The increase of blood pressure during exercise is due partly to signals from contracting muscles; the ascending pathways for this response are unclear. Other investigators have shown that type III afferent fibers respond to muscle contractions, to squeezing of tendons, and to injections of bradykinin (BK) and capsaicin (CAP); the terminals for these fibers are called ergoreceptors. Others have found that bilateral lesions of lateral reticular nuclei (LRN) in the medulla block this somatopressor response.

We have recorded with carbon fiber microelectrodes the activity of neurons located in laminae IV to VII on the left side of L_7 & S_1 segments in 16 anesthetized cats. Antidromic stimuli were applied ipsi- and contralaterally to both nucleus reticularis gigantocellularis (NGc) and LRN. The 28 spinoreticular (SR) neurons projected predominantly contralaterally (20) and then bilaterally (7), with similar numbers to NGc and LRN. Their conduction velocity was 42.6±2.3 ($\bar{x}$±S.E.) m/sec. Amplitudes with optimal localization were often only 0.1-0.5 mV, which precluded study of inputs to them in some cases.

Of 11 SR neurons tested for somatic input, 5 were excited by squeezing the left gastrocnemius muscle or pinching the skin and one was inhibited; one was inhibited from the right gastroc.; 2 were inhibited by squeezing two or three limbs; and 2 were unresponsive. Eighteen of 25 SR neurons tested were fired by electrical stimulation of limb nerves; activity from medial gastronemius, lateral gastrocnemius, and sural nerves had minimum afferent conduction velocities of 7-30 m/sec, indicative of type III afferent fibers. About 15% of nerves also gave faster conduction velocities of type I or II afferents. Four SR neurons were also fired by stimulation of the left sympathetic chain with minimum afferent conduction velocities of about 2 m/sec.

Six neurons were tested for responses to close arterial injection of BK and/or CAP into the left gastroc. muscle. Injection of 10 μg/kg increased firing from 1-10/sec to 10-30/sec in two cells by BK, in two cells by CAP, and in one cell by both. One neuron that was inhibited by both chemicals was also inhibited by somatic stimulation of three limbs. This may represent a small population of SR neurons that are inhibited by ergoreceptors and other stimuli.

Lumbar SR neurons that respond to muscle ergoreceptors also respond to input from sympathetics, skin (sural nerve), and wide somatic fields. Consequently, information about muscle contraction is integrated with other sensory inputs before transmission to the medulla. (Supported by American Heart Assoc. and Tulsa Chapter of Oklahoma Affiliate).

31.2 DISTENTION SENSITIVITY AND MOTOR CONNECTIONS OF VENOUS AFFERENTS: BASIS FOR A NEW ORTHOSTATIC MUSCLE TONUS - VENOPRESSOR MECHANISM. F. J. Thompson, B. J. Yates and J. P. Mickle. Dept. of Neuroscience, Univ. of Fla. Coll. of Med. and Vet. Med., Gainesville, FL 32610.

Recently, details of a peripheral venous afferent input to the spinal cord (Thompson and BArnes, 1979), to the cerebral cortex (Thompson, Lerner, Fields and Blackwelder, 1980) and to somatic motoneurons (Thompson, Barnes, Wald, 1982) were reported. The connections revealed in these studies were proposed to provide the neural substrate for venous afferent elicited influences on skeletal muscle tone. This model proposed that afferents from receptors in the long limb veins contribute to reflex control of the capacitance of the skeletal muscle venous reservoir through modulation of skeletal muscle tone (i.e., muscle tonus-venopressor mechanism).

In the present series of experiments central neural responses to distention of segments of the femoral-saphenous veins were examined to determine the presence and properties of distention sensitive receptors in the walls of these veins. Stretch of the vein wall produced by increasing the intravenous pressure of isolated segments of the femoral-saphenous vein elicited evoked potentials in the sixth lumbar spinal cord segment. Threshold response appeared with distention pressures of 2-3 mmHg. Maximal responses were elicited with perfusion pressures of 18-20 mmHg; and half maximal responses were observed with pressures of 6-10 mmHg. In a second series of experiments, mechanical stretches of the vein wall with a mechanical transducer produced spinal evoked potentials with threshold stretches of 5 microns/mm. Single fiber recordings have revealed that the venous afferent fibers which entered the sixth lumbar cord segment included fibers which conduct action potentials at 57 meters per second. These recordings were made with glass pipette microelectrodes with impedances of 50-60 meg ohms, inserted intracellularly into the fibers in the region where they entered the dorsal horn.

Distention of a segment of the vein wall either by perfusion or by mechanical stretch elicited negative ventral root potentials. These potentials are known to be produced in the ventral root fibers as a result of excitatory postsynaptic potentials which are conducted electrotonically down the axons of motoneurons which course as ventral root fibers and provide a sensitive population assay of facilitation to motoneurons.

The properties of the venous afferent fibers observed in these experiments taken together with influence of venous afferents to motoneurons shown in this and previous experiments, are proposed to provide the functional basis for a VENOUS AFFERENT ELICITED MUSCLE TONUS - VENOPRESSOR MECHANISM. Supported by NIH grant RO1 HL 25619.

31.3 RESPONSES OF UPPER THORACIC SPINOTHALAMIC NEURONS TO GALL BLADDER DISTENTION IN THE MONKEY. Robert D. Foreman, W. Steve Ammons, and Robert W. Blair. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.

Chest pain has a number of causes, and is a particularly important indicator of ischemic heart disease. However, chest pain may also be the result of abdominal disease. A likely explanation for chest pain of abdominal origin is convergence of abdominal afferents onto spinothalamic neurons in the upper thoracic spinal cord whose somatic receptive fields are in the chest region. In support of this hypothesis is our recent finding that electrical stimulation of the greater splanchnic nerve activates T_2 to T_5 spinothalamic neurons of the monkey (Federation Proc. 42:582, 1983). The goal of the present study was to determine if these neurons respond to a more natural stimulus, distension of the gall bladder. Experiments were performed on 10 monkeys (Macaca fascicularis) which were anesthetized with α-chloralose. The gray matter of the T_1 to T_5 segments of the spinal cord was searched for cells which were antidromically activated by stimulation of the contralateral ventral posterior lateral nucleus of the thalamus. Thus, each neuron in this study was determined to be a spinothalamic tract neuron. The somatic field of each neuron was mapped. Most somatic fields were on the left chest and forearm. Thirty-two neurons were tested for responses to distention of the gall bladder with saline for 30 seconds at pressures ranging from 10 to 100 mm Hg. Twelve neurons (38%) responded to gall bladder distention with an increase in cell activity. Both tonic and phasic responses were observed. Pressure threshold for cell response varied from 20 to 80 mm Hg. In 7 cases there was a relationship between the magnitude of the cell response and the gall bladder distending pressure. Maximal responses were usually obtained at 80 mm Hg. The most effective distending pressure resulted in an increase in cell activity from 13 ± 4 to 24 ± 3 impulses per second. The liklehood of cell response was not related to laminar location or to the type of somatic input. The results demonstrate that increased gall bladder pressure activates upper thoracic spinothalamic neurons. This finding may explain the number of clinical observations of chest pain resulting from gall bladder disease. Supported by National Heart, Lung, and Blood Institute Grants HL22732, HL07430, and HL00557.

31.4 LEFT VENTRICULAR SYMPATHETIC AFFERENT FIBER DISCHARGE AND RECEPTIVE FIELD DIMENSION CHANGES ASSOCIATED WITH CARDIAC STIMULATION BY BRADYKININ, CORONARY ARTERY OCCLUSION, AND ARRHYTHMIAS. H. Rodney Holmes, J. Andrew Armour, C. Dale Chapman*, Robert D. Foreman, Univ. of Okla. Hlth. Sci. Ctr., Okla. City, OK 73190.

Receptors in the heart respond to mechanical and chemical stimuli. Comparison of afferent activity with measurements of the cardiac receptive fields demonstrates a relationship of the afferent discharge to the location and tensions of the receptors. To examine the relationship of the afferent discharge to receptive field dimensions during noxious chemical stimulation by bradykinin (BK), coronary occlusions (CAO), and abnormal beats associated with premature ventricular contractions (PVC), we recorded single afferent fiber activity from the thoracic sympathetic rami communicantes of 34 alpha-chloralose (80 mg/kg) anesthetized dogs of either sex. After mapping the receptive field by probing, we placed sonomicrometer crystals around the field on its vertical, horizontal, and wall thickness axes. We measured conduction velocity and continuously recorded afferent activity, EKG, left ventricular (LVP) and aortic blood pressures, and the length dimensions of the receptive field. Waiting 20 min between each manipulation, we electrically induced PVCs, placed a BK soaked pledget on the receptive field, administered BK (1 μg/kg) into the heart, and reversibly occluded the left anterior descending and circumflex coronary arteries. We determined no functional differences between Aδ and C fibers. Bradykinin always stimulated afferent activity. Intracardiac BK always resulted in length changes and pressure decreases, but the BK pledget on the epicardium only once resulted in length and LVP changes. With only one exception CAO stimulated activity of left ventricular receptors. CAO never stimulated activity of the two right ventricular receptors. Following CAO, the ventricle sometimes changed its dimensional characteristics during contraction. This resulted in new receptive field length changes and afferent discharge characteristics. The PVCs always caused length changes during the compensatory pause and the next heart beat. Associated with these length changes were increases in afferent activity. However, LVP was near baseline during the pause, and the next beat was variably higher, lower, or the same as control pressure. These data further elaborate the relationship of afferent activity to receptive field dimensions during and following abnormal cardiac events such as PVC and CAO. The role of the BK stimulation of receptors is unclear. The magnitudes of the discharge rates were much higher than following the other maneuvers. Furthermore, when LVP decreases and receptive field dimension changes comparable to these induced by BK were acheived by the other maneuvers, afferent activity decreased. (Supported by NIH Grants HL07430, HL00557 and HL27260.

31.5 RESPIRATORY-RELATED VARIATIONS IN HEART RATE DURING SLEEP AND WAKING STATES IN AGED CATS, R. M. Harper, M. H. Chase, E. Lucas*, D. Taube* and R. B. Trelease*. Depts. of Anatomy and Physiology, and the Brain Research Institute, UCLA, Los Angeles CA 90024; and Dept. of Anatomy, University of Arkansas, Little Rock AK 72201.

The principal source of heart rate variability during quiet sleep in cats is a respiratory-related sinus arrhythmia that is manifested as an increase in rate during inspiration and a decrease during expiration. The amplitude of sinus arrhythmia appears to be indicative of vagal tone, and extreme loss of amplitude during quiet sleep may indicate a cardiorespiratory disorder in the infant or a number of pathologies, including vagal neuropathy, in the adult. Sinus arrhythmia exhibits a distinct developmental course over the first 6 months of life in humans. However, only incomplete data are available for the development of such heart rate variability in later life.

We examined the extent of sinus arrhythmia in 5 cats aged 9 - 19 years and 5 young adult cats during sleep and waking states. The cats were implanted with diaphragmatic EMG electrodes (which also provided a source of EKG signals), and with electrodes to monitor the EEG, eye movement and nuchal musculature in order to assess the animal's behavioral state. After at least 10 days of recovery, the cats were placed in an isolated temperature-controlled, sound-attenuated chamber for a 5 day period. Food and water were automatically provided. Data were digitized in real time on a large computer disk and magnetic tape.

Cardiac R-R interbeat intervals were calculated with an accuracy of 1 msec and plotted on an incremental plotter. Analysis included 1) an assessment of maximum and minimum R-R intervals at peak cardiac acceleration and deceleration values with each breath, and 2) spectral estimates of the amplitude of sinus arrhythmia calculated from an interpolated rate curve of the R-R intervals which were estimated at the respiratory frequency. The amplitude of respiratory-related sinus arrhythmias was greatly exaggerated in aged cats, with the amplitude in some aged cats exceeding that of young adult cats by a factor of 3.

These data indicate that vagal tone is enhanced in aged cats. The mechanism of this enhancement is not known; it may result from increased sensitivity of the baroreflex with age, or from altered patterns of forebrain or brain stem control of vagal efferent mechanisms. We conclude that respiratory-related heart rate variability increases in aged cats and that it provides an easily quantifiable assessment of vasomotor activity that appears to be age dependent.

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31.6 TEMPORAL PATTERNING OF THE BAROREFLEX IN RESPONSE TO INDUCED TRANSIENT HYPERTENSION. J.D. Marks*, R.C. Frysinger, R.B. Trelease* and R.M. Harper (SPON: R. Saneto). Dept. of Anatomy and Brain Research Institute, UCLA, Los Angeles, CA 90024

Transient hypertension elicits a baroreflex which tends to reduce elevated arterial pressure. The temporal development of the baroreflex is important in analyzing its underlying neural control mechanisms. We analyzed beat-to-beat changes in the relationship between arterial pressure and intervals between systolic pulses in order to characterize this pattern.

Adult cats were instrumented with electrodes for monitoring sleep-waking states and with concentric bipolar stimulation electrodes in the central amygdala. Venous and arterial catheters were introduced via femoral vessels. Transient hypertension was induced in the awake animal using three different methods: 1) phenylephrine infusion, 2) aortic occlusion with a Swan-Ganz catheter, 3) stimulation of the central amygdala. Mean arterial pressure calculated on a beat-to-beat basis was paired with the interbeat interval. X-Y plots were made with arterial pressure on the abscissa and interbeat interval on the ordinate. The points were plotted with a vector connecting sequential points.

This plot allows differentiation of baroreflex phases through 1) the trend of the sequentially ordered points, and 2) the temporal "distance" into the plot where slope changes occur. The slope is analogous to the "gain" of the baroreflex, which constitutes the change in interbeat interval per unit change in arterial pressure.

The pressure-interval plot of a transient hypertensive episode is ellipsoidal in shape, consisting of 1) an ascending limb corresponding to the rise in arterial pressure, 2) an inflection point, and 3) a descending limb corresponding to the fall in arterial pressure. Central amygdala stimulation imparts an initial negative slope to the ascending limb followed by a sharp increase in interbeat interval, indicating that tachycardia accompanies the initial arterial pressure rise and is replaced by bradycardia. These findings suggest that central amygdala stimulation transiently inhibits the bradycardic component of the baroreflex. For a given trial, the descending limb is vertically shifted along the ordinate with respect to the ascending limb. Thus, during a transient hypertensive episode, the baroreflex exhibits hysteresis: a given arterial pressure maps onto a different interbeat interval depending on whether the pressure is ascending or descending.

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31.7 RENAL NERVE-DEPENDENT DOPAMINERGIC ACTIVITY IN THE HYPOTHALAMUS IN THE RAT. K. P. Patel and R. L. Kline (SPON: T. Vilis). Dept. of Physiol., Univ. of Western Ontario, London, Ontario, CANADA N6A 5C1.

Electrophysiological studies have demonstrated that renal afferent nerves project to hypothalamic sites involved in the regulation of arterial pressure and fluid balance. In addition, renal denervation has been shown to alter the concentration of catecholamines in the hypothalamus. In the present study we measured the turnover of dopamine (DA) and norepinephrine (NE) in the anterior hypothalamus and posterior hypothalamus in conscious Wistar rats by measuring the decline of [DA] and [NE] 90 min after administration of α-methyl tyrosine. [DA] and [NE] at 90 min were expressed as a percent of the initial values for each tissue and the difference between renal denervated and intact groups was assessed using a t-test.

Mean arterial pressure (measured by indwelling catheter in the femoral artery of the unrestrained rat) and heart rate were not significantly different between intact and renal denervated groups. There was a significant decrease in DA concentration of the posterior hypothalamus of the renal denervated group compared to animals with intact renal nerves. [NE] was not significantly different between the intact and renal denervated groups. There was a significantly lower DA turnover in the anterior and posterior hypothalamus of the renal denervated group compared to similar hypothalamic sections of animals with intact renal nerves. However, there was no significant difference in the turnover of NE in the hypothalamic sections between intact and renal denervated groups. These results suggest that dopaminergic activity but not noradrenergic activity in the hypothalamus is dependent on intact renal nerves. It is conceivable that renal afferents influence dopaminergic activity involved in physiological responses related to kidney function and cardiovascular adjustments such as modulation of vasopressin release.

(Supported by Ontario Heart Foundation).

31.8 INTRACAROTID INFUSIONS OF α-ADRENERGIC DRUGS ALTER FIRING RATE OF SUPRAOPTIC (SON) NEURONS IN THE RAT. M.F. CALLAHAN, L.D. MITCHELL, S.I. BELLIN, & A.K. JOHNSON. Depts. of Psychology and The Cardiovascular Center, The University of Iowa, Iowa City, Iowa 52242.

Controversy currently exists as to the role of central adrenergic neurons in the firing rate of SON neurons and the secretion of vasopressin. In this respect, Poulain and Waverley (Neuroscience, 7(4):773, 1982) reviewed evidence suggesting that β-adrenergic receptor stimulation decreases, and α-adrenergic stimulation increases the firing of SON neurons. We have recently initiated studies designed to assess the effects of α-adrenergic compounds on firing of SON neurons.

Male albino rats (~350g) were anesthetized with urethane. Arterial blood pressure (and heart rate) were monitered by a catheter placed in the femoral artery. Another catheter (PE 50) was placed into the right external carotid artery and secured into place with the tip at the bifurcation of the internal and external carotid arteries. Single unit activity was recorded from antidromically identified neurons in the SON. At the conclusion of the experiments, animals were infused with 1.0 cc black ink to ensure patency and selectivity of drug delivery to the right hemisphere. All drug concentrations are expressed as the salt.

Intracarotid infusions of the α_2-adrenergic antagonists, Yohimbine and Rauwolscine (100-300 μg) resulted in an inhibition or total block in the firing of spontaneously active cells which occured with a 5-10 sec. latency. These infusions were usually associated with a small (10mm Hg) decrease in b.p. Infusions of low doses (50 μg) failed to affect SON neurons. Infusions of the α_2-adrenergic agonist clonidine (5-7 μg) had no effect on firing of SON neurons except for an inhibition of activity which appears to accompany the initial increase in b.p. caused by clonidine. Infusions of the agonist at higher doses (10-20 μg) results in immediate excitation (latency = 5 sec.) followed by inhibition which is usually accompanied by a long period of excitation.

Infusions of the selective α_1-adrenergic antagonist, Prazocin (25-75 μg), has little effect on SON neurons except for a period of excitation (beginning approximately 20 sec. post infusion) which accompanies a dramatic fall in blood pressure.

The results of these preliminary experiments suggests that the α-adrenergic modulation of magnocellular neurons occurs via α_2-adrenergic mechanisms, perhaps in the CNS. Further studies are being conducted to extend these dose-response relationships, and to examine the possible interactions of these adrenergic mechanisms in the regulation of magnocellular neurons during osmotic and angiotensin challenges.

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31.9 MAGNOCELLULAR RESPONSES TO BOTH HYPERTONIC SOLUTIONS AND ANGIOTENSIN II. L.D. Mitchell, S.I. Bellin, M.F. Callahan and A.K. Johnson Dept. of Psychology and The Cardiovascular Center, Univ. of Iowa, Iowa City, Iowa 52242.

The activity of magnocellular neurons of the supraoptic nucleus (SON) in male Sprague-Dawley rats was used to determine the appropriate parameters for exciting neurosecretory cells by vascular administration of hypertonic saline or angiotensin (AII). Single units were identified by constant latency following stimulation of the neurohypophyseal tract, the ability of antidromically-induced action potentials to follow stimuli of 70-100 Hz, and electronic discrimination to distinguish between induced and spontaneously occurring action potentials. Central blood pressure was monitored through the femoral artery and experimental solutions were infused through small bore tubing (PE 50) implanted into either the bifurcation of the carotid artery or the femoral vein. Following experimentation, placements of microelectrodes were confirmed by histological examination, and the patency of the carotid system was proved by the injection of ink staining the right hemisphere.

When hypertonic saline solutions (1cc, 1-4M NaCl) were infused into the femoral vein of three rats, no alterations in the activity of magnocellular neurons were observed. Infusion of solutions (0.1cc, 167 mM - 1.5M NaCl) into the carotid artery of 15 rats (27 neurons) increased neuronal activity in a dose-dependent fashion. The most effective dose proved to be 100 uL of 0.7M NaCl for 10 sec., which was repeatedly applied without desensitization. Accompanying the enhanced excitation of the neuron was a brief rise in blood pressure (10-20 mm Hg) which appeared to terminate magnocellular activity. Comparable application of Locke's, Krebs or normal saline solutions did not affect either neuronal activity or blood pressure.

Infusions of AII (1ng-300ug) in 100 uL bolus (10 sec), were also administered through the carotid artery. The magnocellular response was variable. AII increased firing in a dose-dependent manner, with a peak at 30ng. Above this dose, there was a reduction in the response, with a concommitant rise in blood pressure. At a very high dose (15 ug), AII was observed to excite all magnocellular neurons in the face of a 50 mm Hg rise in blood pressure. These data suggest that the magnocellular neurons may be excited by both hypertonic saline and AII, although the response to the latter is more variable.

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31.10 REM SLEEP ATTENUATES THE PRESSOR RESPONSE TO AMYGDALA STIMULATION IN THE CAT. R. C. Frysinger, J. D. Marks*, R. M. Harper and R. B. Trelease*. Dept. of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

The central nucleus of the amygdala (ACE) projects heavily to cardiorespiratory areas, including the hypothalamus, parabrachial pons and the nucleus of the solitary tract, while receiving input from a variety of limbic and cortical structures. The ACE is thus strategically located to modulate forebrain influences on lower brainstem cardiovascular areas.

Stimulation of a region of the central amygdala that includes the central nucleus and the dorsomedial basolateral nucleus produces a rapid rise in arterial pressure in the awake cat. Quiet sleep reduces the magnitude of this pressor response (Trelease et al., Neurosci. Abstr. 8:723, 1982). The question remains whether REM sleep also influences this response.

Four adult cats were surgically prepared with concentric bipolar stimulation electrodes in the central amygdala and with chronic implants for monitoring arterial pressure, ECG, neck and crural diaphragmatic EMG, and EEG from motor cortex, hippocampus, and lateral geniculate. Following recovery from surgery, the animals were given 0.5 sec, 100 Hz trains of 0.5 msec pulses at a current level sufficient to produce a profound rise in arterial pressure in the alert animal. Identical stimulus trains delivered during quiet sleep produced pressor responses 50% - 80% of the amplitude of the waking response. During REM sleep, the effect of the stimuli was almost completely abolished, only rarely attaining 20% of the values associated with the alert state. There was no consistent difference in the degree of attenuation between active REM periods (i.e., during eye movement episodes) and tonic REM periods (i.e., without eye movement).

These results suggest a reduction in the effect of forebrain activity on the visceromotor system during REM sleep.

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31.11 LESIONS OF DORSOMEDIAL THALAMUS, BUT NOT INSULAR CORTEX, ATTENUATE STIMULATION-ELICITED CARDIOVASCULAR CHANGES FROM ANTERIOR MEDIAL CORTEX. Shirley L. Buchanan, and D. A. Powell, Neuroscience Lab., VA Hospital and University of South Carolina, Columbia, S.C.

We have previously reported that electrical stimulation in anterior medial cortex elicits heart rate (HR) decelerations and blood pressure (BP) depressor responses, along with increased ^{3}H-2-deoxyglucose (2DG) uptake (i.e., increased functional neural activity) in dorsomedial (DM) nucleus of the thalamus. Anterior insular cortex also appeared to have slightly increased ^{3}H-2DG uptake; additionally, this area is cardioactive to electrical stimulation. Stimulation of DM elicits large HR decelerations, but accompanied by BP pressor responses.

In an effort to further trace this pathway, rabbits were implanted bilaterally with bipolar stimulating electrodes in anterior medial cortex and lesion electrodes in either DM nucleus or insular cortex. Animals received 1-2 second trains (100 Hz, ~100-400 μA) of electrical stimulation. Animals with positive electrodes (i.e., those eliciting HR changes of 10% or greater from prestimulus baseline) were cannulated for blood pressure measurement; electromyographic activity (EMG) and respiration were also measured. Animals received four stimulation trials and two trials during which no stimulation was delivered ("zero" trials). Lesions were then made in either insular cortex, or DM nucleus of the thalamus. The animal was allowed to recover overnight, and the stimulation regimen was repeated.

Lesions of insular cortex had no effect on the autonomic changes elicited by stimulation of anterior medial cortex. DM lesions, however, appeared to attenuate the HR decelerations and BP depressor responses elicited by stimulation of anterior medial cortex. These findings suggest that the stimulation-elicited changes from anterior medial cortex are routed through the dorsomedial nucleus of the thalamus. Animals with DM lesions were also more reactive to anterior medial cortical stimulation, as evidenced by the occurence of consistently larger EMG responses after the DM lesions. This finding may indicate that a generalized increased sensitivity to midline cortial stimulation occurs as a result of DM lesions leading to movement-biased HR increases. Although insular cortex is cardioactive to electrical stimulation, this pathway appears to be distinct from that arising in anterior medial cortex.

31.12 INSULAR CORTEX PARTICIPATES IN CARDIOVASCULAR INHIBITION, BUT IS NOT NECESSARY FOR PAVLOVIAN CONDITIONED BRADYCARDIA IN RABBITS. D. A. Powell, Linda L. Hernandez, and Shirley L. Buchanan, Neuroscience Lab., VA Hospital and University of South Carolina, Columbia, S.C.

A series of ongoing experiments using 2-deoxyglucose autoradiography revealed that electrical stimulation of anterior midline cortex, which elicits cardiovascular inhibition, resulted in increased metabolic activity in two areas of the forebrain. One of these was the dorsomedial nucleus of the thalamus; the other was the anterior insular cortex. In the present series of experiments we studied the effects of electrical stimulation and lesions of insular cortex on cardiovascular mechanisms. New Zealand albino rabbits of both sexes were implanted with bilateral stimulation electrodes in the anterior or posterior insular cortex. After a 1 week recovery period heart rate (HR) and blood pressure (BP) as well as other selected variables were assessed subsequent to 1 to 5 sec trains of biphasic electrical stimulation at 100 pulses per sec. Electrical stimulation of insular cortex produced bradycardia accompanied by vasodepressor responses similar to those elicited by anterior midline cortical stimulation. However the thresholds were much lower and the bradycardia and depressor changes elicited were considerably greater than those produced by anterior midline cortical stimulation.

In separate groups of rabbits radio frequency lesions were made in anterior and posterior insular cortex and the animals compared with a group of sham operated animals during differential Pavlovian conditioning. Unlike lesions of the anterior midline cortex, lesions of the insular cortex did not attenuate or abolish the bradycardia associated with classical conditioning contingencies. In fact, the magnitude of the response appeared to be somewhat enhanced in lesioned animals. These findings are thus comparable to those in which septal lesions have also been found to enhance the magnitude of the Pavlovian conditioned bradycardiac response even though electrical stimulation of the septum elicits BP and HR decreases.

These findings support the contention that the insular region is a cortical area involved in autonomic activities. However, it does not appear to be necessary for the integration of the bradycardia associated with classical conditioning. Instead the present findings suggest that insular cortex may participate in cardiac changes by normally holding inhibitory cardiovascular mechanisms in balance.

31.13 EFFECTS OF NALOXONE AND TRH ON PLASMA CATECHOLAMINES AND ARTERIAL PRESSURE IN NORMAL AND ENDOTOXEMIC RATS. J.B. Long, C.R. Lake[†], A. Reid*[†], B.A. Ruvio*, and J.W. Holaday. Neuropharm. Br., Dept. of Medical Neurosciences, Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307, and [†]Depts. of Psychiatry and Pharmacology, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20014.

Recent work in this and other laboratories has revealed the importance of sympatho-medullary function in the therapeutic cardiovascular effects of naloxone (NX) and thyrotropin releasing hormone (TRH) in treatment of endotoxic shock. In order to further investigate sympatho-medullary involvement in the therapeutic actions of these compounds, plasma norepinephrine (NE), epinephrine (E), and dopamine (DA) were evaluated during iv NX and TRH treatment of endotoxemia.

Male S.D. rats (400-600g) were implanted with external jugular vein and tail artery catheters. 24 hours following surgery, mean arterial pressure (MAP) was monitored and a pre-treatment venous blood sample was withdrawn (1.5 ml over 1 min). Immediately following withdrawal of this and subsequent equal-sized venous blood samples, rats were reinfused with an equal volume of blood taken from a common donor pool to alleviate hypovolemia otherwise accompanying repeated sampling. The unrestrained, unanesthetized rats were injected with E. coli endotoxin (30 mg/kg, iv) or saline. When MAP declined by 20 mm Hg (or 15 min post-saline), a venous blood sample was withdrawn and followed immediately by iv injection of NX (3 mg/kg), TRH (4 mg/kg), or saline (1 ml/kg) and reinfusion of donor blood. Venous blood samples were additionally withdrawn 15 and 45 min following drug treatment. This schedule of blood sampling allowed measurement of plasma catecholamines (CA) during different stages of the MAP response to endotoxemia.

In the absence of endotoxemia, naloxone altered neither MAP nor CA. In contrast, 15 min following TRH administration, a significant ($p<0.05$) increase in MAP was associated with selective increases in plasma NE and E, but not DA. As previously demonstrated, endotoxin elicited an initial hypotension followed by a partial recovery at 15-30 min and a secondary hypotension at 45 min.; NX and TRH produced significant improvements in MAP of endotoxemic rats. In all cases, endotoxin evoked a large CA increase which progressively increased through 45 minutes irrespective of therapeutic treatment. Neither NX nor TRH significantly altered plasma CA levels from those measured in endotoxic rats treated with saline. These results indicate that therapeutic pressor effects of NX and TRH do not simply arise from enhanced sympatho-medullary outflowing of CA. Furthermore, these results reveal a dissociation between the changes induced by endotoxin in MAP and CA levels.

31.14 THE EFFECT OF BARODENERVATION ON THE RESPONSE TO HEMORRHAGE AND SUBSEQUENT OPIATE RECEPTOR BLOCKADE IN CONSCIOUS RABBITS. J. C. Schadt, R. R. Gaddis*, M. D. McKown*, and D. Franklin*, Dalton Research Center, U. Missouri, Columbia, MO 65211

We have previously shown that opiate receptor blockade with naloxone reverses the hypotension and tachycardia associated with hemorrhage. The pressor effect is due primarily to a sympathetically-mediated vasoconstriction and is accompanied by an increase in plasma norepinephrine (NE). In these same studies, we observed that the transition to hypotension during hemorrhage is abrupt and is accompanied by a decrease in heart rate (HR). The simultaneous drop in mean arterial blood pressure ($\overline{BP}$) and HR suggests the loss of normal baroreflex function. If the transition to hypotension is mediated by a loss of baroreflex function, then naloxone could produce this pressor effect by increasing baroreflex mediated sympathetic vasoconstriction. Petty and Reid (Hypertension 3:I-142-I-147, 1981) have recently shown that naloxone increases baroreflex sensitivity in the conscious rabbit. Therefore, our hypothesis was: Naloxone requires an intact baroreceptor reflex to increase $\overline{BP}$ and sympathetic release of NE. In order to test this hypothesis we examined the cardiovascular (n=6) and plasma catecholamine (n=3) effects of hemorrhage and subsequent naloxone treatment in conscious rabbits before (Intact) and after chronic sinoaortic barodenervation (SABD). The experimental protocol involved hemorrhage until $\overline{BP}$ fell below 40mmHg. 60 sec after $\overline{BP}$ reached this level, naloxone (3 mg/kg) was injected IV. $\overline{BP}$, HR, plasma epinephrine (E) and NE were measured at three points: 1) Control, before hemorrhage; 2) Hypotensive, after $\overline{BP}$ fell below 40mmHg; and 3) Recovered, 2 min postnaloxone. The results are shown below:

	$\overline{BP}$ (mmHg)	HR (bpm)	E pg/ml	NE pg/ml
Control				
Intact	72±3	191±9	30±16	113±42
SABD	81±6	215±15	20±20	307±99
Hypotensive				
Intact	29±2	230±10	1461±878	140±37
SABD	32±2	218±12	169±50	291±109
Recovered				
Intact	76±5	194±13	2088±234	248±24
SABD	120±7	206±9	2872±414	606±138

In summary, a functional baroreflex appears to be required for the increase in HR with hemorrhage, the postnaloxone bradycardia, and the large increase in adrenal medullary output during the hypotensive stage. However, SABD potentiates the postnaloxone increases in $\overline{BP}$ and plasma NE. Indeed, the baroreceptors appear to buffer these effects. Therefore, the sympathetically-mediated pressor effect of naloxone does not require an intact baroreflex.

31.15 LESIONS OF PERIVENTRICULAR TISSUE OF THE PREOPTIC RECESS ATTENUATE PLASMA VOLUME RESTITUTION FOLLOWING HEMORRHAGE. S. L. Bealer. Dept of Physiology, Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

Electrolytic ablation of the periventricular tissue surrounding the anteroventral third ventricle (the AV3V region) results in profound alterations in fluid and electrolyte metabolism and cardiovascular regulation. Animals with lesions in this brain area have an attenuated capacity to increase sodium excretion to a number of experimental treatments, and fail to develop some forms of experimental hypertension. In addition, following recovery from the acute effects of AV3V lesions, rats have chronically decreased blood volume and plasma volume, while total extracellular fluid volume is increased compared to control animals. In order to further characterize the role of this brain region in cardiovascular regulation and maintenance of plasma volume, these experiments were designed to determine the effect of AV3V ablation on plasma volume restoration following hemorrhage. Animals were allowed at least two weeks to recover from the acute effects of either AV3V ablation or control surgery. Rats were then implanted with catheters in the femoral artery in order to monitor mean arterial blood pressure, inject the radioisotope, take blood samples, and perform the hemorrhage. The following day, the conscious, unrestrained rats were injected intraarterially with 125-I labelled serum albumin, and plasma volume was subsequently calculated by radioisotope dilution. Following this treatment, 20% of the estimated blood volume was removed from the animal via the femoral artery catheter. Two hours following the hemorrhage, plasma volume was again determined, and the percent of shed plasma which was restored during this period was calculated for both groups. There was no significant difference in mean arterial pressure between groups either prior to, or at any time following hemorrhage. However, control operated rats restored a significantly greater proportion of shed plasma volume (75±8%) than animals with AV3V lesions (49±8%), during this time. These data indicate that the periventricular tissue surrounding the AV3V region is important for the acute restoration of plasma volume which follows hemorrhage. (Supported in part by USPHS Grant HL-25877)

31.16 ROLE OF THE VENTROLATERAL MEDULLA IN THE CONTROL OF THE CEREBRAL ISCHEMIC RESPONSE IN RABBITS. William G. LeBlanc,* Philip M. McCabe, James R. Haselton,* Carole L. Haselton,* Howard H. Ellenberger,* and Neil Schneiderman. Program in Behavioral Medicine, Dept. Psych., Univ. of Miami, Coral Gables, FL 33124

The Cerebral Ischemic Response (CIR), produced by occlusion of the vertebral and carotid arteries, consists of elevated arterial blood pressure, bradycardia, and apnea. Previous research has demonstrated that the CIR is integrated in the medulla, but discrete electrical stimulation failed to elicit all three components of the CIR from a single location. However, in our laboratory we have found a site centered at the retrofacial nucleus (RFN) and the rostral extremity of the nucleus ambiguus (NA) which when stimulated (100 pps, .5 msec duration, 75 μA, 10 second trains) produced a pressor response, bradycardia, and apnea for the duration of the stimulation.

Stimulation sites producing cardiovascular and respiratory responses were mapped throughout the ventrolateral medulla (VLM) of the rabbit. Pressor responses (30-60 mmHg) could be obtained from most regions of the VLM. Stimulation near or in NA elicited a profound primary bradycardia (40-100 bpm), whereas more rostral sites (RFN) were associated with bradycardia that was reflexive to the pressor response. Respiratory responses exhibited a medial-lateral organization, such that stimulation of regions lateral to RFN/NA elicited increases in respiratory frequency and amplitude. Sites medial to RFN/NA produced brief apnea followed by increasing respiratory amplitude for the remainder of the stimulation interval. Stimulation near or in RFN/NA produced inspiratory apnea for the duration of the stimulation. Ventilated animals exhibited attenuated bradycardia during stimulation suggesting that apnea enhanced the bradycardia response. This effect was observed for reflexive bradycardia, but the apnea did not affect the primary bradycardia response to stimulation.

We have also attempted to abolish the CIR by lesioning large regions of the VLM. Lesions near the ventrolateral surface reduced the pressor component of the CIR but did not affect the bradycardia. Lesions that did not include RFN did not greatly affect spontaneous breathing. However, lesions that destroyed the full rostral-caudal extent of RFN resulted in respiratory failure. We have been unable to abolish all three components of the CIR with selective lesions in the VLM.

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31.17 EFFECT OF PCO_2 ON THE GLUCOSE UTILIZATION OF CEREBRAL ARTERIES OF THE CAT USING ^{14}C-2-DEOXYGLUCOSE.
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CO_2 is known to be a potent vasodilator of cerebral blood vessels. In addition, glucose is thought to be a major substrate for vascular smooth muscle metabolism. Since vasoconstriction probably requires more energy than vasodilatation, the effect of CO_2 on the glucose utilization of vascular smooth muscle in cerebral blood vessels was studied in anesthetized cats. Six adult cats 2-3 kg were anesthetized with pentothal administered with an infusion pump at the rate of 20 mg/hr. The animals were intubated and placed on positive pressure ventilation with 100% O_2 using a solenoid controlled ventilator that maintained tidal volume, inspiratory time, and expiratory time constant. In 3 animals the arterial blood gases were maintained at an arterial PO_2 of 350-500 Torr, PCO_2 20-25 Torr, and pH 7.3-7.4. In the other group of three animals 9% CO_2 was added to the inspiratory gas mixture yielding arterial blood gas measurements of PO_2 of 350-500 Torr, PCO_2 of 55-60 Torr, and pH of 7.0-7.4 Once the blood gas measurements were stable, a single bolus of ^{14}C-2-deoxyglucose, 100 uCi/kg (Pathfinders Labs), was injected i.v. Arterial samples were taken at regular intervals during the 45 min experiment for measurements of glucose, scintilation counting and blood gas measurement. The animals were then sacrificed and the brains were frozen and sectioned at 20 um and prepared for autoradiography. After 12 days exposure, the autoradiographs were developed and some tissue sections were stained with cresyl violet or H & E for identification of cell bodies or vascular structures respectively. Once cerebral vessels were identified from histological sections, the autoradiographs were scanned using a computerized densitometer with aperature settings of 10 or 25 um. These densitometer readings were then converted to glucose utilization values using Sokoloff's equation and the lumped constant for the cat brain. The cerebral arteries of the animals with low arterial PCO_2 had glucose utilization measurements that were between 30% and 400% greater than those measurements in similar cerebral arteries of the animals subjected to high PCO_2. This data suggests that the ^{14}C-2-deoxyglucose technique may possibly be useful for detecting vasoconstriction and vasodilatation of cerebral blood vessels, thereby indicating changes in cerebral blood flow of major cerebral arteries in the same animals that are used for brain mapping studies using this technique. (Supp. by Dept. of Anesth., VA, NIH RCDA 00959, HLBI 27968, and NINCDS 18037).

31.18 REGIONAL CEREBRAL VASOCONSTRICTION ELICITED BY ELECTRICAL STIMULATION OF THE MEDIAL PARABRACHIAL NUCLEUS IN RAT. S. Mraovitch, C. Iadecola, D.A. Ruggiero and D.J. Reis, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

The parabrachial nucleus of the pons (PBN) plays an important role in the regulation of the systemic circulation and autonomic nervous system (Mraovitch et al., Brain Res. 232:57, 1982). Stimulation of the PBN in cat or rat will elicit a marked elevation of arterial pressure resulting from differentiated excitation of preganglionic sympathetic neurons. In the present study, we sought to examine in rat, whether electrical stimulation of the PBN would also modify regional cerebral blood flow (rCBF), and if so, whether the changes are mediated through neural pathways intrinsic to brain.

Rats were anethetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. In all animals, the adrenal glands were removed. Arterial pressure (AP), heart rate and body temperature were continuously monitored and blood gases were measured and controlled. PBN was electrically stimulated with intermittant trains (1 sec on/1 sec off) at 70 Hz and with an intensity (50-100 uA) corresponding to 3x the threshold current required for a 10 mmHg AP elevation. At all times, AP was maintained within the autoregulated range for rat rCBF. rCBF was measured in right and left samples of 9 brain regions by the 14C-iodoantipyrine technique with regional dissection (Nakai et al., Am. J. Physiol. 243:H226, 1982). In all animals, histological localizations of stimulated sites were carefully reconstructed.

In unstimulated rats (n=5), rCBF ranged from 58 $\pm$ 6 (ml/100 g x min) in corpus callosum to 103 $\pm$ 8 in hypothalamus and parietal cortex. During stimulation of the medial PBN (PBNm) (n=5) rCBF decreased bilaterally in several brain regions ranging from -33% in control in occipital cortex to -26% in hippocampus ($p<0.05$). rCBF was also significantly reduced in frontal (-29%), parietal (-28%) cortex and caudate n. (-28%). In contrast, rCBF was unchanged in medulla, hypothalamus, thalamus and corpus callosum. The cerebral vasoconstriction persisted after unilateral transection of the cervical sympathetic trunk without differences in rCBF between innervated and denervated sides. In 6 rats, stimulation of sites adjacent to the PBNm, including the caudal part of the nucleus and central grey matter, failed to modify rCBF.

We conclude that: (a) electrical stimulation of PBNm reduces rCBF; (b) the response is anatomically specific and restricted primarily to cerebral cortex and basal ganglia; (c) the vasoconstriction is not mediated by the sympathetic fibers to cerebral vessels nor by adrenal hormones. Excitation of projections originating in or fibers passing through the PBNm elicits a powerful cerebrovascular vasoconstriction which appears to be mediated by intrinsic neural pathways. (Supported by Grant HL18974).

PERIPHERAL AUTONOMIC NERVOUS SYSTEM I

32.1 THE MORPHOLOGY OF GRANULE-CONTAINING CELLS AND THEIR ASSOCIATION WITH NEURONS IN THE GUINEA PIG (GP) CELIAC GANGLION (CG).
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Small granule-containing cells (SGC) reside within sympathetic ganglia singly or in highly vascular groups. The single cells are interneurons (SIF cells) which receive preganglionic signals and inhibit the principal neurons. The clustered SGC are sparsely innervated and apparently do not make synaptic contacts upon neuronal elements. Instead, they may serve as endocrine systems for releasing neurotransmitters/neuromodulators into the ganglion environment. However, the exact nature of contacts from the SGC onto the ganglion neurons and their role in ganglion functions are not fully understood. This study will illustrate the morphological association between SGC and neurons in hopes of offering structural correlates for at least some of the postsynaptic potentials which emanate from the GP CG.

Four adult guinea pigs were anesthetized and perfused with 3% glutaraldehyde in 0.1M phosphate buffer. The celiac ganglia were removed and processed for light and ultrastructural study following secondary fixation in osmium tetroxide.

Histologically, the CG was a composite of large often binucleate neurons, myelinated and unmyelinated nerve fibers, many blood vessels, connective tissue, satellite and Schwann cells as well as conspicuous SGC occurring singly or clustered. Two SGC clusters were noteworthy with respect to size, displaying 40 and 62 nuclear profiles. Ultrastructurally, certain SGC (Type A) showed a large complement of dense core granules 150-170 nm in diameter; a second SGC (Type B) was neuronal-like and contained granules measuring only 70-90 nm. This cell was positioned directly against neurons and only a thin rim of satellite cell cytoplasm intervened between the two. The clusters predominantly contained Type A cells, nevertheless B types also were a component. Nerve terminals with numerous small, clear core vesicles (and a few larger dense vesicles) synapsed upon the soma of Type A cells. The A cells also gave rise to noticeable granule-rich processes which traveled through the ganglion and closely approached the principal neurons, but synapses were not seen.

Based upon morphology, the following conclusions are offered: 1) small, neuronal-like cells (Type B) containing many granules are closely associated with the principal ganglion neurons; 2) distinct synapses from either type of SGC upon neurons were not seen but certainly could exist; 3) B cells as well as A cell processes, because both are close to and minimally separated from neurons, may act as modulators by releasing their granule content(s) onto the neuronal surface.

32.2 RESPONSES IN CUTANEOUS VESSEL TONE AND RESPIRATION TO TRANSIENT HYPOXEMIA IN MAN. M. Kollai* (SPON: C. McC. Brooks). Dept. of Physiol., Semmelweis Med. Univ., Budapest, Hungary 1082.

The cutaneous vascular bed has been shown to be the target of a number of reflexes, but is role in cardiovascular adaptation to hypoxia is still a matter of controversy. In response to chemoreceptor activation or arterial hypoxia, skin vasoconstriction, as well as vasodilation has been reported in different species. The aim of the present study was to determine the changes in skin vessel tone and skin sympathetic activity in response to transient systemic hypoxemia in man and also to study the relation of cutaneous vasomotion to concomitant changes in respiration.

The studies were performed on young, healthy volunteers. Arterial oxygen tension was measured transcutaneously ($TcPo_2$), photoplethysmography was employed to monitor changes in cutaneous vessel tone, and respiratory tidal volumes were measured by a Fleisch pneumotachograph. End tidal Co_2 concentration, ECG, heart rate, finger skin resistance, and finger skin temperature were monitored continuously as well. Multiunit skin sympathetic activity was recorded in the median nerve at wrist level by using Tungsten microelectrodes. Informed consent was obtained from each participant.

Breathing 8% O_2 in N_2 for 90 sec reduced $TcPo_2$ from the control value of 95.4 $\pm$ 1.8 mm Hg to 51.7 $\pm$ 2.8 mm Hg. Respiratory tidal volume increased with hypoxia from the resting value of 582 $\pm$ 22 ml to 746 $\pm$ 38 ml, while photoplethysmographic pulse amplitude decreased to 60% of control, together with a 0.22 $\pm$ 0.03°C drop in skin temperature. The above values are means $\pm$ SE (n = 18). Occasionally skin resistance was reduced as well. Skin sympathetic activity increased during hypoxia and each major burst was followed by a reduction in pulse amplitude. The respiratory and plethysmographic responses exhibited similar time courses, with corresponding peaks. Voluntary hyperventilation for 90 sec with room air also produced an initial reduction in pulse amplitude, however, it recovered within 60 sec.

It is concluded that in conscious human subjects transient systemic hypoxia leads to constriction of cutaneous vessels in the hand, and that the vasoconstriction is the result of increased traffic in sympathetic efferent fibers. Skin vasoconstriction can develop independently of respiratory changes; usually the concomitant hyperventilation facilitates the cutaneous response.

32.3 THE EFFECT OF PROPRANOLOL, ATENOLOL AND MINOXIDIL ON PLASMA NOREPINEPHRINE AND EPINEPHRINE CONCENTRATION IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). James S. Hall*, Ana M. Biediger* and T. Kent Keeton* (SPON: R. Allan Buchholz). Dept. of Pharmacology, The Univ. of Texas Health Science Center, San Antonio, Tx. 78284.

To test the hypothesis that beta-adrenergic receptor antagonists (β-blockers) exert their antihypertensive effect by decreasing sympathetic nervous system (SNS) activity, the plasma concentration of norepinephrine (NE) and epinephrine (EPI) was measured in SHR after the administration of the β-blockers propranolol or atenolol (10 mg/kg, s.c.) or drug vehicle (control animals). The plasma concentration of NE and EPI also was measured in SHR treated with the vasodilator minoxidil (0.5 mg/kg, i.p.) to determine the effect of a decrease in blood pressure per se on plasma catecholamines. Mean arterial pressure (MAP) and heart rate (HR) were measured in conscious, unrestrained rats via a chronically indwelling aortic catheter. Blood samples (0.8 ml) for the measurement of plasma catecholamines were drawn through the aortic catheter immediately before and at 0.5, 2, 4 and 8 hours after drug administration. Both atenolol and minoxidil produced a significant decrease in MAP by 0.5 hour post-injection, and MAP continued to fall until a maximal response of 15% occurred at 4 hours post-injection with atenolol and at 6 hours with minoxidil. In contrast, propranolol produced an initial increase ($p < .01$) in MAP of 10% at 1 hour post-injection followed by a depressor response which reached a peak of 15% at 4 hours. Plasma NE, but not EPI, concentration was increased significantly in the minoxidil-treated rats, when compared to the control rats, at 2 and 4 hours after administration when MAP was reduced. In contrast, neither propranolol nor atenolol produced an increase in plasma NE concentration at 4 hours post-injection, the time of the peak antihypertensive response to the drugs. Propranolol produced a 6-fold increase in plasma EPI ($p < .001$) and a smaller increase in NE ($p < .001$) concentration at 0.5 hr post-injection, corresponding with the early rise in MAP caused by propranolol, whereas atenolol had no effect on plasma catecholamines at this time. These results suggest that either SNS activity or NE release from sympathetic neurons is inappropriately low relative to the fall in MAP produced by the β-blockers as compared to minoxidil. Furthermore, the early rise in MAP produced by propranolol may be related to its ability to increase plasma EPI concentration. (Supported by NIH grant HL 25681.)

32.4 ADRENAL TYROSINE HYDROXYLASE ACTIVITY: EFFECTS OF 6-HDA, UNILATERAL ADRENALECTOMY AND STRESS. Steven J. Fluharty*, Gretchen L. Snyder*, Edward M. Stricker and Michael J. Zigmond. Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Stress increases adrenal tyrosine hydroxylase (TH) activity, presumably in order to couple catecholamine (CA) synthesis with accelerated secretion. Damage to the sympathetic nerves similarly increases the demand for adrenal CA biosynthesis and elevates TH activity. In the present experiments we examined the effect of partial damage to the sympathoadrenal system on adrenal TH activity during stress.

Male, Sprague Dawley rats (250-300 g) were anesthetized with Equithesin and received either 6-hydroxydopamine (6-HDA; 100 mg/kg, iv), unilateral adrenalectomy (UAdx), or both. Some animals from each group were then treated acutely with insulin (20 U/kg, sc, 1 hr prior to sacrifice) or chronically exposed to cold (5°C for 4 days). Animals were anesthetized and adrenal gland and heart were removed for determinations of TH activity and tissue CA content.

Four days after 6-HDA treatment, CA content of heart was reduced by 90%. At this time adrenal TH activity was increased by 54% with no change in adrenal CA content. By 8 days TH activity had returned to normal despite the fact that cardiac CA levels remained low. Insulin and cold exposure resulted in still further increases in TH activity (by 105% and 168%, respectively).

UAdx also increased TH activity in the remaining adrenal, by 60%, within 4 days without a change in adrenal CA content. However, by 8 days TH activity was still elevated and CA levels had increased by 70%. Both insulin and cold exposure further increased TH activity, by 173% and 150%, respectively.

The combination of 6-HDA and UAdx produced an additive increase in TH activity in the remaining adrenal, at both 4 days (126%) and 8 days (55%). Moreover, insulin and cold stress each produced further increases in enzyme activity. These findings demonstrate that there is a substantial range of plasticity of CA biosynthesis within the adrenal medulla which permits enhanced synthesis and secretion during stress despite partial damage to the sympathoadrenal system. (Supported by MH-29670 and MH-08758.)

Adrenal TH Activity (pmols/min/gland)

	4 Days		8 Days	
	Basal	Insulin	Basal	Cold
Control	423 ± 67	706 ± 44	425 ± 55	817 ± 86
6-HDA	649 ± 70	857 ± 53	350 ± 40	1134 ± 127
UAdx	678 ± 100	1154 ± 68	673 ± 60	1055 ± 88
Comb.	955 ± 140	1241 ± 153	655 ± 62	1845 ± 94

*Mean ± SE (n=3-5 per group)

32.5 DOPAMINE MAY BE A NEUROHORMONE IN THE RAT ADRENAL CORTEX. R. McCarty, R. F. Kirby and R. M. Carey*. Departments of Psychology and Internal Medicine, University of Virginia, Charlottesville, VA. 22901

In the periphery, dopamine (DA) is a precursor for norepinephrine (NE) and epinephrine (EPI) biosynthesis and is itself a neurotransmitter in sympathetic ganglia. In addition, DA may function as a neurohormone in providing maximum tonic inhibition of aldosterone secretion from the adrenal cortex (J. Clin. Invest. 66: 10, 1980). For this study, we have quantified the amounts of DA, NE and EPI localized in the adrenal cortex and have examined factors that regulate concentrations of DA. Adult male Sprague-Dawley rats (199-266g) were assigned to one of four groups: (1) adrenal demedullated (ADM), (2) adrenal denervated (ADN), (3) adrenal demedullated-denervated (DMN) and (4) sham-operated controls (SHAM). Following surgery, rats were housed in groups of 4/cage with food and water ad lib for 10 days. Rats were then sacrificed by decapitation and the adrenals were removed, weighed and homogenized in 0.1N $HClO_4$. Catecholamine concentrations in adrenal homogenates were quantified by a radioenzymatic-thin layer chromatographic assay (see Table)

Catecholamine concentrations (μg/pair) in rat adrenals:

	SHAM	ADM	ADN	DMN
DA	3.03 ± 0.13	1.26 ± .05	2.50 ± 0.09	1.31 ± .10
NE	10.38 ± 0.47	0.46 ± .08	7.74 ± 0.64	0.75 ± .12
EPI	33.11 ± 1.19	0.47 ± .10	26.12 ± 1.36	1.93 ± .28

Compared to SHAM, ADM and DMN reduced NE and EPI levels by 92-99% but DA levels by only 57-58%. ADN reduced levels of each catecholamine by 18-26%. These findings indicate that the adrenal cortex contains approximately 40% of the total gland content of DA and less than 8% of the gland content of either NE or EPI. Further, DA in the adrenal cortex does not appear to require an intact nerve supply to the adrenal. In a second experiment, we examined the effects of inhibition of catecholamine biosynthesis with alpha methyl-p-tyrosine (AMPT, 250 mg/kg X 2) on DA content in SHAM and ADM rats. In saline-injected controls, ADM reduced NE and EPI levels by 96-98% but DA levels by only 75%. In SHAM rats, AMPT reduced adrenal DA by 44% and heart NE by 37%. In contrast, treatment of ADM rats with AMPT increased adrenal DA levels by 49% but decreased heart NE by 36%. Thus, DA in the adrenal cortex does not appear to require de novo catecholamine biosynthesis. We suggest that adrenal cortical cells acquire DA from extracortical sites. Cells of the adrenal zona glomerulosa may utilize this substance as an inhibitor of aldosterone secretion.

32.6 SYMPATHECTOMY INHIBITS COMPENSATORY ADRENAL CORTICAL GROWTH. N. Kleitman and M. A. Holzwarth, Neural and Behavioral Biology Program and Department of Anatomical Sciences, University of Illinois, Urbana, IL 61801.

Compensatory adrenal growth, a proliferative response of the contralateral adrenal cortex after unilateral adrenalectomy, is known to be neurally mediated, but the neural substrate for the response is unknown. In the present experiment we have found that neonatal sympathectomy blocks compensatory adrenal growth in adult rats, suggesting an important role for the sympathetic nervous system in the mediation of this response.

Newborn rats were sympathectomized with repeated subcutaneous injections of 6-hydroxydopamine (6-OHDA, 50 mg/kg every 48 hrs for the first 15 days of life; n=18), or guanethidine (20 mg/kg, same schedule; n=22). Controls were vehicle-injected littermates (n=19). Anatomical evidence for successful sympathectomy included a reduction in catecholamine histofluorescence (de la Torre method) in the adrenal cortex, and decreased size and cell number in the superior cervical ganglion of rats injected with 6-OHDA or guanethidine. Rats underwent either left-adrenalectomy or sham operation at 40 days of age and were sacrificed 72 hrs later. Adrenals were cleaned, weighed, and assayed for DNA (Burton method) and RNA (Orcinol method).

In agreement with previous reports, the mean weight of the right adrenals of left-adrenalectomized vehicle-injected animals was 32±9% greater than that of sham operated animals ($p=.001$). This weight increase was accompanied by a 14±5% increase in DNA content ($p=.02$), suggesting that this was a cell proliferative response. After sympathectomy, the weight increase in right adrenals of left-adrenalectomized animals was attenuated in comparison to the vehicle control group, and not statistically different from sham operated sympathectomized animals (15±5% for 6-OHDA; 19±3% for guanethidine; p's>.05). There was no difference between the DNA content of right adrenals of left-adrenalectomized vs. sham operated animals (102±7 μg vs. 103±8 μg/gland for 6-OHDA; 102±9 μg vs. 104±8 μg/gland for guanethidine) thus the observed growth was not the result of cell proliferation. The weight increase in both sympathectomized and control group glands was accompanied by elevated RNA content which may have been the result of increased secretory activity and cell hypertrophy.

Thus, in sympathectomized rats, we observed no evidence of the proliferative compensatory adrenal growth response. Based on this observation and histofluorescence evidence, we conclude that the adrenal cortex is innervated by sympathetic catecholaminergic nerves, and that this innervation is involved in the mediation of compensatory growth in response to unilateral adrenalectomy. (Supported by NSF Grant PCM-8109756 and NIH PHS-5T32GM07.)

32.7 ORGANIZATION AND DISTRIBUTION OF THE RAT SUBDIAPHRAGMATIC VAGUS AND ASSOCIATED PARAGANGLIA J. C. Prechtl* and T. L. Powley. Lab. Regulatory Psychobiology, Purdue Univ., W. Lafayette, IN 47907

Although an extensive literature exists on surgical and electrophysiological manipulations of the vagus of the rat, no detailed surgical anatomies of the nerve are available for the species. Thus, specific conclusions pertaining to the vagus often involve problematic assumptions about the vagal distribution, connectivity, and composition. To provide a more detailed, microscopic anatomical description of the rat vagus, we prepared tissue blocks of the upper abdominal viscera taken from male (200-250g) Sprague Dawley rats. After the tissue was stained according to the pyridine silver method of Ranson and Davenport (1931), it was double embedded with celloidin and paraffin, and sectioned transversely at 7μ. Beginning at the diaphragm and proceeding to the cardiac sphincter, every 4th 70μ length was examined at 120X and 300X magnification. A section from each length was projected (140X) and traced onto a polar coordinate grid. All size and position information on the tracings was digitized and plotted with a computer program that normalized distances as percentages. The resulting plots summarized the sizes and locations of all fascicles and paraganglia having cross sectional areas greater than $700\mu^2$.

Of the 23 specimens studied, 17% had double anterior vagal trunks at the level of the diaphragm, 26% double posterior trunks and 4% both double posterior and anterior trunks. The hepatic branch comprised the first bifurcation below the diaphragm in 67% of the cases, and occurred on average at 18% of the distance from the diaphragm to the cardia. At 32% of the distance to the cardia, the accessory coeliac branched from the anterior trunk; it moved first laterally and then continued to spiral over 180°, traversing the posterior trunk before arriving to a peripheral part of the posterior fat mass of the lesser curvature. In 35% of the cases, the accessory was part of a common anterior-hepatic trifurcation. At 77% of the distance to the cardia, the posterior vagus arborized into numerous (19±1) bundles, the majority of which exited onto the left gastric artery immediately following the departure of the fascicles of the accessory coeliac. All paraganglia noted on the esophagus ($\bar{X}$=8±1) were associated with the vagus, particularly at or after a branch point. Approximately 33% of all paraganglia occurred at the coeliac branching and 15% at the hepatic branch point. Gastric and accessory coeliac branches also contained paraganglia although in lower densities. Coeliac branch points contained the largest paraganglia ($\bar{X}$=2803±487μ^2) as compared to the overall average (1755±110μ^2); and it was these large paraganglia that most commonly contained neurons (from 1 to 6) having soma diameters ranging from 14 to 22μ.

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32.8 TONIC VAGAL INHIBITION AND ENHANCEMENT OF PORTAL PLASMA INSULIN CONCENTRATION. King C. Lee* and Ralph E. Miller. Departments of Pharmacology and Medicine, Univ. of Ky., Lexington, KY 40536.

The aim was to: 1) Determine if cutting a mainly afferent neural pathway between liver and brain, the hepatic vagus nerve, would alter portal venous plasma immunoreactive insulin (IRI) concentrations, and 2) find what role the celiac vagus nerve might have in IRI changes induced by hepatic vagotomy. (The celiac vagus is a major efferent nerve trunk supplying the pancreas).

In unanesthetized, spinalized (C7), adrenalectomized, male Sprague-Dawley rats (300 gms) the hepatic vagus was cut or sham cut prior to cutting or sham cutting the celiac vagus. The four groups, in the table below, were examined.

GROUP	HEPATIC VAGUS	CELIAC VAGUS	# ANIMALS
1.	sham cut	sham cut	9
2.	sham cut	cut	9
3.	cut	sham cut	7
4.	cut	cut	8

Portal plasma glucose concentrations were not different among the groups. Basal glucose (33 rats) was 125±22 mg/dl (mean ± sem). Basal IRI concentrations for group 1 through group 4 were: 3.5±1.1, 2.5±.7, 2.6±.9, 1.8±.5 ng/ml (mean ± sem). The changes in % are tabulated below. (Hepatic vagus cut/sham cut, 0 min, celiac vagus cut/sham cut, 21 min).

	TIME (min)					
GROUP	-6	0	11	21	26	30
1.	100	90± 7	63±11	44±10	26± 9	32± 7
2.	100	78± 6	91±33	75±14	105±19	110± 16
3.	100	124±30	145±31	186±58	240±97	459±224
4.	100	94± 5	129±18	207±33	171±33	90± 24

Group 1: IRI fell through most of the experiment ($p<.01$). Group 2: IRI rose after celiac vagotomy at 21 min ($p<.01$). Group 3: IRI rose after hepatic vagotomy ($p<.01$). Group 4: IRI was not different from that of group 3 before, but was lower 10 min after, celiac vagotomy ($p<.01$).

In summary: 1) Hepatic vagotomy (group 3,4) caused a rise in IRI which was reversed by celiac vagotomy (group 4). 2) Celiac vagotomy caused a rise in IRI in hepatic vagus intact rats (group 2), but a fall in hepatic vagotomized animals (group 4). 3) The changes were independent of the portal plasma glucose concentrations.

The results are consistent with the hypothesis that signals from the liver affect portal venous plasma insulin concentrations via a tonically active vago-vagal pathway. The celiac vagus may be responsible for inhibitory as well as stimulatory influences on portal insulin concentrations. (Supported by BRSG Grant SO7-RR-05374 NIH).

32.9 VAGAL REGENERATION MAY MEDIATE THE SPARING OF VMH OBESITY OBSERVED WITH PRIOR VAGOTOMY. E. A. Fox* and T. L. Powley (SPON: F. R. Brush). Lab. of Regulatory Psychobiology, Purdue University, West Lafayette, IN 47907.

Although subdiaphragmatic vagotomy (VAGX) performed after ventromedial hypothalamic (VMH) lesions eliminates much of the obesity typically produced by such damage (e.g. Powley and Opsahl, 1974), VAGX performed prior to the lesions spares more of the obesity (e.g. King et al., 1978). To quantify the extent of such sparing and to characterize more completely the role of the vagus in the VMH syndrome, we gave 50-day-old female Sprague-Dawley rats VAGX with pyloroplasty (N = 42), sham VAGX with pyloroplasty (N = 20), or sham VAGX with sham pyloroplasty (N = 20). The VAGX consisted of resecting each of the branches leaving the subdiaphragmatic trunks and then resecting both the anterior and posterior vagus between the diaphragm and the cardia. After a 100-day recovery period during which the animals were fed a wet mash diet, two-thirds of each group were given VMH lesions (1ma, 18sec) and the remaining third, sham operations. Following the lesion, all groups were fed in succession a wet mash diet (6 wks), a high fat diet (10 wks), and a supermarket diet (4 wks) before they were sacrificed. Vagus function was assessed at the end of the experiment by (1) cell loss in the dorsal motor nucleus of the vagus (DMN), (2) microscopic analysis of the vagus stained with pyridine silver, and (3) basal and stimulated insulin secretion. Based on these analyses, complete VAGX was defined by cell loss in excess of 23.6% and an absence of normally formed vagal trunks. Forty-two animals satisfied these criteria; they averaged 52.7% cell loss in the DMN (range 23.9% to 77.1%). Lesioned animals with prior VAGX displayed 80% of the obesity observed in lesioned animals without VAGX. This expression of VMH obesity by the animals with prior VAGX was associated with anatomical and physiological evidence suggesting that the vagus may have regenerated (see also Evans and Murray, 1954). All forty-two VAGX animals exhibited a consistent anatomical pattern: each proximal vagal trunk formed a neuroma below the diaphragm and immediately above a scar of the periesophageal tissues. The distal stumps of the hepatic, gastric and coeliac vagal branches had survived and contained axons. Axons issued from the neuroma and could also be found connecting the distal portion of the scar to surviving hepatic, gastric and coeliac stumps. Finally, insulin secretion occurred in response to electrical stimulation of the cervical vagus in 10 of 31 vagotomized rats that were tested. Additional research will need to determine the time course of vagal regeneration and to assess whether prior VAGX prevents VMH obesity when regeneration is blocked. This research was supported by NIH grant AM 27627.

32.10 ANDROGEN-ESTROGEN SYNERGY IN RAT LEVATOR ANI MUSCLE: HORMONAL REGULATION OF GLUCOSE 6-PHOSPHATE DEHYDROGENASE. S. R. Max, Dept. of Neurology, Univ. of Maryland Sch. Med., Baltimore, MD 21201.

The effects of castration (GDX) and hormone administration on the activity of glucose 6-phosphate dehydrogenase (G6PD) in the rat levator ani muscle of adult male rats were studied. GDX caused a decrease in G6PD activity. Chronic administration of testosterone propionate (TP) increased G6PD activity in the levator ani muscle of castrated rats; the magnitude of the increase was related to the length of time the animals were castrated and to the length of time of exposure to hormone after GDX. The longer the period of GDX before exposure to hormone, the greater the effect. For example, 7 days after GDX, treatment with TP (GDX) caused G6PD to increase by 50%; 30 days after GDX, TP caused G6PD to increase by 100% with respect to uncastrated controls. Dihydrotestosterone (DHT) was less effective than TP in enhancing G6PD in the levator ani muscle from castrated rats; estradiol (E2) was ineffective. Combined treatment with E2 and DHT, however, was as effective as TP alone (Table).

Treatment*	G6PD (% Control)
None	100
GDX	50
GDX + TP	200
GDX + DHT	100
GDX + E2	50
GDX + E2 + DHT	200

*GDX - 30 days, followed by 14 days of hormone treatment.

Apparently E2 is necessary for the maximal effect of androgen on G6PD activity, although in itself it is ineffective. TP may be maximally active because it can act as an androgen and can also be aromatized to E2 by muscle. DHT may be less effective because although it is a potent androgen, it cannot be aromatized to estrogen. Thus androgens and estrogens may exert synergistic effects on muscle. [Supported by grants from NIH (NS 15760) and NASA (NAG 2-100)].

32.11 INFLUENCE OF THE SACRAL SYMPATHETIC CHAIN ON URETHRAL FUNCTION IN THE CAT. J.W. Downie, D. Pillay*, D.M. Nance and J.A. Champion*, Departments of Pharmacology and Anatomy, Dalhousie University, Halifax, N.S., Canada, B3H 4H7.

The sympathetic nervous input to the urethra which is involved in lower urinary tract function is usually assumed to be carried in the hypogastric nerves (HGN). Nerve tracing experiments investigating the distribution of nerves to specific regions of the urinary tract revealed a substantial projection to the urethra from sacral sympathetic chain (SC) ganglia. These observations were then followed by electrophysiological and functional studies. In male cats 2% solutions of fast blue, nuclear yellow or bisbenzimide were injected into the lateral aspect of the bladder (B) above the ureteric orifices or into the preprostatic urethra (U) at least 1 cm below the bladder neck under aseptic conditions. 28 to 68 h later the cats were deeply anesthetized and perfused with buffered formaldehyde/glutaraldehyde. The inferior mesenteric ganglion (IMG) and SC ganglia were cut in 48 μm sections on a cryostat and viewed in a fluorescence microscope. The SC ganglia provided 55% of the nerves reaching U but only 21% of nerves reaching B. Most of the remaining contribution arose from the IMG. Electrophysiological tracing of action potentials evoked by stimulation of the sympathetic chain was carried out in chloralose-anesthetized cats. Potentials evoked by stimulation of SC were slowly conducted to all major branches of the pelvic plexus. In contrast, potentials evoked from the HGN could not easily be found on the major plexus nerves. The synaptic junction in the SC pathway was in the lower lumbar SC ganglia or more distal. In a separate series of experiments lumen perfusion pressure was monitored as an index of functional urethral responses. SC stimulation at L_4 to L_5 induced urethral constriction which was of smaller magnitude than HGN stimulation. Urethral dilatations were also observed in some experiments. The constriction response was sensitive to prazosin (0.1 mg/kg) and to hexamethonium (2 mg/min). In conclusion, an extensive projection of neurons from the lower SC to the urethra can be demonstrated by both retrograde tracing and electrophysiological techniques. These neurones could represent a second sympathetic pathway (in addition to the HGN) involved in controlling urethral activity.

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32.12 SUBSTANCE P IN THE FEMALE REPRODUCTIVE SYSTEM AND THE EFFECTS OF CAPSAICIN. H. Traurig*, J. Cotton*, R. Papka, A. Saria* and F. Lembeck*. Department of Anatomy, University of Kentucky, School of Medicine, Lexington, Ky 40536 and Institute Pharmacol., University of Graz, Austria.

The female reproductive system possesses an abundant and functionally significant innervation by adrenergic, cholinergic and sensory fibers. Recently, peptidergic nerves, including substance P(SP)-immunoreactive fibers, have been demonstrated in various parts of this system. There is strong evidence available that SP is the transmitter of certain primary sensory neurons conveying nociceptive information centrally as well mediating vasodilation and plasma extravasation peripherally. Immunohistochemical and radioimmunoassay methods were used to determine the distribution and concentration of SP in female reproductive organs of Sprague-Dawley rats. Capsaicin is known to deplete certain peptidergic fibers in adults and induce degeneration of certain primary sensory neurons in neonates. Some rats were injected (s.c.) with capsaicin to determine if the SP in the reproductive tract is attributable to primary sensory neurons and if SP depletion resulted in diminished reproductive function. Immunohistochemical whole-mount preparations and an indirect immuno-fluorescence technique revealed SP-like immunoreactive nerves throughout the reproductive system. Regional differences in the number of SP fibers were: vagina$\rangle$cervix $\cong$ uterine horns $\cong$ oviducts$\rangle$ovary. RIA of SP correlated well with histochemical observations revealing the highest concentration in the vagina (8.9 pmoles/g wet wt.) followed by cervix, uterine horns and ovaries. There was no significant change in SP concentration during the estrous cycle. Pretreatment of rats as neonates with capsaicin caused a permanent depletion of assayable SP in all organs studied. Additionally, in capsaicin pretreated rats the time of vaginal opening was delayed by 3-4 days (36.9 ∓ 0.27 VS 41.0 ∓ 0.57) and while pretreated rats cycled normally they were less efficient in mating (sperm-positive vaginal lavage) and maintaining pregnancy compared to age matched, vehicle injected controls. Assay of tissues of pregnant rats revealed a decline in SP concentration but no change in total organ SP content during pregnancy. These results suggest that SP-containing nerves may play a role in female reproductive function, e.g., by modulating neurogenic organ hemodynamics; neonatal capsaicin treatment may result in a disturbance of some aspects of reproduction; and the afferent side of certain reproductive reflex mechanisms may be SP-dependent. Supported by BRSG 5482; Austrian Scientific Research Funds 4402 and 4952 and the Austrian Academy of Science.

REGULATION OF AUTONOMIC FUNCTION I

33.1 CATECHOLAMINE LEVELS IN ADRENAL VENOUS AND PERIPHERAL PLASMA FOLLOWING STIMULATION OF BEHAVIORALLY IDENTIFIED HYPOTHALAMIC SITES IN THE CAT. S.L. Stoddard-Apter, V. Bergdall* and B.E. Levin. Indiana Univ. Sch. of Med., Fort Wayne, IN 46805 and New Jersey Med. Sch., Newark, NJ 07103

This study was designed to determine the sympatho-adrenal (SA) concomitants of hypothalamically elicited aggressive behavior in the cat. Electrodes were lowered into the brain of awake cats to elicit aggressive behavior: escape, defense, or attack. Experimental measurements were made in the acute preparation. Cardiovascular (CV) parameters of mean arterial blood pressure (MAP) and heart rate (HR) were monitored continuously through an indwelling arterial cannula. For each behaviorally identified site, concurrent, continuous blood samples were taken from either the adrenal vein ipsilateral to the side of stimulation, or both adrenal veins, and from the atrium during four 1 min periods -- two prior to stimulation and two following stimulus onset. Each site was stimulated (biphasic, square-wave pulses; 60 Hz; 30 sec) at intensities which evoked behavior in the awake animal (0.2-0.5 mA). Plasma levels of norepinephrine (NE) and epinephrine (E) were determined by radioenzymatic assay. Effects of hypothalamic stimulation on adrenal catecholamine (CA) secretion (ng/ml/kg/min) were determined by comparing ratios of the change in E from the baseline average of E to the change in NE from its baseline average. In all instances, stimulation resulted in an increase in adrenal E that was greater than the increase in adrenal NE. However, the magnitude of this differential increase varied with the individual behavior, with the greatest differential increase ($\Delta E/\Delta NE = 10.74 \pm 2.94$) observed following stimulation of a site in the ventromedial nucleus which elicited attack behavior. Stimulation of defense sites resulted in an increase in E about 3 times greater than the increase in NE, while escape behavior was accompanied by approximately equal increases in both CAs. The greatest activation of the peripheral sympathetic nervous component of the SA system appeared to be associated with attack, since stimulation of this site resulted in an average 10-fold increase in peripheral NE levels. Although both MAP and HR generally increased, the changes did not parallel those for adrenal CAs. Stimulation of an escape site elicited the greatest increase in HR (35.0 ± 7.1), while stimulation of an attack site elicited the smallest CV responses. These data suggest that different hypothalamically elicited aggressive behaviors are accompanied by characteristic profiles of SA activation. (Supported by Grant #5-S07-RR-5371-Biomedical Research Support-DRR-NIH, and the V.A. Medical Research Service.)

33.2 SYMPATHO-ADRENAL ACTIVATION MAY BE MODULATED BY POSTSYNAPTIC (D_2) DOPAMINE RECEPTORS IN THE HINDBRAIN. Stephen P. Arnerić* and John P. Long*, (SPON: W.J. Steele), Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

Dopamine (DA) analogs can stimulate the release of epinephrine from the adrenal medulla through central mechanisms to produce hyperglycemia (Arnerić et al., 1982, Soc. for Neurosci. ABST. 8: 421). The central site of action and the DA receptor subtypes involved in this response have not been previously determined. Experiments reported here were performed using male Sprague-Dawley rats.

To eliminate descending neural pathways from the hypothalamus a midbrain transection (MBT) was performed. MBT did not attenuate apomorphine-induced hyperglycemia. Nerve transections, together with central injections of pimozide (a relatively selective antagonist of D_2 receptors) suggest that DA analogs activate DA receptors in the hindbrain to stimulate epinephrine release.

The ranked ordered potencies of a number of experimental and clinically active DA receptor agonists were calculated for the compounds to increase serum glucose (↑ SG), inhibit the accumulation of DOPA using in vivo γ-butyrolactone procedure (↓ DOPA) and inhibit food intake (↓ FI). Positive correlation were found for the following: ↓ FI versus ↓ DOPA, r=0.96; ↑ SG versus ↓ DOPA, r=0.98; ↑ SG versus ↓ FI, r=98. Despite the positive correlation for the ranked ordered potencies to ↑ SG and ↓ DOPA, the dose-response relationships to ↑ SG and produce other dopaminergic actions suggest it is unlikely that presynaptic DA receptors mediate the hyperglycemic effect. Consistent with this idea the putative DA autoreceptor agonist TL-99 [2-dimethylamino-6,7-dihydroxytetralin] does not produce hyperglycemia which is dependent on the sympatho-adrenal system. Experiments using DA agonists devoid of D_1-agonist properties still produce hyperglycemia which is dependent on sympatho-adrenal activation. The actual potency (dose-response curves) of DA analogs to activate the sympatho-adrenal system correlates more closely with postsynaptic mediated actions [i.e. hyperactivity and rotational behavior] than with presynaptic mediated actions [inhibition of DOPA accumulation].

Together these data suggest that postsynaptic DA receptors of the D_2 subtype located in the hindbrain may modulate the activity of the sympatho-adrenal system. (Supported by NIH grant GM-22365, a grant from Eli Lilly and NIMH grant #MH-08857.)

33.3 Testosterone & Estrogen Effects on Thermosensitive Neurons in Hypothalamic Tissue Slices. N.L.Silva and J.A.Boulant. Dept. of Physiology, Ohio State Univ., Columbus, Ohio 43210.

The preoptic area and anterior hypothalamus, PO/AH, plays an important role in the regulation of both body temperature and reproductive activity. In vivo and in vitro electrophysiological studies have demonstrated that approximately 40% of the PO/AH neurons may be classified as temperature-sensitive. Other studies show that many PO/AH neurons possess binding sites for estrogen and testosterone. Futhermore, these two steroids have been shown to alter the activity of certain PO/AH neurons. The purpose of the present study was to examine the specificity of in vitro PO/AH neurons for their responses to temperature, testosterone and estrogen.

Male Sprague-Dawley rats were decapitated, their brains were removed and their hypothalamus blocked. PO/AH tissue slices (300-400 μm thick) were maintained in a humidified chamber which was constantly perfused with an oxygenated nutrient medium (7.4 pH, 300 mOsm/kg, 10 mM glucose). The medium perfusing the slices was maintained at 37°C, but could be rapidly changed between 32°-42°C. During perfusion with a normal nutient medium, each PO/AH single unit was characterized according to its spontaneous firing rate and thermosensitivity. Following this determination, the tissue perfusion was switched to a nutrient medium containing either 0.1 nM testosterone or 0.1 nM estradiol. The spontaneous firing rate and thermosensitivity were again examined during these steroid perfusions to determine the type of cell affected and the interactions between neuronal thermosensitivity and testosterone/estrogen-sensitivity.

In the present study, the same proportions of thermosensitive and insensitive neurons were found as were found in previous studies. Testosterone and estradiol affected the firing rates of 30-40% of both the temperature-sensitive neurons and the temperature-insensitive neurons. Of the neurons that were affected by these two steroids, the predominent effect was excitation; however, testosterone and estradiol tended not to excite the same neurons. Therefore, while there appear to be separate populations of testosterone-sensitive and estrogen-sensitive neurons, there does not appear to be a major distinction between the steroid-sensitive neurons and the neurons that are either temperature-sensitive or temperature-insensitive.

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33.4 EFFECTS OF BARORECEPTOR DEAFFERENTATIONS ON CIRCULATORY AND THERMAL RESPONSES TO BILATERAL COMMON CAROTID ARTERY OCCLUSION IN THE COLD-EXPOSED HINDLIMB OF ANESTHETIZED CATS. Carl A. Ohata. US Army Research Institute of Environmental Medicine, Natick, MA 01760

Previous experiments in this laboratory demonstrated that cold-induced vasodilation is commonly associated with a concomitant pressor response. Due to individual variations in the pattern of cold-induced vasodilation, bilateral common carotid occlusion (BCCO) was used as an alternate technique to induce a large and reproducible pressor and vasodilatory response. Responses in mean arterial blood pressure, heart rate, mean femoral arterial blood flow, footpad temperature and heat loss, and calculated femoral arterial vascular resistance and footpad thermal insulation during a 5 minute BCCO were determined when the hindlimb was exposed to room air, after hindlimb immersion in a 0°C bath, after each baroreceptor deafferentation with the hindlimb still exposed to cold, and after re-exposure to room air in 16 cats anesthetized with chloralose. In order to determine which baroreceptors were mediating the responses during BCCO, the following baroreceptor deafferentations were performed in sequence: sympathetic deafferentations were performed by bilateral stellate to T_4 ganglionectomies, aortic deafferentation was performed by applying phenol to the aortic arch and bilateral denervations of the vagus nerves, and carotid deafferentations were performed by applying phenol to both carotid sinuses and sectioning the carotid sinus nerves. A large pressor response during BCCO was observed in the phases prior to carotid denervation, and was attenuated thereafter. A tachycardia during BCCO occurred in the neurally intact phases, but was attenuated by sympathectomy, enhanced by vagotomy, then attenuated after carotid denervation. An initial femoral arterial vasoconstriction during BCCO occurred in the first 1-3 minutes and was followed by a decline in vascular resistance. A secondary vasodilation was observed in the neurally intact cold phase, after sympathectomy, and after vagotomy. A large hyperemia during BCCO occurred in the phases prior to carotid denervation, and was attenuated thereafter. A large reduction in footpad thermal insulation during BCCO occurred only in the neurally intact cold condition, after sympathectomy, and after vagotomy; thermal insulation decreased slightly after carotid denervation, but was unchanged during BCCO in both room air exposures. Consequently, a warming and increased heat loss of the footpad during BCCO occurred only in the neurally intact cold condition, after sympathectomy, and after vagotomy. These responses were attenuated by carotid denervation and were not observed during BCCO with the hindlimb in room air. In conclusion, BCCO elicits responses similar to that observed during cold-induced vasodilation suggesting that carotid baroreflexes mediate cold-induced vasodilation. The pressor response, peripheral vasodilation, hyperemia, and footpad warming during BCCO was mediated principally by the carotid sinus nerves and required exposure of the hindlimb to cold.

33.5 THERMOREGULATORY ALTERATIONS IN CATS WITH LESIONS IN THE PONTINE TEGMENTUM. L. Amini-Sereshki* and A.R. Morrison. (SPON: A. Rosenquist). Dept. of Animal Biology, Univ. of Penn., Sch. of Vet. Med., Phila. Pa 19104.

Bilateral pontine tegmental lesions abolish the atonia of paradoxical sleep (PS). Cats in PS without atonia do not shiver, piloerect or curl their bodies protectively when placed in the cold (Hendricks,'82), which supports the idea that during PS there is a state-related change in thermoregulatory control (Parmeggiani,'77). The present experiments were done to study the alterations in thermoregulatory responses of pontine-lesioned cats in wakefulness. Thresholds for shivering and panting, and the body posture at different environmental temperatures, were noted in 4 cats before and after placement of pontine lesions. Intact cats started to shiver, showed slight piloerection and curling of the body at ambient temperatures (T_a) of 8-10° C. When placed in a cage at T_a=35° C they did not pant, extend their bodies, exhibit vasodilation in the pinnae, or show obvious signs of thermal discomfort. On the contrary, following pontine lesions that induced PS without atonia, cats were more sensitive to thermal changes. They shivered, piloerected and curled their bodies at T_a=15-17° C. When placed in a cage at T_a=35° C, they panted within a few minutes (respiratory rate =120-150/min.), extended their bodies and exhibited vasodilation in the pinnae. A normal cat did not begin panting until 2.5 hours after being placed in T_a=36.5° C. These preliminary results suggest an alteration of the thermoregulatory control in wakefulness of pontine-lesioned cats that exhibit PS without atonia. This alteration is most likely due to the disruption of the central mechanisms involved in thermoregulation because of the discreteness of the lesions.The heightened sensitivity of cats with pontine lesions to thermal load during wakefulness makes the apparent insensitivity to cold of cats in PS without atonia all the more striking.

(Supported by NS 13110.)

33.6 AUTORADIOGRAPHIC LOCALIZATION OF MONOAMINE RECEPTOR SITES IN THE DORSAL VAGAL COMPLEX OF THE DOG. H.A. Robertson, R.A. LESLIE, and K.M. Murphy*. Depts. of Pharmacology and Anatomy, Dalhousie University, Halifax, N. S. Canada B3H 4H7

Nuclei in the lower brainstem play a central role in the regulation of autonomic function. For example, the nucleus tractus solitarius (NTS), which has a dense noradrenergic innervation, appears to be involved in the regulation of blood pressure. The antihypertensive agent clonidine may exert its effects via activation of α_2-adrenoreceptors in this area. Another nucleus in this area, the area postrema (AP), is a chemoreceptor trigger zone for the emetic response to dopaminergic agents such as apomorphine.

Because of the obvious importance of monoaminergic mechanisms in the autonomic functions in this brain area, we have examined the distribution of α_1 and α_2-adrenoreceptors and dopamine receptors in the dog medulla oblongata using light microscopic antoradiographic techniques. The dog was chosen because, unlike some species (rat, for example), the dog shows an emetic response. Four adult male dogs were anaestetized with sodium pentobarbital and perfused with 4 L. of 0.1% formalin in 0.1 M sodium phosphate buffer (pH 7.3). Frozen sections (16 μm) were thaw-mounted on slides and incubated with ^{3}H-prazosin, ^{3}H-p-aminoclonidine, or ^{3}H-spiroperidol. Specific binding for α_1 and α_2-adrenoreceptor and D_2 dopamine receptors was determined using 100 μM phentolamine and 10 μM domperidone, respectively. The slides were opposed to LKB ultrofilm in x-ray cassettes and exposed for at least 3 months.

The most dramatic finding was the very heavy labelling of the dorsomedial NTS with the α_2-adrenoreceptor ligand ^{3}H-p-aminoclonidine. α_2-Adrenoreceptors were concentrated in the dorsomedial NTS, and were found throughout the rostrocaudal extent of the nucleus and including commissural, gelatinous, dorsomedial, dorsolateral and medial subnuclei. There was little or no labelling in the solitary tract itself. The AP was also labelled but the receptor density was less than one half that in the NTS. Only very light labelling was seen with the α_1-adrenoreceptor ligand ^{3}H-prazosin and the D2-dopamine receptor ligand ^{3}H-spiroperidol.

The remarkably high density of α_2-adrenoreceptors in this region of the brain reinforces the idea that this could be the locus of the antihypertensive actions of clonidine.

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33.7 COMPARISON OF THE TOPOGRAPHIC DISTRIBUTION OF INSULAR CORTICAL AND CENTRAL AMYGDALOID INPUTS TO THE NUCLEUS TRACTUS SOLITARIUS AND DORSAL MOTOR NUCLEUS IN THE RABBIT. J.S. Schwaber, B.S. Kapp[1], G.A. Higgins* and P.A. Driscoll-Mendes*[1]. E.I. du Pont de Nemours & Co., Glenolden, PA 19036, and [1]Dept. Of Psychology, The Univ. of Vermont, Burlington, VT 05405.

In this study we have compared the pattern of distribution of descending inputs from the insular cortex to the nucleus tractus solitarius (NTS) and dorsal motor nucleus (DVN) to those arising from the central nucleus of the amygdala (CE).

Tritiated proline/leucine (50-100 nl) injections were placed into the insular cortex at various anterior posterior levels. In a representive case, a 100 nl posteriorly located injection site covered the ventral portion of the dorsal and the ventral agranular cortices and excluded the dorsally located granular insula.Anterogradely transported label was followed in autoradiograms into the medulla bilaterally and produced the heaviest terminal field in the NTS and DVN, extending throughout the rostro-caudal extent of both nuclei. The label is distributed in a topographic pattern in both structures bilaterally, but is much heavier on the contralateral side. At rostral levels fibers appear to reach the NTS/DVN from two descending bundles, including a component traveling in the pyramidal tract ipsilateral to the injection site. At intermediate levels of the DMN label is present laterally and additionally completely encapsulates the nucleus. In the NTS, particularly heavy label is present in the dorsomedial, lateral and ventrolateral subdivisions. The CE projection to the medulla appears to be entirely ipsilateral and more heavily innervates the reticular formation ventrolateral to the NTS/DVN. Within the NTS, as compared to the insular input, heavy terminal labeling is present within the parvocellular and intermediate subnuclei and is relatively light within the lateral and ventrolateral subnuclei. It is particularly interesting that the posterior agranular cortical input, in common with the input from CE, is pronounced within the dorsomedial NTS, a region receiving baroreceptor inputs in the rabbit. These findings further indicate that rabbit insular cortex is involved in the central system integrating emotional, as well as cardiovascular and other autonomic events.

33.8 THE ORGANIZATION OF INSULAR CORTEX PROJECTIONS TO THE AMYGDALOID CENTRAL NUCLEUS AND AUTONOMIC REGULATORY NUCLEI OF THE DORSAL MEDULLA. B.S.Kapp, J.S.Schwaber[1], & P.A.Driscoll-Mendes*. Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405, & [1]Dupont Central Research Neurobiology Group, Glenolden, PA 19036.

We have presented evidence in the rabbit suggesting that (a) the amygdaloid central nucleus (ACE) may contribute to cardiovascular regulation (Kapp et al., 1983), (b) projections from the ACE to medullary cardiovascular regulatory nuclei exist which may mediate this contribution (Schwaber et al., 1982), and (c) the ACE may be an important component within a larger interconnected system, including the insular cortex, involved in cardiovascular/autonomic regulation (Kapp et al., 1982). The present experiment was performed to further examine the anatomical organization of this system. Since the insular cortex has been demonstrated to project to the ACE and to the nucleus of the solitary tract (NTS) (Saper, 1982; Kapp et al., 1982), the present study was designed to determine if insular cortex neurons which project to the ACE also project via collaterals to the NTS.

Nineteen New Zealand rabbits received injections of True Blue (5%, 40-600 nl) aimed at the ACE. Three to four weeks later, injections of Bisbenzimide (10%, 400 nl) were made along the anterior-posterior extent of the NTS and the vagal dorsal motor nucleus. From two to three days thereafter the animals were sacrificed, and the insular cortex was sectioned (30 μm) and examined using fluorescent microscopy for the presence of single and double labeled neurons.

The majority of labeled insular cortex neurons were found to be single labeled, and less than five percent contained both Bb and TB. Furthermore, Bb and TB labeled neurons demonstrated different topographical distributions which varied along the anterior-posterior extent of the insular cortex. At anterior levels Bb labeled neurons were found in the innermost region of layer V, and extended in an uninterrupted continuum from the dorsal portion of the dorsal agranular insula dorsally through the granular insula and into the lateral pre-central cortex. True Blue labeled neurons at this anterior level were located within layer V of the agranular insular cortex but primarily lateral to Bb labeled neurons. At more posterior levels, both Bb and TB labeled neurons demonstrated similar, overlapping distributions and were located within layer V of the agranular and granular insula.

These results suggest that primarily separate populations of neurons within the insular cortex project to the ACE and the dorsal medulla autonomic regulatory nuclei, and that these populations demonstrate partially different topographical distributions.

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33.9 DIRECT PROJECTION FROM RAT MEDIAL PREFRONTAL CORTEX TO THE SOLITARY NUCLEUS. R.R. Terreberry* and E.J. Neafsey (SPON: A.J. Castro). Dept. of Anatomy, Loyola Univ. Med. Ctr., Maywood, IL 60153.

A recent study has reported a substantial projection from the lateral prefrontal cortex to the solitary nucleus (NTS) in the rat (van der Kooy et al, Neurosci. Lett. 33:123-127, 1982). A small projection from the medial prefrontal area to the NTS was also mentioned. The present study investigated the cortical projections from the medial prefrontal cortex to the NTS complex in the rat by utilizing both retrograde and anterograde transport of horseradish peroxidase conjugated with wheat germ agglutinin (HRP-WGA). The rats were anesthetized with Ketamine HCl (100mg/kg,IP) and placed in a stereotaxic frame. The cisterna magna was opened to prevent cortical swelling and a small piece of bone (2x5mm) was removed just rostral to bregma on one side. In one series of experiments (n=4), stereotaxic injections of .03-.04 μl of a 1% solution of HRP-WGA (Sigma) in physiological saline were made into the medial prefrontal cortex (3.5mm rostral, .75mm lateral to bregma, depth of 3.5mm) using a 1 μl Hamilton syringe fitted with a 50 μm tip. In the second series of experiments (n=3), stereotaxic injections of .03-.04 μl of 1% HRP=WGA were made into the NTS complex (.5mm rostral, .5mm lateral to obex). After survival periods of 2 days, the animals were reanesthetized, transcardially perfused with 1.25% glutaraldehyde and 1% paraformaldehyde, and the tissue was processed for HRP histochemistry according to the TMB procedure of Mesulam (J. Histochem. Cytochem. 26:106-117, 1978).

Following brainstem injections retrogradely labeled neurons were found in the paraventricular nucleus of the hypothalamus, central nucleus of the amygdala, insular cortex and in the infralimbic, prelimbic and anterior cingulate regions of the medial prefrontal cortex. The infralimbic labelling consisted of a dense band of labeled neurons and was bilateral. Cells in the prelimbic and anterior cingulate regions were less densely packed. Injections of the prelimbic-infralimbic region of prefrontal cortex resulted in anterograde labelling within the NTS. Label was found throughout the rostral-caudal extent of the NTS and was bilateral but heavier contralateral to the injection site. The projection from the medial prefrontal cortex to the NTS suggests that this area of cortex may function primarily as a "visceral motor" cortical region that may play a role in regulating autonomic activities. Preliminary data from our lab indicate that electrical stimulation in this cortical region alters the activity of respiratory neurons in the NTS complex. Supported by NIH grant NS16146 and BRSG grant RR05368 from Loyola University.

33.10 NEURAL CONTROL OF INSULIN AND GASTRIC ACID SECRETION: FUNCTIONAL ORGANIZATION OF VAGAL PREGANGLIONIC NEURONS. W.B. Laughton,* H.R. Berthoud and T.L. Powley, Lab. of Regulatory Psychobiol.,Purdue University, W. Lafayette IN 47907

Recent HRP studies have substantiated that the vagal efferent preganglionic perikarya are distributed in the dorsal motor nucleus of the vagus (DMV) and the nucleus ambiguus. Although the issue of organ and response specificity has been addressed in several of these studies by selectively injecting HRP into vagal target organs or peripheral vagal branches (e.g. Laughton and Powley, Neurosci. Abs. 5:46,1979; Luiten et al, Neurosci Lett. 33(10):S310,1982; Kalia and Mesulam, J. Comp. Neurol. 193:467, 1980), the results do not unequivocally support the existence of a viscerotopic organization within the motor nuclei. Since there are different vagal motor functions in many organs, and since there is no simple relationship between the vagal branches and motor functions, the present study addressed the specificity issue using a more functional approach.

Male Sprague-Dawley rats bearing preimplanted gastric fistulae were overnight food deprived, anesthetized, and equipped with arterial and venous catheters. Incidental activation of sympathetic adrenergic mechanisms was suppressed with α and β blockers. After surgical exposure of the dorsal medulla, semi-microelectrodes (<50μ) were introduced into the brainstem region of the DMV with a sampling procedure chosen to yield a systematic map. Plasma insulin and glucose concentration, gastric acid secretion, heart rate and blood pressure were continuously monitored before, during, and after a period of electrical stimulation (50μA, 50Hz, 1 msec. for 10 min.). Gastric acid was measured using a saline pressure-perfusion system and a Radiometer Copenhagen on-line titrator. Blood sampled was continuously replaced with an identical volume from similarly deprived donor rats.

45 percent of the stimulation sites yielded significant insulin responses (37% increased, 8% decreased); 49% of the sites yielded changes in gastric acid output (34% increased, 8% decreased, 4% biphasic response). There was no correlation between vagal activation of the two subdiaphragmatic responses, gastric acid and insulin (r=-0.05). Generally, the responses studied tended not to covary, however there were low but significant (0.2 to 0.3) correlations between insulin and glycemia, heart rate and blood pressure, and gastric acid and blood pressure, while stimulation of the cervical vagus yielded strong correlations (>0.9). These results indicate that individual electrode placements within the DMV are capable of selectively activating one or more vagally mediated responses and support the idea that the responses examined are independently organized in the DMV.

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33.11 COMPARISON OF GASTRODUODENAL AND CARDIOVASCULAR RESPONSES PRODUCED BY ELECTRICAL STIMULATION OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS AND NUCLEUS AMBIGUUS IN THE CAT. F.D. Pagani*, W.P. Norman, D.K. Kasbekar*. and R.A. Gillis*. Depts. of Pharmacol., Physiol., and Anat., Georgetown Univ. Schs. of Med. and Dent., Washington, D.C. 20007

Studies were performed to compare the role of the dorsal motor nucleus of the vagus (DMV) and nucleus ambiguus (NA) in gastroduodenal and cardiovascular control in cats. Animals, fasted for 24 hrs., were anesthetized with alpha-chloralose, and artificially ventilated. A midline abdominal incision was made and extraluminal force transducers were sutured to the antrum, pylorus, and proximal duodenum oriented to record circular smooth muscle activity. Gastroduodenal motility was analyzed by calculation of the minute motility index (MMI). Arterial pressure (AP) and heart rate(HR) were also monitored. A stimulating electrode (David Kopf SNE 100) was placed in either the DMV or NA, and verification of placement was made at the termination of the experiment by reacting Na ferrocyanide with iron deposited by the electrode tip. Stimulation parameters were 100-133 uA, 50 Hz, and 0.2 msec pulse duration, unless otherwise stated.

Stimulation of the DMV(n=15) produced significant increases ($p<0.01$) in the MMI of the antrum, pylorus, and proximal duodenum of 17+3, 28+3, and 17+5, respectively. These changes occurred with bradycardia of $\bar{}$-11.3+3.4 beats/min and with no significant change in AP. Altering the frequency of stimulation from 50 to 100 Hz did not diminish the cardiovascular or motility responses. Stimulation of the NA (n=13) produced comparable effects on antral, pyloric, and duodenal motility. Associated with these responses was a large decrease in HR of 81.9+12.5 beats/min and a decrease in AP of 19.8+5.2 mmHg. Altering the frequency from 50 to 100 Hz greatly attenuated the motility response evoked by stimulation of the NA. Increasing the frequency had no effect on HR or AP.

These data indicate that stimulation of the DMV and NA produce comparable effects on gastroduodenal motility. However, motility responses evoked by stimulation of the NA appear to be mediated by several synaptic connections as increasing the frequency from 50 to 100 Hz greatly attenuated the motility response. This is in agreement with anatomical findings which do not find the NA as an origin for preganglionic parasympathetic vagal fibers innervating the stomach. In addition, activation of the DMV pathway appears to produce selective effects on the gastrointestinal tract, while activation of the NA pathway poduces both motility and cardiovascular effects. (Supported by AM NS 29975).

33.12 ADRENERGIC VAGAL EFFERENT NEURONS PROJECT TO THE STOMACH. T.C. Ritchie, D.G. Gwyn, M.C. Sullivan*, P.J. McKinney*, R.B. Leonard and J.D. Coulter. Marine Biomedical Institute, Univ. Tex. Med. Branch, Galveston, Texas and the Dept. of Anatomy, Dalhousie Univ. Med. School, Halifax, Nova Scotia, Canada.

We have previously reported the existence of catecholamine (CA)-containing vagal efferent cells in the dorsal motor nucleus (DMN) of the rat (Ritchie et al., Neurosci., 1982, 7: 1471-1482). These cells were identified by double labeling for dopamine-B-hydroxylase (DBH) and for HRP retrogradely transported from the cervical vagus nerve. The cells exhibiting DBH-immunoreactivity probably contain either norepinephrine or epinephrine. We now report that at least a portion of the CA vagal efferent cells project to the stomach.

HRP (Miles; 15% solution) was injected into the anterior and posterior stomach walls at multiple sites (10 µl/site) and a 2 or 3 day survival period was used. Transverse frozen sections (25 µm) were taken through the medulla and stained first for HRP ($NiNH_4SO_4$-$CoCl_2$-DAB) and then for DBH (immunocytochemistry, PAP method). Cells stained with both labels contain black HRP reaction granules on a homogenously brown stained cytoplasm.

HRP-positive cells were distributed bilaterally throughout the rostrocaudal extent of the DMN and both within and ventral to the rostral nucleus ambiguus (NA) in agreement with earlier studies (Leslie et al., Br. Res. Bull. 1982, 8: 37-43). Neurons doubly stained from HRP and DBH were bilaterally distributed in the rostral one third of the DMN. The distribution of CA-containing vagal efferent cells which project to the stomach coincides with the distribution of HRP-DBH cells identified previously as projecting through the cervical vagus. In addition, a few HRP-DBH cells were located near, but not within, the NA. This finding confirms our earlier suggestion that a second population of CA-containing vagal efferent cells is associated with the A1 cell group. Since the DMN may have collateralized projections, it is possible that the DBH-positive vagal efferent cells project to thoracic and subdiaphragmatic structures in addition to the stomach. Supported by grants from the Medical Research Council, Canada, and the NIH NS12481, NS11255 and NS07185.

33.13 EFFECT OF TRH AND GABA ON VAGAL EFFERENT ACTIVITIES IN THE RAT WITH RESPECT TO GASTRIC ACID STIMULATION. Y.GOTO*, Y.Taché, H.Debas*, and D.Novin, Center for Ulcer Research and Education, VA Wadsworth, Los Angeles, CA and Psychology Department, UCLA, Los Angeles, CA.

TRH and GABA have been shown to act within the brain to stimulate acid secretion in the rat through vagal dependent pathways, because vagotomy completely abolished acid output induced by TRH or GABA.

This study was performed to obtain direct evidence for vagal stimulation by neural-acting substances. Experiments were performed in urethane-anesthetized male rats. TRH (0.1-1µg, intracisternally) or ß-(p-chlorophenyl)-GABA (PCPGABA, 1-8mg/kgs.c.), a lipophilic GABA derivative, dose-dependently stimulated acid output in gastric fistula rats. The maximal acid responses to TRH and PCPGABA were 25+4 and 38+3µEq.H^+/10min, respectively and comparatively much higher than those to pentagastrin (32µgkg^{-1}h^{-1}i.v.) or histamine (10mg/kg,s.c.). The effect of TRH and PCPGABA on vagal efferent discharges were studied. A pair of Pt-wire electrodes were placed upon a fine vagal bundle dissected from the proximal portion of the divided vagus. Basal vagal discharges averaged about 10 spikes per second. Intracisternal TRH (0.1-1µg) increased vagal efferent discharges immediately after its application and this response persisted for 30 min. PCPGABA potentiated vagal efferent firings within 15 min of s.c. injection and showed plateau levels 30 min later. The time-course and dose-dependency of the effects of TRH and PCPGABA were coincident to those of acid responses. It was also found that TRH action appears to be different from GABA effect, because substance P (10-50µg/kg i.v.) inhibited only the vagal impulses stimulated by PCPGABA.

These results indicate that the hypersecretion of acid by TRH and PCPGABA can be explained by centrally-induced changes in parasympathetic nerve activity. This experimental method for monitoring vagal efferent impulses will be useful for studying the modulation of the autonomic nervous system.

33.14 AMYGDALOID LESIONS AND INTRACISTERNAL BOMBESIN: EFFECTS ON GASTRIC SECRETION. Carlos V. Grijalva, Yvette Taché, Mark W. Gunion and John H. Walsh*. Dept. of Psychology, Brain Research Institute and Center for Ulcer Research and Education, UCLA, Los Angeles, CA 90024

In a recent study we reported that intracisternal bombesin attenuated the increase in gastric acid secretion induced by lateral hypothalamic (LH) lesions (Taché et al. Life Sci. 31: 2485-2491, 1982). The present experiment examined the effects of amygdaloid lesions alone or in combination with intracisternal (IC) bombesin on gastric secretion and blood gastrin. Two groups of male rats were given large anodal electrolytic lesions of the amygdala (encompassing the central and medial nuclei but also encroaching upon the lateral and cortical regions) and two other groups were give control operations. After at least 2 months postoperative recovery all rats were food but not water deprived for 24 h, anesthetized with methohexital, and then one amygdala group (AmB, N=6) and one control (CB, N=9) group were injected (IC) with bombesin (500 ng). The remaining amygdala (AmS, N=7) and control (CS, N=7) groups were injected with an equal volume of saline (10 µl). Immediately following the injections the pylorus was ligated. The rats, all conscious, were decapitated 2 h later and gastric contents and trunk blood collected.

Bombesin significantly reduced gastric acid output and concentration and increased pH in CB rats and induced a small but nonsignificant reduction in gastric volume in both the AmB and CB groups. Interestingly, rats in Group AmS exhibited significantly higher gastric pH levels and lower gastric acid output and concentration levels than rats in Group CS and did not differ from Group AmB on these parameters. The lack of a bombesin effect in Group AmB may have been due to the fact that amygdaloid lesions alone induced changes similar to those seen following bombesin injections in nonlesioned control animals [means: **pH**; CS = 1.8, CB = 6.8, AmS = 4.3, AmB = 4.4, **Acid concentration** (mEq/l); CS = 67.0, CB = 5.7, AmS = 18.3, AmB = 14.9, **Acid output** (µEq/2h); CS = 99.9, CB = 1.3, AmS = 22.9, AmB = 6.7]. Although there was a trend for bombesin to increase gastrin levels in control animals and to reduce these levels in amygdaloid rats these results were quite variable and nonsignificant.

The present findings of increased pH and decreased gastric acid following amygdaloid lesions are consistent with recent studies showing that the severity of gastric ulceration in rats produced by physical restraint (Henke, P.G. Neurosci. Biobehav. Rev. 6:381-390, 1982) or by LH lesions (Geiselman, P.J. & Grijalva, C.V. 5th Eur. Neurosci. Meeting, Liege, 1981) can be attenuated by prior lesions of the centromedial amygdala.

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33.15 ANALYSIS OF HEART RATE VARIABILITY IN DIABETIC PEOPLE. Walter N. Tapp*, Mark Wiesen*, Gerald A. Curtis* and Benjamin H. Natelson. Depts. of Neurosciences and Medicine, VAMC and New Jersey Medical School, East Orange NJ 07018.

Heart rate varies considerably in normal people but becomes much more regular in diabetics with severe disease. This is usually attributed to loss of respiratory sinus arrhythmia due to peripheral autonomic neuropathy in diabetics. Typically, heart rate variability has been assessed by gross measures of variability, such as the standard error. In this study, we used spectrum analysis to evaluate the sources of variability in heart rate data in greater detail. The spectrum decomposes variability into its constituent frequencies, and recent evidence suggests that particular frequencies reflect the activity of different cardiovascular control systems. We recorded heart rate from 14 diabetics and 12 normals. Subjects were asked to lie quietly for 15 min, then their ECG was recorded for 10 min and the digitized record of heart rate was stored on a computer. Spectrum estimates were computed by fast fourier transform. As predicted by the clinical literature, diabetics exhibited significantly less heart rate variability at the respiratory frequency (0.3 Hz) than normals ($p < 0.01$). This is consistent with other evidence of parasympathetic dysfunction in the control of diabetic heart rate. Surpisingly however, diabetics exhibited significantly more heart rate variability in the low frequency band (< 0.06 Hz) than normals ($p < 0.01$). This means that diabetics actually exhibited larger slow changes in heart rate than normals. Heart rate variability along this slow time course can reflect parasympathetic and sympathetic influences. However, because of their frequent parasympathetic deficits, it may be reasonable to regard the low frequency band of most diabetics as predominantly sympathetic. This interpretation is supported by the finding that diabetics with signs of frank sympathetic dysfunction, such as orthostatic hypotension, had markedly reduced low frequency power. These patients exhibit flat spectra with low power (i.e., variability) across all frequencies that would be consistent with loss of both parasympathetic and sympathetic inputs to the heart. In contrast, patients with increased low frequency variability may have early renal disease which is manifested by subtle changes in the renin-angiotensin system. In pharmacological experiments, inhibition of angiotensin converting enzyme increased amplitude in the low frequency band. If this hypothesis can be supported, it will mean that spectrum analysis of heart rate may be a useful non-invasive tool in the detection of early renal dysfunction in the diabetic patient. Regardless of this, however, these data indicate that changes in heart rate variability in diabetics are far more complex than was previously appreciated. (Supp. by VA med. res. funds, HL 26760, HL 24498)

ENDOCRINE AND AUTONOMIC REGULATION: NEURAL CONTROL OF IMMUNE SYSTEM

34.1 DECREASED NATURAL KILLER CELL ACTIVITY IN ALZHEIMER'S DISEASE. L.J. Kraus* (SPON: M. O. Berman), Department of Neurology, Boston University School of Medicine, Boston, MA 02118

Growing evidence suggests that altered immune function occurs in Alzheimer's disease and senile dementia of the Alzheimer's type (AD/SDAT). In this study we report substantial changes in one measure of cellular immunity, natural killer cell activity (NKCA), in late stage patients with AD/SDAT.

NKCA is a particularly appropriate measure to study immune function in AD/SDAT. NK cells are regulated by interferon (IF), a serum factor produced in response to viral infection. Viral infection has been suggested in AD/SDAT. AD/SDAT is associated with Down's syndrome (trisomy-21). The gene for the species specific interferon response, IF-Rec, occurs on chromosome 21. NKCA is under genetic control and is influenced by HLA type. A strong genetic component, including HLA associations, exists in AD/SDAT. Finally, NKCA is influenced by a variety of neuroendocrine hormones and peptides which may be altered due to cholinergic loss in AD/SDAT.

NKCA was measured in a standard 4 hr ^{51}Cr release assay against K562 targets. Lymphocyte effectors were tested at multiple effector/target ratios from 1.5:1 to 60:1. We first studied 7 carefully diagnosed, medication free, late disease stage SDAT patients and 7 matched controls. NKCA was depressed and the shape of the lytic curve was altered in all SDAT patients tested. These 7 subjects plus 12 additional late stage AD/SDAT patients were tested with matched normal controls and controls with other neurologic disorders in subsequent experiments. Again NKCA was depressed in AD/SDAT patients. However, in 10 AD/SDAT patients tested in the early stages of disease NKCA was elevated compared to controls. Subjects with other disorders including Huntington's disease and multi-infarct dementia did not differ from normal controls. Monoclonal antibodies were used to determine the percent of various cell subsets including NK cells in the lymphocyte preparations from 7 patients. Interestingly, NK cell number was elevated in the four patients who had the most depressed functional response.

In recent experiments lymphocytes were incubated for 18 hrs with purified α or γ IF 1000 units VSV/ml/$6x10^6$ cells, then tested for NKCA. 3 late stage AD/SDAT patients showed no increase in NKCA with αIF. 1 patient showed no change with γ IF while 2 patients had decreased NKCA. NKCA was augmented with both α & γ IF in controls.

Depressed NKCA & hyporesponsiveness to IF in AD/SDAT may reflect immune exhaustion resulting from chronic viral infection, or may be a secondary effect of cholinergic loss, or may be due to an unidentified suppressive factor. Furthur studies of this phenomenon may result in the development of new diagnostic tools and ultimately in strategies for the management and prevention of disease.

34.2 VARIATION IN SERUM PROLACTIN LEVELS DURING THE PRIMARY IMMUNE RESPONSE. B.L. Spangelo*, N.R.S. Hall, J.P. McGillis* and A.L. Goldstein. Dept. of Biochemistry, The George Washington University Medical Center, Washington, D.C. 20037.

Evidence exists that suggests an involvement for prolactin (PRL) in modulating the immune response. Hypophysectomized rats have been shown to have significantly reduced antibody titers to SRBC (Berczi, I., et al., Acta Endocrinol., 98: 506, 1981) unless PRL was administered at a concentration of 200 ug/day for 10 days. This treatment restored the immune response to control levels. Furthermore, immunodeficient athymic mice have been found to have abnormally low serum PRL levels compared to normal littermates (Pierpaoli, W., et al., Clin. Exp. Immunol., 24: 501, 1976). PRL was restored to normal levels by thymic implantation at birth. The present studies were performed to establish the relationship of PRL to the primary immune response.

Male Swiss Webster mice housed individually were injected i.p. with SRBC (0.2 ml of 10% packed cells). This T-cell dependent antigen elicited an immune response which was quantified by measuring hemagglutination titers in individual animals. Serum levels of PRL were determined by radioimmunoassay. Peripheral PRL levels were found to decrease significantly during the course of the immune response as analyzed by one-way analysis of variance ($p < 0.025$). Twenty-four hours after SRBC injection (day 1), PRL decreased approximately 30% as compared to uninjected controls. PRL remained depressed throughout the logarithmic phase of the immune response and as antibody titers fell from peak levels on days 5 through 7, the concentration of PRL gradually returned to control values. The possibility of a direct influence on lymphocytes was examined by incubating various concentrations of PRL (10^{-5} to 10^{-14}M) in mitogen-stimulated splenic cell cultures. PRL was found to enhance significantly the mitogenic potentials of Con-A and PHA (T-cell mitogens). However, there was no observed effect using the B-cell mitogen LPS.

The enhancement of T-cell mitogenesis coupled with the significant depression of serum antibody titers during the immune response to a T-cell dependent antigen suggests a potential role for PRL in modulating the immune response. Whether this modulatory influence is exerted upon subpopulations of lymphocytes will require further experimentation.

34.3 EFFECT OF SUPERFUSED THYMOSIN FRACTION 5 AND THYMOSIN α_1 ON IN VITRO PITUITARY ACTH RELEASE. J.P. McGillis*, N.R. Hall and A.L. Goldstein*. Dept of Biochemistry, The George Washington University, Washington, DC 20037

Results from various studies have suggested that peptides produced by components of the immune system constitute part of an immune-neuroendocrine circuit. In particular, studies have shown that intracerebroventricular injection of Thymosin Fraction 5 (TSN-5), a partially purified thymic extract, and Thymosin α_1 (TSN α_1), a 28 amino acid constituent of TSN-5, cause a specific increase in serum corticosterone in mice. Intra-peritoneal injection of TSN-5 also caused an increase, but at much higher doses, while i.p. injection of TSN-α_1 at doses up to 100 ug/animal had no significant effect on serum corticosterone. Since these materials are active in the brain at 100 fold lower doses, and since they have been shown to have no effect on corticosterone production by adrenal fasciculata cells in vitro, it was hypothesized that their effect is at the level of the hypothalamic-pituitary axis. An in vitro superfusion system was used to test this hypothesis.

In these studies, pituitary glands were dissected from male Wistar rats. Four animals were sacrificed between 0800 and 0900. The pituitaries were quartered and placed in the superfusion apparatus. The pituitaries were superfused with Medium 199 at a rate of 0.5 ml/min. They were then pulsed for 1 min with the peptides to be tested. Two ml fractions were collected and analyzed for ACTH by RIA. In these experiments there was no change in ACTH production when superfused with TSN-5 (1 pg-1mg), or TSN α_1 (10^{-10} to 10^{-6}M). CRF was used as a positive control and was found to be effective from 10^{-9} to 10^{-6}M. Based on these results, we believe that the site of action of these materials is either at the hypothalamus or at some site influencing the hypothalamus.

34.4 PLASMA B-ENDORPHIN CONCENTRATIONS IN PATIENTS WITH HEREDITARY ANGIONEUROTIC EDEMA (HANE). R.Perricone+°, C.Moretti+°°, C.De Carolis+°, L.Fontana+°, G.De Sanctis+°, L.Gnessi+°°, A.E.Panerai and F.Fraioli+°°. Dept. Pharmacology, School of Medicine, University of Milano, +VI Clinica Medica, University of Rome and °°V Clinica Medica, University of Rome, Italy.

Angioneurotic edema is manifested by attacks of swelling of the extremities, face, trunk, airways or abdominal viscera occurring after psychological and physical stress or secondary to trauma. Cutaneous attacks are characterized by being non pitting and pruritic, differently from what observed in other edematous conditions such as urticaria, moreover HANE differentiates from similar conditions also for the complete absence of pain. The etiopathology of HANE is referred to C1 inhibitor (C1INH) deficiency due to a decreased production of C1IN or by the production of functionally inactive C1INH. Two orders of considerations led us to investigate a possible role of circulating B-endorphin in the pathophysiology of the disease. First, the existence of close links between the immune-system and endogenous opiates such as the presence of speficic binding sites for B-endorphin on the terminal complex of human complement; second, the clinical observation of the absence of pain in these patients during attacks. We measured B-endorphin and B-lipotropin plasma concentrations using an HPLC-RIA coupled method in patients before, dueing and after the HANE attack. Moreover, we evaluated plasma B-endorphin and B-lipotropin in patients during remission or in relatives of HANE patients. Plasma B-endorphin increased dramatically during a HANE attack without a concomitant increase in B-lipotropin, thus indicating that the increase we observed was not dependent on the presence of stress. During remission B-endorphin concentrations decreased although in some patients they remained high, but again in presence of low B-lipotropin concentrations. Our results suggest a link between B-endorphin plasma concentrations and HANE. In vitro studies in order to better characterize this relationship are in progress.

34.5 INNERVATION OF A LACRIMAL GLAND. B. Walcott,[1] Kent T. Keyser,*[2] and John Mclean.*[3] [1]Dept. of Anatomical Sciences, [2]Dept. of Psychiatry, SUNY, Stony Brook, New York and [3]Department of Zoology, University of Melbourne, Parkville, Australia.

The Harderian gland of the bird is the major lacrimal gland and the tears produced by it are complex, containing lysozyme, IgA, and other bacteriosides in a serous fluid. The serous fluid and other components are produced by a tubular secretory epithelium while the IgA is produced by a very large population of plasma cells. Tear production is modulated by the autonomic nervous system and the gland is richly innervated. We have examined the innervation pattern to the two cell populations using histochemical techniques. We have confirmed a dense acetylcholinesterase network using a modified Coupland-Holmes technique. This network extended throughout the gland and appeared to be associated with both cell populations. Using the Falck-Hillarp technique we found varicose fibers around superficial blood vessels and among the plasma cells. There was very little positive staining associated with the secretory epithelium. There was no suggestion of the presence of serotonin by this method or by immunocytochemistry. The glyoxalic acid method also showed an extensive noradrenergic innervation primarily among the plasma cells. Ultrastructurally, the chromate/dichromate method showed dense chromaffin reaction product in vesicles in nerve varicosities in the region of the plasma cells but such endings were never seen associated with the secretory epithelium. Immunocytochemical methods revealed the presence of Substance-P-like, L-Enkephalin-like, and Vaso-Intestinal-Peptide-like immunoreactivity in a fibrous staining pattern throughout the gland. These anatomical data suggest that the release of all the components of tears, including the IgA, may be neurally modulated and that there are many putative transmitters present. Supported by GM 28804 and NS 19350.

34.6 SYMPATHETIC INNERVATION OF THE SPLEEN. S.Y. Felten, R.K. Malone*, D.J. Madura* and D.L. Felten. Dept. of Anatomy, Univ. of Rochester, Sch. of Med., Rochester, NY 14642 and Dept. of Anatomy, Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Recent anatomical, physiological, immunological and behavioral studies have supported the hypothesis that the nervous system can modulate responses of the immune system. While humoral agents, such as corticosteroids, can influence the immune response, the possibility that neurotransmitters can also play a modulatory role has been investigated. This study was undertaken to further explore the noradrenergic sympathetic innervation of the spleen in the rabbit and the rat. Postganglionic sympathetic fibers and varicosities were demonstrated using the SPG (glyoxylic acid) method of de la Torre. Acetylcholinesterase was localized by the procedure of Karnovsky and Roots. In addition, norepinephrine levels were measured using HPLC with electrochemical detection (LCEC). The following conclusions were drawn: 1.) Both the rabbit spleen and the rat spleen are extensively innervated by noradrenergic fibers. These fibers enter the spleen through vascular plexuses which follow the central arteries, through a diffuse subcapsular plexus, and through the trabeculae. 2.) Noradrenergic varicosities are present primarily around arteries in the white pulp. Numerous fibers exit from these plexuese and enter the parenchyma of the white pulp, where they end in proximity to lymphocytes. 3.) The vascular and parenchymal varicosities are often associated with yellow fluorescent cells. 4.) Acetylcholinesterase is present in the rabbit spleen but we were not able to demonstrate its presence in the rat spleen. In the rabbit, AChE seems to be independent of neural elements, and is generally associated with the germinal zones of the white pulp.

In an additional study on the noradrenergic innervation of the spleen in the streptozotocin diabetic rat, norepinephrine levels and density of varicosities were found to be increased in almost all groups of rats with diabetes. The higher content of norepinephrine suggests that the lymphocytes in the spleen of the diabetic rat may be subjected to higher levels of the transmitter. We hypothesize that this relationship may in part explain the altered immune responses in the diabetic state, exacerbated by an enhanced presence of a substance thought to be inhibitory.

(Work on the diabetic rats was supported in part by a Juvenile Diabetes Foundation grant.)

34.7 EFFECTS OF 6-HYDROXYDOPAMINE AND RESERPINE ON THE GROWTH OF LPC-1 PLASMACYTOMA. C. G. Frondoza*, R. Grzanna and R. L. Humphrey*. Oncology Center and Department of Cell Biology and Anatomy, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

There is increasing evidence for a functional relationship between the nervous system and tumor development and growth. In particular, the sympathetic nervous system has been implicated in the neural regulation of tumor growth. We have studied the effects of chemical sympathectomy with 6-hydroxydopamine (6-OHDA) and of long-lasting depletion of peripheral catecholamine stores with reserpine on the growth of LPC-1 plasmacytoma.

BALB/c mice were treated with 6-OHDA postnatally (100 mg/kg s.c. every other day for 2 weeks). The effectiveness of the drug treatment was verified by assays of dopamine-β-hydroxylase, the biosynthetic enzyme of norepinephrine, in samples of heart and spleen tissue. Reserpine (100 µg/kg) was injected i.p. into adult mice. One to two million LPC-1 cells were injected into the calf. Tumor growth was monitored by caliper measurements of the calf diameter and the median survival time for each group of mice was determined. Seven to 10 mice were used in each set of experiments. A significantly slower tumor growth rate was observed in mice treated with 6-OHDA along with an increase in the median survival time from 23 to 28 days. Injections of a single dose of reserpine on the day of tumor transfer did not alter the tumor growth curve or median survival time. Repeated injections of reserpine on day 10, 15 and 20 were toxic. A striking decrease in the rate of tumor growth and a profound increase in the median survival time from 22 to 33 days were observed when reserpine was given 12 days after tumor transfer, a time at which the tumor mass is established. Reserpine showed no direct cytotoxic effects on LPC-1 tumor cells in tissue culture.

Analysis of the tumor growth curves revealed that the time of appearance of the tumor mass (day 12) was not altered by either drug treatment but that the slope of the growth curves in treated mice was less steep than that measured in control mice, suggesting that the log-phase of tumor growth rather than the latency period is primarily affected. Since both 6-OHDA and reserpine treatment deplete peripheral norepinephrine stores, the data may be taken as evidence for an involvement of the sympathetic nervous system in the modulation of LPC-1 tumor growth.
(Supported by USPHS grants CA-31499 and NS-15199)

34.8 EFFECTS OF STRESS AND MORPHINE ON GROWTH OF A MAMMARY ASCITES TUMOR (13762B) IN RATS. J.W. Lewis, Y. Shavit, G.W. Terman, R.P. Gale* & J.C. Liebeskind, Depts. of Psychology and Medicine, University of California, Los Angeles, CA 90024.

Stress has been shown variously to accelerate and retard tumor development in laboratory animals. The type of tumor studied, amount or type of stress experienced, and timing of stress exposure relative to tumor induction or implantation appear to be important factors in determining which outcome is obtained. We have reported that daily exposure to 10 min of inescapable footshock for 4 days prior to tumor injection enhances growth of a mammary ascites tumor (MAT 13762B) in rats. This effect is blocked by naltrexone suggesting mediation by opioid peptides. In the present experiment we: A) compared the effects of footshock stress given before or after injection of this tumor; B) assessed the effects on tumor development of morphine administration and of stress in animals made tolerant to morphine; and C) determined whether tolerance develops to the effect of stress with chronic (14 days) exposure.

Groups of 45 day old female Fischer 344 rats (n=15 to 20) were studied. Footshock stress was 10 min of intermittently applied (on 1 sec every 5 sec) 2.0 mA, 60 Hz sine waves. All rats were injected i.p. with 10^4 MAT 13762B cells, and survival was recorded for 30 days.

Compared to nonstressed controls, the percent of animals surviving and the median survival time was decreased equally in groups exposed to footshock daily for 4 or 14 days before tumor injection. Daily footshock for 4 or 14 days after tumor injection also enhanced tumor development although these effects were less marked. Morphine treatment (daily s.c. injections of 30 mg/kg for 2 days, then 50 mg/kg for 12 days) before tumor administration did not affect survival. This same morphine regimen also did not prevent the tumor enhancing effect of stress in animals receiving 4 days of footshock before tumor injection. This regimen given after tumor injection, however, did significantly decrease survival time and percent survival.

Thus, exposure to stress either before or after tumor injection enhances the development of MAT 13762B. Although our previous work indicates involvement of opioid peptides, stress was still capable of enhancing tumor development in animals made tolerant to morphine and no evidence was seen for development of tolerance to this effect after 14 footshock sessions. These results are paralleled by our findings on the effects of these stress procedures and morphine on an index of immune function, cytotoxic activity of natural killer cells (Shavit et al., this volume). (Supported by NIH grant NS07628 and a gift from the Brotman Foundation)

34.9 THE EFFECTS OF STRESS AND MORPHINE ON IMMUNE FUNCTION IN RATS. Y. Shavit, J.W. Lewis, G.W. Terman, R.P. Gale* and J.C. Liebeskind. Departments of Psychology and Medicine, UCLA, Los Angeles, CA 90024.

Stress can adversely affect both cellular and humoral aspects of immune function. Natural killer (NK) cells appear to act in immune surveillance against viral disease and neoplastic growth. NK activity is reduced after such stresses as food deprivation and surgery. We have suggested that the effect of stress on NK activity in rats is mediated by opioid peptides. Intermittent footshock stress causes opioid-mediated analgesia, whereas an equal amount of continuous footshock causes analgesia independent of opioid systems. We found that NK activity was suppressed in rats exposed to the "opioid" footshock paradigm, and this effect was blocked by naltrexone. NK activity in rats given the "nonopioid" stress did not differ from control values. To investigate further the involvement of opioids in this phenomenon we sought to determine if the effect of the opioid stress would develop tolerance with repeated exposure and cross-tolerance in morphine tolerant rats.

In the first experiment, rats were subjected daily to the opioid form of footshock (2.0 mA; on 1 sec every 5 sec for 10 min) for either 4 or 14 days. A third group served as nonstressed controls. Three hours after the last stress session, rats were anesthetized and their spleens removed and dissociated into a single cell suspension. Lymphocytes were co-cultured with YAC-1 target cells labelled with chromium-51, and NK activity was measured in a 4 hr chromium release assay. In the second experiment, one group of rats was treated with morphine for 14 days (30 mg/kg for 3 days, then 50 mg/kg); a second group received no drug. Ten days after the beginning of morphine treatment half of each group was subjected to the opioid form of stress for 4 days. Activity of NK cells was measured as described above.

The percent of specific NK cytotoxicity was equally reduced after 4 or 14 days exposure to the opioid form of stress ($P<.05$ compared to nonstressed controls). Morphine treatment alone (14 days) had no effect on the activity of NK cells, nor did it attenuate the suppressant effect of the opioid form of stress. That is, the activity of NK cells was equally suppressed after 4 days of stress in both morphine treated and no drug groups. These findings of naltrexone antagonism but lack of tolerance and cross-tolerance are paralleled by our findings on the effects of this same footshock stress on tumor growth in rats (Lewis et.al., this volume). (Supported by NIH grant #NS07628 and a gift from the Brotman Foundation).

34.10 INNERVATION OF THYMUS TRANSPLANTS IN NUDE MICE: AN ULTRASTRUCTURAL STUDY. M.R. Cullen* and K. Bulloch, Department of Neurology, SUNY Stony Brook, Stony Brook, New York 11794.

The developing thymus, prior to the onset of its functional and structural organization, is innervated by the autonomic nervous system. (ANS) In a recent light and fluorescent histochemical study a similar temporal and distributive pattern was seen in the establishment of functional thymus transplants under the kidney capsule of nude mice (Bulloch et al, 1983). The present study extends these earlier findings by analysing at the ultrastructural level, the size, type and distribution of ANS nerves within the established thymic transplant. Thymuses were removed from E-18 embryos of B10 mice and placed under the kidney capsule of syngeneic nudes. Mice were sacrificed during the fourth post-operative week and the transplanted thymus was fixed and embedded in epon-araldite. Ultrathin sections were stained with uranyl acetate and lead citrate. Ultrastructural analysis demonstrated that myelinated and non-myelinated fiber bundles penetrate the thymic capsule and the interface between the kidney and the thymus. The myelinated fibers measured 2 um microns or less in diameter while the non-myelinated fibers were found to be lum in size. Myelination did not accompany the nerve fibers in the parenchyna of the gland. However, non-migratory thymic cells were found to engulf these nerves in a fashion similar to that seen in support cells of the nervous system. Surface nerves formed a complex network among the thymocytes directly under the capsule while inter-thymic nerves enter the parenchyma in bundles along the vasculature and interlobular septa. Some larger nerve fibers terminate in encapsulated receptor-like structures at the cortico-medullary boundaries and in the interlobular septa. Smaller fibers formed enpassant boutons near parenchymal cells. Mature lymphocytes were common in the vicinity of these nerve bundles. Ultrastructural analysis verifies that the types and distribution of nerve fibers innervating functional thymic transplants is comparable to those nerves found innervating normal mouse thymus. These data further support a role for the ANS innervation in the development of thymic competency. Supported by NIH grant NS 18401

35.1 INTRAVENTRICULAR ARGININE VASOPRESSIN INCREASES CEREBROSPINAL FLUID ABSORPTION IN THE ANESTHETIZED RABBIT P. McL. Black, A. Tsouras,* L. Foley.* Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. 02114. Ventriculo-cisternal perfusions in rabbits anesthetized with pentobarbital were used to evaluate the effect of arginine vasopressin (AVP) on cerebrospinal fluid (CSF) formation and absorption. The baseline CSF formation rate Vf was calculated during 3 hours of perfusion by the relationship $\dot{V}f=\dot{V}i\ ((Ci/Co)-1)$ where $\dot{V}i$ was the infusion rate, Ci the inflow concentration of the indicator dextran blue and Co the outflow concentration of dextran blue as measured spectophotometrically. The absorption rate $\dot{V}a$ was calculated by the equation $\dot{V}a=\dot{V}f+\dot{V}i-\dot{V}o$ where $\dot{V}o$ is the outflow rate. Synthetic arginine vasopressin was then infused at a rate of 250 uU/minute for 3 hours and CSF formation and absorption rates were recalculated under these conditions.

The CSF formation rate did not change with arginine vasopressin infusion: the control value was 10.25 $\pm$ 5.1 ul/min. (mean $\pm$ S.D.); the value during AVP infusion was 12.2 $\pm$ 6.7 ul/min. The pressure was kept constant during these infusions at the cisternal opening pressure of the animal. The CSF absorption rates increased significantly with arginine vasopressin infusion into the ventricles. For nine animals the mean absorption rate was 5.7 $\pm$ 3.7 ul/min. before arginine vasopressin administration: during the administration it rose to 19.1 $\pm$ 12.2 ul/min. This increase was significant at $p<0.02$ by the 2 tailed t test. A similar increase was not seen with infusion of oxytocin or lysine vasopressin at the same concentration and rate. These data show that intraventricular arginine vasopressin increases the CSF absorption rate in rabbits. This is a novel finding and one whose mechanism requires further study.

35.2 REVERSAL OF EXPERIMENTAL DIABETES MELLITUS BY INTRACRANIAL ALLOGRAFT OF FETAL PANCREAS. Alan Fine. Department of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Embryonic neural tissue can be transplanted into the brain of unrelated rats, where it may survive indefinitely, differentiate anatomically and biochemically, and influence the behavior of the host. Juvenile adrenal medullary grafts can also survive in, and influence, the brain (Freed, W.J. et al., Nature 292:351, 1981). Such findings suggest that other endocrine allografts might survive within the immunologically "privileged" confines of the CNS. If their hormones continued to be produced and released appropriately, and could pass out of the brain with the bulk flow of CSF, such grafts might be useful for the treatment of certain endocrine diseases.

I have tested this hypothesis in an animal model of insulin-dependent diabetes. Normal young adult male PVG/Hooded rats (blood glucose less than 150 mg/dL) were made diabetic by single i.v. or i.p. injections of streptozotocin (60 mg/kg). From 5 to 10 fetal pancreases, of 15-16 day gestation WF (major histocompatability barrier) or SPD (histocompatability undefined) embryos, were injected into the cisterna magnum of diabetic rats (blood glucose at least 300 mg/dL). Control diabetic rats received equivalent intracisternal injections of vehicle alone; a second group of control animals received equivalent fetal pancreas grafted into the kidney capsule. Urine volume and glucose, water intake, blood glucose, and weight were monitored before and throughout the experiment. Within the first week after grafting, fetal pancreas recipients, but not vehicle-recipient controls, returned to normal levels in all measured parameters. At the time of abstract submission, intracisternally-grafted animals have remained non-diabetic beyond 1 month. This result indicates that, despite the blood-brain barrier, hormones from intracranial endocrine grafts can pass from the CSF to the periphery in physiologically and therapeutically significant amounts, and that such grafts can survive for a prolonged period without rejection. (Supported by a Bantrell Fellowship, with assistance from Ames Division, Miles Laboratories.)

35.3 CHOROID PLEXUS EPITHELIAL CELLS IN CULTURE: BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERISTICS. D. H. Gabuzda*, E. J. Hunnicutt, C. J. Owen* and J. A. Nathanson. Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02144.

The epithelial cells of the choroid plexus are involved in the secretion of cerebrospinal fluid (CSF). Recent physiological evidence indicates that the production of CSF from these cells may be influenced by catecholamines, either blood-born or released from adrenergic nerve terminals present in the choroid. Previous biochemical studies from this laboratory (Nathanson, Mol. Pharmacol. 18, 199-209) have identified in presence, in choroid secretory cells, of a very active adrenergic-stimulated adenylate cyclase with beta$_2$-receptor characteristics. Unfortunately, physiological investigations of the possible function of this enzyme have been hampered because of the inaccessability of the choroid plexus in situ.

We now report studies investigating the biochemical and pharmacological characteristics of epithelial cells separated and purified from intact bovine lateral and fourth ventricle choroid plexus. These cells are isolated in artificial CSF by selective, graded enzymatic digestion with trypsin combined with special mechanical agitation, followed by sieve filtration and washing under gentle conditions. For calf choroid obtained from the slaughterhouse, additional precautions must be taken to avoid fungal and bacterial contamination. Acutely isolated cells demonstrate 70-95% viability and, when placed in artificial CSF, are able to accumulate cyclic AMP from endogenously synthesized ATP. Such cyclic AMP accumulation is markedly stimulated in the presence of isoproterenol.

We have also been able to maintain these cells in viable condition in culture for periods up to four days. After day three, precautions must be taken to prevent significant fibroblast contamination. Cell survival in modified MEM is enhanced using collagen-coated substrates, high cell density, and fetal calf serum. Cells also survive in suspension culture. After several days in culture, such cells are still able to synthesize cyclic AMP from ATP and display receptor sensitivity to adrenergic agonists.

35.4 TRACER PROTEIN ENTERS THE BRAIN AROUND PENETRATING BLOOD VESSELS; EVIDENCE FOR AN ACTIVE MOVEMENT OF HORSERADISH PEROXIDASE (HRP) THROUGH THE PERIVASCULAR SPACES AND MICROVASCULAR BASAL LAMINAE M.L. Rennels, T.F. Gregory*, O.R. Blaumanis*, K. Fujimoto* and P.A. Grady, Departments of Neurology and Anatomy, University of Maryland School of Medicine, Baltimore, MD 21201

After administration into the cerebrospinal fluid (CSF), HRP enters the perivascular spaces surrounding penetrating arterioles and moves rapidly into the brain substance through the basal laminae around capillaries arising from these vessels. Solutions of HRP (4.0%) were infused into the lateral ventricles of anesthetized cats and dogs and allowed to circulate in the CSF spaces for 4 min. to 2 hrs. The animals were then fixed by intravascular perfusion of aldehydes. Intracerebral tracer distribution was studied in Vibratome sections of the brain after incubation in tetramethylbenzidine. After HRP circulation for 4 min., penetrating arterioles and capillaries were surrounded by tracer reaction product but venules were not. After 6 or 10 min. HRP circulation, however, the intraparenchymal microvasculature was outlined, in toto, throughout the brain. The tracer thus appears to move along penetrating arterioles to capillaries and thence to venules and veins. After incubation of adjoining sections from the same animals in diaminobenzidine, electron microscopy showed dense reaction product in the perivascular spaces, filling the basal laminae around capillaries and dispersed throughout the extracellular spaces of the surrounding parenchyma. In further experiments this rapid HRP influx along the intraparenchymal microvasculature was abolished if the heart was stopped prior to tracer infusion. After 10 min. HRP circulation, reaction product was found only along the ventricular and pial borders and in open perivascular spaces around the largest penetrating vessels. Similarly, perivascular tracer penetration did not occur if the intracranial arterial pulse was damped by partial ligation of the dog brachiocephalic artery prior to HRP infusion and circulation for 6 min. The exchange of solutes between the CSF and cerebral extracellular spaces is generally attributed to diffusion. However, HRP movement through the perivascular spaces and within the basal laminae around the microvascular network occurred too rapidly to be explainable solely on this basis. This tracer influx may represent a convective process facilitated by travelling pulsations of the cerebral arterioles in their perivascular spaces and of capillaries surrounded by astroglial endfeet. A reasonable interpretation of these data is that this rapid HRP influx reveals an active extravascular fluid circulation in the brain.

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35.5 COMPARISON OF ELECTRICAL RESISTANCE, BUBBLE WITHDRAWAL AND COORDINATE METHOD FOR CANNULATION OF VENTRICLES. B.H. Herman, S. Berger* and S.G. Holtzman.* Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.

A comparison was made of three methods of lateral intraventricular (LV) cannulation in the rat brain. Rats in a control group (n = 8) were cannulated using traditional stereotaxic coordinates. In a second group (n = 8), electrical resistance was used to localize the ventricle and differentiate it from surrounding brain areas. In a third group (n = 8), a bubble withdrawal technique was employed where movement of a bubble in polyethylene tubing was monitored as the cannula was lowered in brain.

Differences in electrical resistance in rat brain was an accurate method for locating the LV. The LV showed significantly lower resistance than areas immediately above it (corpus callosum) and the fiber tracts immediately below it. Although histological analyses indicated that all three methods produced accurate LV cannulation (all 24 rats had LV placements), the electrical resistance technique was associated with optimal placement within the LV. Using the resistance procedure, greater access was obtained to posterior portions of the ventricular system. Electrical resistance in rat brain was about 25% greater than in physiological saline, presumably due to the presence of additional ions in CSF.

Also described is a novel stereotaxic cannula holder that may be used in the bubble withdrawal procedure or in obtaining acute samples of cerebrospinal fluid.

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BEHAVIORAL PHARMACOLOGY: DOPAMINE

36.1 SERINE POTENTIATES NEUROLEPTIC ACTIVITY IN THE RAT. F. Petty, B. Proctor* and A.D. Sherman*. Vet. Admin. Med. Cent. and Dept. Psychiat., Univ. Ia. Col. Med., Iowa City, IA 52242.

Three animal behavioral paradigms -- catalepsy, antagonism of amphetamine induced stereotopy, and inhibition of the conditioned-avoidance response (CAR) -- demonstrate a high (.75 - .95) rank-order correlation with antipsychotic drug potency in humans. Pharmaceutical firms have used these on a routine basis to screen neuroleptic activity for well over 20 years. Since elevated levels of serine have recently been reported in psychotic humans (Waziri et al., Brit. J. Psychiat., in press), we examined interactions of L-serine, D-serine and D and L-methionine with trifluoperizine in these three behavioral paradigms. Pilot experiments with the CAR had demonstrated a potentiation of neuroleptic effect, so we chose a dose of trifluoperazine (0.53 mg/kg catalepsy, 0.25 mg/kg stereotopy and CAR) intended to optimize finding potential potentiation of neuroleptic effect. L-serine was administered with trifluoperazine at a dose of 200, 300, and 400 mg/kg IP. A dose related potentiation of neuroleptic activity was seen in all three behaviors, whereas no significant effects were noted with 800 mg/kg of L-serine administered alone. D-serine had no significant effect on catalepsy or stereotopy but did potentiate inhibition of the CAR, although significantly less than L-serine. D and L-methionine were without effect in any of the three behaviors.

These data are difficult to interpret in light of numerous reports that methionine exacerbates psychosis in about 1/3 of psychotic patients (Cohen et al., Biol. Psychiat., 8:209, 1974) and that serine induces psychotic symptomatology in patients with episodic psychosis in remission (Pepplinkhuizen et al., Lancet, May 1, 454, 1980) and would seem to be at odds with the "abnormal methylation" hypothesis of schizophrenia.

Clinical trials with acute and chronic serine administration in psychotic patients are in progress and will also be discussed.

36.2 EFFECTS OF IMIPRAMINE, BUPROPION, CHLORPROMAZINE, CLOZAPINE, DIPHENHYDRAMINE, AND PREFEEDING ON RATS RESPONDING UNDER A DIFFERENTIAL-REINFORCEMENT-OF-LOW-RATE 72-SEC SCHEDULE (DRL 72). G.T. Pollard and J.L. Howard, Department of Pharmacology, Wellcome Research Laboratories, Research Triangle Park, NC 27709

Under a DRL schedule, a response is reinforced only if a specified period has elapsed since the previous response. A DRL schedule requiring a relatively long pause, 72 sec, was proposed as a screening method for antidepressant drugs: tricyclics (e.g., imipramine), monoamine oxidase inhibitors (e.g., phenelzine), and some atypical antidepressants (iprindole, mianserin, trazadone, fluoxetine, one dose of bupropion) were shown to decrease responses and increase reinforcements, whereas non-antidepressants (alcohol, chlordiazepoxide, morphine, pentobarbital, chlorpromazine, diphenhydramine) did not produce this pattern of change; the antidepressants nomifensine and bupropion (at higher doses), which increase locomotor activity in rodents, produced false negatives by increasing responses and decreasing reinforcements (O'Donnell, J.M. and Seiden, L.S., Psychopharmacology, 78:214, 1982; O'Donnell, J.M. and Seiden, L.S., JPET, 224:80, 1983; Seiden, L.S., Fed. Proc., 1983, in press). These results were obtained in albino rats responding for water. In the present study, in which hooded rats responded for food on a DRL 72, imipramine (5.0, 10, 20 mg/kg i.p.) produced a true positive; chlorpromazine (0.5, 1.0, 2.0 mg/kg i.p.), clozapine (10, 20 mg/kg i.p.) and prefeeding produced false positives (decreased responses, increased reinforcements); bupropion (10, 20 mg/kg i.p.) produced a false negative (increased responses, decreased reinforcements); and the behaviorally active dose of diphenhydramine was found to be very close to the convulsant dose. Preliminary results from a DRL 36, which yielded baseline reinforcement rates about the same as those of O'Donnell and Seiden, suggested also a pattern of false positives for non-antidepressants which reduce operant responding and false negatives for antidepressants which increase operant responding. Strain of rat, type of reinforcer, and the adequacy of testing of response-decreasing non-antidepressants are discussed as possible explanations for the disagreement with previous results.

36.3 APOMORPHINE-INDUCED PECKING IN THE CHICK IS MEDIATED BY D-2 RECEPTORS. Giovanni Marzullo and Arnold J. Friedhoff. Millhauser Labs., New York University School of Medicine, New York, NY 10016.

Systemic injections of apomorphine in newly-hatched chicks induced prolonged pecking at conspicuous spots located on the cage walls. This response was mediated by mature and stereospecific dopamine receptors since it was blocked by pretreatment with various dopamine antagonists, including (+)-butaclamol, but not by other classes of antagonist drugs or by (-)-butaclamol. The specificity of the chick dopamine receptor was further investigated using a series of antagonists having different relative affinities for the D-1 (adenylate cyclase-linked) and D-2 types of mammalian dopamine receptors. Drug efficacy in blocking the pecking response was unrelated to the affinity for the adenylate cyclase-linked receptor, but was highly correlated with the affinity for the D-2 receptor site. An even higher correlation was found between anti-pecking activity in the chick and clinical efficacy of the above drugs as antipsychotics. Therefore, the receptor mediating the pecking behavior is similar to the mammalian D-2 site and may be useful as a model for investigating antipsychotic drug action.

36.4 THE EFFECTS OF NALTREXONE AND AMPHETAMINE ON SQUIRREL MONKEY BEHAVIOR IN GROUPS. James T. Winslow*, Arnold Kozak* and Klaus A. Miczek. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Amphetamine has been shown to produce a profound disruption of social behavior in primates. Opiate antagonists have been reported to increase affiliative behavior. Catecholamines and endogenous opioids may exert opposing functions on social behavior. In the following studies we examined the effects of several doses of naltrexone on social and non-social behaviors exhibited by treated individuals in established groups of adult male, female and juvenile squirrel monkeys (Saimiri sciureus). The colonies formed stable social relationships and are reproductively active. Temperature, humidity and lighting mimic the natural habitat.

A catalogue of social and aggressive behaviors and postures, olfactory and sexual behaviors, and non-social behaviors such as walking and feeding was used, and a continuous behavioral record was collected. Using the focal animal technique, the frequency and duration of behaviors were summarized over a two hour observation period. The method of data collection permitted the identification of the focal monkey as the recipient or initiator of social interactions.

Naltrexone (0.1, 1.0, 10.0 mg/kg, IM) produced dose-related increases in the frequency and duration of social encounters initiated by untreated monkeys toward treated monkeys. Treated monkeys were touched, investigated, huddled with, threatened and grasped more often by untreated monkeys following administration of naltrexone. Selected doses also increased walking and marking behavior. The frequency of social initiatives by treated monkeys decreased as dose of naltrexone increased. Feeding was reduced at the highest dose, which also produced nausea.

The increase in affiliative behavior following naltrexone treatment appears to be an indirect effect on the social behavior of untreated members of the group directed toward the treated monkey. Similar indirect drug effects on social behavior have previously been reported for amphetamine and alcohol, and may be related to changes in the treated monkey's behavior, appearance or odor.

We have shown earlier that amphetamine leads to social withdrawal of treated monkeys in established groups. Naltrexone (0.1 mg/kg IM) enhanced amphetamine (0.3 mg/kg IM) induced increase in locomotion and grooming. Naltrexone reduced the frequency of limb flicks and head jerks induced by amphetamine, and increased feeding behavior suppressed by amphetamine. Naltrexone did not antagonize the suppressive effects of amphetamine on social behavior in the monkeys studied.

The findings confirm the disruptive effects of catecholamine activation, but indicate a complex relationship with opioids.

36.5 **SELF-INJECTION OF NEUROTENSIN INTO THE VENTRAL TEGMENTAL AREA (VTA).** Paul W. Glimcher*, Adrienne A. Giovino* and Bartley G. Hoebel. Department of Psychology, Princeton University, Princeton, NJ 08544.

Earlier work by our group has demonstrated that neurotensin (NT) is a potent reinforcer when administered into the VTA in the conditioned place preference paradigm (Glimcher et al., NY Acad Sci., 400:422, 1982). In order to cross-validate this finding we utilized the intracranial self-injection technique to further demonstrate the reinforcing properties of NT in the VTA. Ten animals were unilaterally implanted with 22 gauge stainless steel guide cannulas which intercepted the VTA (A 2.2, L 0.6, V 7.5). Animals were then placed in an operant chamber which contained two levers. One delivered NT, while the other delivered nothing and served as a control for nonspecific activity. Animals were tested in 4-hour sessions every third day. After an initial training period they reliably self-administered NT at regular, repeatable rates. Animals typically received 2.5 ug of NT in 50 nl of saline during 0.5 sec per bar-press. They consistently preferred the active bar to the inactive one, pressing on the average 5 to 20 times per hour for NT and typically less than once per hour on the blank lever.

In order to demonstrate that NT was in fact responsible for the self-administration behavior, 3 animals which had demonstrated consistent operant performance over an extended period were tested for response extinction by substituting saline for NT. These animals demonstrated a decrease in rate to less than half of their NT levels after 3 to 5 sessions of saline substitution.

We conclude from these data and our earlier work that NT is a potent reinforcer in the VTA in both operant and nonoperant paradigms.

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36.6 **PHENCYCLIDINE (PCP) GENERATES CONDITIONED REINFORCEMENT IN THE NUCLEUS ACCUMBENS (ACC) BUT NOT IN THE VENTRAL TEGMENTAL AREA (VTA).** Adrienne A. Giovino*, Paul W. Glimcher*, Cruz A. Mattei* and Bartley G. Hoebel (SPON: C.R. Gallistel). Department of Psychology, Princeton University, Princeton, NJ 08544.

Several groups have suggested that PCP may exert some of its effects on dopaminergic brain substrates. Involvement of some dopaminergic neurons in brain reward processes led us to investigate the possibility that the mesencephalic dopamine (DA) system might be a central substrate for reinforcing effects of PCP. This hypothesis suggested that PCP might generate reinforcement in the VTA or in the ACC, two important poles of the mesolimbic DA system. Twenty-eight animals were stereotaxically implanted with 22g s.s. guide cannulas in the VTA (A 2.2, L 0.6, V 7.5) and 17 with cannulas in the ACC (A 9.8, L 0.8, V 5.8). The conditioned place preference paradigm of Rossi and Reid (Physiol. Psychol., 4:269, 1976) was used. The experimental chamber was a rectangular tilt cage, one end of which was covered, the other left open to bright illumination. Animals were placed in the cage for 900 sec on the first 4 days of testing and allowed to demonstrate their place of preference, measured as time spent in each environment. On the next 4 days animals were confined to the bright environment after receiving injections of drug or vehicle. On the final day animals were allowed to express their preference after a saline injection, thus acting on their memory of the drug's effects. An increase in time spent on the bright side, when compared to baseline data generated on the first 4 days, indicated a conditioned preference. This paradigm eliminates confounding performance effects associated with some drugs by performing the test in an nondrugged condition.

VTA animals were divided into 3 groups receiving PCP (10 ug or 20 ug) or saline. Neither of these two doses produced a significant shift in preference for the bright side, suggesting that the VTA is not a site at which PCP generates its rewarding effects. The ACC animals were divided into 2 groups which received either PCP (20 ug) or saline. Animals which received PCP demonstrated a statistically significant increase in preference for the bright environment with which injections had been paired ($p<0.005$). We conclude from this that the ACC is a brain site sufficient for generating PCP reward in the place preference paradigm.

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36.7 **REWARDING EFFECTS OF AMPHETAMINE AND COCAINE IN THE NUCLEUS ACCUMBENS AND BLOCK BY FLUPENTHIXOL.** Edward F. Aulisi* and Bartley G. Hoebel. Department of Psychology, Princeton University, Princeton, N.J. 08544.

The nucleus accumbens (ACC) is an area rich in dopaminergic terminals; rats will self-inject amphetamine directly into this region (Hoebel, B.G., Monaco, A.P., Hernandez, L., Aulisi, E.F., Stanley, B.G., and Lenard, L., Psychopharmacology, in press). This suggests that stimulants can act in the ACC to generate reinforcing effects by way of dopamine (DA) receptors. To test this hypothesis, amphetamine (AMPH) and cocaine (COC) were tested for reward effects in the ACC using a different reinforcement paradigm along with isomers of a DA receptor blocker, cis-flupenthixol (c-FLU) and trans-flupenthixol (t-FLU).

Seventy rats were implanted with 26 gauge canulas in the ACC (A 9.8, L 0.8, V 5.8). They were tested in a tilt cage with one half covered and the other half exposed to bright light. Rats were tested for 900 sec/day for 4 days and found to prefer the dark side. The next four days, they received ACC injections of either AMPH, AMPH & c-FLU, AMPH & t-FLU, COC, COC & c-FLU,or COC & t-FLU and were confined to the bright side of the cage. On day nine, they received saline injections only and were free to choose their place of preference.

AMPH and COC caused a conditioned shift in preference toward the bright side. Thus, rats preferred to spend additional time in an environment associated with ACC injections of these DA agonists. This place preference was significantly attenuated when the DA receptor blocker, c-FLU, was added to either AMPH or COC. There was no significant decrease in place preference when the inactive isomer, t-FLU, was used.

In conclusion, AMPH and COC injections in the ACC caused a conditioned place preference in rats. These effects were blocked by an antipsychotic drug delivered centrally. Given the relative specificity of flupenthixol for DA receptors, these results suggest that the rewarding effects of amphetamine and cocaine in the nucleus accumbens involve a dopaminergic system.

This work was supported by USPHS MH-35740.

36.8 A COMPARISON OF ALCOHOL AND d-AMPHETAMINE EFFECTS ON AGGRESSION OF FEMALE MICE IN DIFFERENT HORMONAL STATES. Klaus A. Miczek and Joseph F. DeBold. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Aggression in females has not been extensively investigated, and little is known about the endocrine and neural processes which may mediate it. It was our objective to challenge female mice pharmacologically in situations that engender aggressive behavior toward other females or toward males. We studied females which were either (1) ovariectomized, (2) ovariectomized and maintained on testosterone, or (3) lactating. The rationale for these drug-hormone interactions derives from their demonstrated effects on male aggression and their potentially common sites of action in the brain.

After the hormonal state of the female CFW mice was experimentally prepared, each female, housed together with a male, confronted either a female or a male intruder mouse that was placed into her home cage. Videorecords of each 5-min confrontation were analyzed with the aid of computer. The frequency, duration and pattern of attack bites, threats, pursuits, tail rattles, as well as non-aggressive behavior such as grooming, rearing, and walking, were determined. d-Amphetamine sulfate (0.63-5 mg/kg) or its saline vehicle were injected IP 30 min before the test, and alcohol (0.1-5.6 g/kg) or its water vehicle were administered orally 15 min before the test.

Female mice attacked, threatened and pursued female intruder mice, after ovariectomy, during prolonged testosterone (7.5 mm capsule, SC) treatment, or during lactation; male opponents were attacked less. Amphetamine disrupts the pattern of female aggressive behavior toward female intruders only at higher doses (2.5, 5.0 mg/kg) regardless of their hormonal state. The low level of aggressive behavior toward males remained unaltered by amphetamine. By contrast, a biphasic action of alcohol on aggressive behavior was seen after alcohol administration to ovariectomized mice, i.e. a 0.1 g/kg dose increased attacks and threats toward female intruders, whereas 1.7 and 3.0 g/kg alcohol doses decreased these responses. Testosterone prevented the enhancing effect of 0.1 g/kg alcohol. Lactating females only showed a suppression of aggression in response to alcohol.

These results demonstrate that the hormonal state can alter the behavioral response to alcohol and to a lesser extent to d-amphetamine. The sex of the opponent also altered the drug effects on aggression. Finally, the pattern of alcohol response seen in females is different from that seen earlier in male mice, but the amphetamine response in females paralleled that of males.

36.9 **DISCRIMINATIVE STIMULUS PROPERTIES OF A LOW DOSE OF APOMORPHINE IN THE RAT.** Andrew H. Tang* and Allegra A. Cangelosi*, (SPON: J. M. Braughler). The Upjohn Company, Kalamazoo, MI 49001.

The direct dopamine (DA) receptor agonist, apomorphine, is thought to have a dose-dependent dual effect on central DA systems. At low doses, apomorphine reduces DA neuronal activities through selective stimulation of DA autoreceptors. At higher doses, post-synaptic DA receptors are also stimulated, enhancing DA neuronal activities. Male Sprague-Dawley rats were trained to discriminate a low dose (0.1 mg/kg s.c.) of apomorphine from a control treatment (s.c. saline injections) in a 2-lever, food reinforcement procedure. The dose of apomorphine was chosen to activate DA autoreceptors predominantly. Other direct-acting DA agonists tested for stimulus generalization shared the discriminative stimulus properties of apomorphine. The doses which produced maximum apomorphine lever choice are as follows: n-propyl norapomorphine (0.003 mg/kg); pergolide mesylate (0.03 mg/kg); apomorphine (0.1 mg/kg); lergotrile mesylate (0.3 mg/kg); (-)3-PPP (1 mg/kg); (+)3-PPP (3 mg/kg); and bromocriptine (10 mg/kg). The greater potency of the (-) over the (+) isomer of 3-PPP supports the premise that DA autoreceptors mediated the discriminative stimulus effects of apomorphine in these experiments. Neither d-amphetamine, methylphenidate nor cocaine generated more than 20% apomorphine-appropriate lever choice.

The selective lever choice produced by the training dose of apomorphine was partially antagonized by haloperidol or sulpiride. On the other hand, some of the antipsychotic drugs produced significant proportion of apomorphine-appropriate lever choice: haloperidol (50%); chlorpromazine (50%); clozepine (80%).

36.10 ANTAGONISM OF APOMORPHINE ENHANCED STARTLE BY α_1-ADRENERGIC ANTAGONISTS. M. Davis, J.H. Kehne*, and R.L. Commissaris* Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508

Considerable data indicate that catecholamines modulate sensorimotor reactivity as measured by changes in the startle reflex. Activation of dopamine (DA) receptors via the direct DA agonist apomorphine increases acoustic and tactile startle (Davis and Aghajanian, 1976; Geyer et al., 1977; Kehne and Sorenson, 1978). The excitatory effects of apomorphine can be blocked by the DA antagonists haloperidol (Davis and Aghajanian, 1976) or pimozide (Kehne and Sorenson, 1978).

Surprisingly, the α_1-adrenergic antagonist phenoxybenzamine also blocked apomorphine excitation of acoustic startle (Kehne and Sorenson, 1978). However, because of the possible nonselective effects of phenoxybenzamine the present study was designed to determine whether other α_1-adrenergic antagonists would also block apomorphine enhanced startle.

Groups of 5 rats each were pretested for acoustic startle and then 1 day later pretreated with a given drug or its vehicle and 15-30 min later injected with apomorphine or water. Startle response amplitude was monitored for 30 min beginning immediately after the second injection. Two days later these procedures were repeated except that rats previously injected with apomorphine now were injected with water and vice versa.

The α_1-adrenergic antagonists WB-4101 or prazosin potently attenuated the normal excitatory effect of 3.0 mg/kg apomorphine, i.p. on startle. The ED_{50} dose of prazosin was 30 µg/kg, i.p. In contrast, much higher doses of prazosin (250 or 1000 µg/kg) only slightly decreased the excitatory effects of the glycine antagonist strychnine or the serotonin agonist 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) on startle. The β-adrenergic antagonist propranolol and the mixed α_1, α_2-adrenergic antagonist phentolamine failed to block apomorphine. The α_2-adrenergic antagonists yohimbine and piperoxane markedly potentiated the excitatory effects of apomorphine.

Taken together the data suggest that DA and norepinephrine may act in concert to enhance acoustic startle. Curiously, however, drugs that decrease central noradrenergic transmission by other mechanisms (clonidine, 6-OHDA) failed to affect apomorphine excitation. Moreover, WB-4101 and prazosin do not the block excitatory effects of d-amphetamine on startle. Changes in the disposition of apomorphine after α_1-adrenergic drugs and/or the participation of peripheral adrenergic catecholamines in modulating the excitatory effects of apomorphine are currently being investigated.

36.11 ANALYSIS OF STEREOTYPED BEHAVIOR USING A COMPUTER SUPPORTED OBSERVATIONAL METHOD. Donna L. McCorkle, Mark H. Lewis, Alan A. Baumeister, Laura Staples*, and Richard B. Mailman. Biological Sciences Research Center, Neurobiology Curriculum and Depts. of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, NC 27514

Stereotyped behavior, while repetitive and invariant, actually involves a variety of differentiated response topographies. It has been customary in the pharmacology literature to assess stereotyped movements along the dimension of intensity, using rating scales, and more recently, with various automated devices. As these methods have a number of disadvantages, we have developed an observational method that employs an electronic data collecting device (Datamyte®, ElectroGeneral Corp., Minneapolis, MN) with TRS-80 Model II microcomputer hardware and software support. This permits us to quantify effects of drugs on multiple response topographies. The method allows for the generation of interval scale data and, hence, the application of powerful parametric statistics. Comparisons between the effects of thioridazine (an "atypical" antipsychotic drug) and mesoridazine (a major thioridazine metabolite) in attenuating apomorphine-induced stereotypy were used to illustrate the utility of the method. Whereas mesoridazine showed some attenuation of apomorphine-induced gnawing, thioridazine surprisingly potentiated this response when apomorphine was administered by the intraperitoneal route. In contrast, no difference was noted between the two compounds in their ability to antagonize stereotyped gnawing when apomorphine was administered subcutaneously. Other topographies analyzed for drug vs. metabolite differences included licking, sniffing, and locomotion. Effects of thioridazine and mesoridazine on the time course of apomorphine-induced behavior were also examined. The present method appears to be sensitive to effects of dose, time, as well as route of administration, and is easily adapted to a wide variety of behavioral models. As these data demonstrate, this represents an improvement over intensity rating scales.

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36.12 SELF-STIMULATION IN REGIONS OF CATECHOLAMINE HYPER OR HYPO INNERVATION PRODUCED BY NEONATAL 6-HYDROXYDOPAMINE. S. Kurumiya, T. Takeichi, M. Umemoto, T. Itakura, and M. E. Olds.* Psychol. Div., Osaka City Univ.; Dept. of Neurosurg., Wakayama Med. College; Calif. Inst. of Tech., Pasadena, CA 91125.

Damage was inflicted to the catecholamine (CA) systems on days 3 and 5 after birth and self-stimulation (SS) tests were given when the rats were 4-5 months old. Each rat pup received bilateral intraventricular injections of pargyline (P, 50 mg/kg, i.p.) followed by 6-hydroxydopamine (6-OHDA, total dose, 400 µg); or desmethylimipramine (DMI, 25 mg/kg, i.p.) followed by 6-OHDA. Control pups were given P plus CSF which contained ascorbic acid (1 mg/ml) or DMI plus CSF that contained ascorbic acid.

In the first experiment, the animals aged 150 days or older were implanted with monopolar electrodes (62 or 100 µm) aimed at the locus coeruleus. Each subject was given first a session of 10-12 h, and then five daily consecutive sessions of 2 h each in operant chambers in which depression of a lever led to the application of a brain stimulus of 60 Hz sine waves, 0.25 sec duration and current intensity selected to produce optimal responding. The test animals learned to SS almost immediately, at very high rates. The controls required several sessions, never reaching the rates of the test animals. Fluorescence visualization of CA in the dorsal pons at the site of SS showed massive loss of A6, A5, and A4 cell bodies, but not A1-A3 perikarya, and the presence of a dense network of CA fibers and terminals in test animals but not in controls. Levels of CA were reduced in tissue samples of frontal cortex, caudate n., hypothalamus, but levels of norepinephrine in the dorsal pons samples were elevated.

In Experiment 2, animals which had received DMI+6-OHDA or DMI+Vehicle were implanted with electrodes in the substantia nigra, or the ventral tegmentum, or the medial prefrontal cortex (MFC), or the n. accumbens. There was no difference in the percentage of SS sites obtained in the substantia nigra and ventral tegmentum of test and control animals, but the rates of SS were lower in the test animals. Fluorescence visualization of the CA in these two regions, at the tip of the probes, showed a near total loss of A9 cell bodies and a reduction of A10 cell bodies. Visualization of CA at the tip of the probes in the MFC showed a loss of CA input to the deep layers but not the shallow layers. The rates of SS in the MFC and the n. accumbens did not differ between test and control animals. In the test animals, the levels of dopamine (DA) in the cortex were 10% of control levels but the levels of NE did not differ from those in control animals.

These results demonstrate the presence of normal or enhanced SS behavior in regions where NE or DA perikarya were greatly reduced, or where the DA input was greatly reduced.

36.13 MICROWAVE RADIATION-INDUCED ALTERATIONS IN OPERANT BEHAVIOR OF RATS: A COMPARISON WITH d-AMPHETAMINE AND BARBITURATE. R.M. Lebovitz and J. Orr*. Dept. Physiology, University of Texas Health Science Center, Dallas, Texas 75235.

This laboratory has shown that microwave radiation (MWR) at macroscopically non-thermogenic dose rates produced a differential effect on ongoing schedule controlled and extinction components of a repeated multiple schedule in rats. It would be useful to have a behaviorally similar pharmacologic model with which to compare the results of acute exposure to MWR.

Male, hooded (Long-Evans) rats weighing from 250-350 g were trained and maintained on a multiple-schedule of fixed-ratio (FR) 25 for 15 minutes alternated with ten minute time-out (TO) periods. The two components were distinguished by the presence or absence of non-contingent food cup illumination. This basic 25 minute multiple schedule was repeated five times producing a continuous operant session duration of 120 minutes. The animals were irradiated (actual or sham) coincident with their operant performance. Each animal received a 6-8 g daily food supplement and was run once per day, five days per week, in its active (dark) phase. Operant trials were carried out in cylindrical irradiation cells under computerized behavioral control, with groups of 32 (16 sham, 16 irradiated) animals run simultaneously. Otherwise, animals were individually housed with water available ad lib.

Exposure to pulse modulated (PM) or to continuous wave (CW) MWR at 3.5 mW/g yielded essentially no change in performance during the successive FR components of the repeated multiple schedule. TO response rates were reduced and the rate of extinction increased. Separate experiments had verified that at this dose rate PM or CW MWR did not significantly elevate whole body temperature during a three hour exposure. IP injection of d-amphetamine sulphate (in saline, with saline controls) yielded a marked decrease in FR response rates with dose rates above 0.5 mg/kg, coupled with a dispersion of the inter-response time histograms. TO response rates were essentially unaffected by d-amphetamine at doses of from 0.25 to 2.0 mg/kg. In contrast, IP injection of Na-pentobarbital (at 0.5 and 1.0 mg/kg, in saline with saline controls) yielded a marked reduction in TO response rates. FR responding was either not affected or slightly elevated, similar to the result with MWR at dose rates near behavioral threshold. Thus, while d-amphetamine reduced high, FR operant response rates, it had little effect on the low rates of TO responding. The result of low doses of pentobarbital was to produce a differential effect on FR and TO response rates of rats that approximated that of non-thermogenic MWR.

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36.14 STRIATAL PHARMACOLOGY OF AN ANIMAL MODEL RESEMBLING HUNTINGTON'S DISEASE (HD). B.I. Diamond, C. Shah*, A. Hitri and R.L. Borison. Psychiatry Dept., Medical College of Georgia, Augusta, GA 30912.

We have previously demonstrated that the one week daily i.p. administration of β,β-iminodipropionitrile (IDPN) to animals results in irreversible spontaneous and unprovoked choreatic head and truncal movements that resemble HD. Furthermore, we demonstrated that this model shows neuroanatomical changes consistent with striatal degeneration, and biochemical changes including increased striatal dopamine release and the reduction of high and low affinity striatal ^{3}H-spiroperidol binding sites; these changes parallel those found clinically in HD. We now discuss our further experiments, including the striatal pharmacology of the IDPN animal model. All subjects were white male Sprague-Dawley rats (200-250g). We first attempted to replicate our animal model via the direct daily injection of IDPN stereotactically into the caudate-putamen nucleus. This treatment failed to affect behavior, implicating a peripherally formed metabolite of IDPN as being responsible for central neurotoxicity. In pharmacological studies, spontaneous stereotyped behavior and locomotor activity were quantified before and after acute drug administration. The cholinesterase inhibitor physostigmine at low doses (0.25mg/kg) failed to affect behavior, but at higher doses (0.5mg/kg) decreased stereotypy without affecting locomotion. The anticholinergic agents benztropine (0.5mg/kg) or trihexyphenidyl (0.5mg/kg) significantly increased all behavioral activities. At low doses (0.05mg/kg) the dopamine agonist apomorphine reduced stereotypy, however at higher doses (0.7mg/kg) there was an increase in behavioral activity. Likewise, the indirect dopamine agonist, d-amphetamine, in a dose per se subthreshold for producing stereotypy, potentiated both stereotypy and locomotion. In contrast, typical dopamine receptor blocking neuroleptics, haloperidol (0.5mg/kg) or trifluoperazine (1.2mg/kg) reduced all behaviors. Atypical dopamine blockers (viz., those with few extrapyramidal actions,) but with intrinsic anticholinergic activity, thioridazine, clozapine, fluperlapine, actually potentiated behavior in 30%, 66% and 66% of animals respectively. An atypical neuroleptic with very low anticholinergic activity, molindone (1.2mg/kg) failed to affect behavior. These studies therefore demonstrate that the IDPN animal model mimics the clinical pharmacology of HD.

36.15 SOME EFFECTS OF NEUROLEPTICS ON OPERANT AND REFLEXIVE LICKING IN RATS. S.E. Gramling* and S.C. Fowler. Dept. of Psych., Univ. of Miss., University, MS 38677

The rate reducing effects of neuroleptics on operant behavior have been variously attributed to motoric, dissociative and anhedonic (reward-reducing) processes. Heretofore, it has proved impossible to separate clearly the degree of involvement of a given process in a given experimental effect. The present research proposes the rats' licking behavior as a model system for separating neuroleptics' motor effects from these other putative effects. In this procedure the motor requirements of the response are equated by requiring a lick response in either an operant or reflexive lick condition; thus it should be possible to observe a neuroleptic's effect on reflexive licking and then use this result to estimate how much of the neuroleptics' effect on operant licking is the result of motor effects per se.

Rats in the operant lick condition (N=9) were trained to lick a dry horizontal disk (FR 15) for water reinforcement delivered elsewhere in the chamber. Rats in the reflexive condition (N=7) licked water from a reservoir. The surface of the disk and the reservoir water level were both 10mm below the aperture in the chamber through which rats extended their tongues to lick. Three neuroleptics, Chlorpromazine (CPZ:0.5, 1.0, 2.0 mg/kg, ip.) Clozapine (CLOZ: 2.5, 5.0, 7.5 mg/kg, ip.) and Haloperidol (HAL: .06, .12, .24 mg/kg, ip.) were administered in an acute dosing regime. Lick rate and lick duration data were collected via a laboratory computer system.

Rats in both lick conditions exhibited dose dependent reductions in average rate for all three neuroleptics. Moreover, operant licking showed greater rate reductions than reflexive licking for each of these drugs. On the measure of lick duration, these three neuroleptics produced dose dependent increases in lick duration in the reflexive lick condition. Neuroleptics' effects on operant lick duration were mixed with some animals showing increases and some decreases on this measure.

The larger decrease in rate exhibited by the operant lickers compared to the reflexive lickers cannot be attributed to a motoric effect since the kinetic requirements for the response were the same for both groups. Thus, the explicit operant contingency, or the "conditionedness as opposed to motoricity" of the response seems to be an important determinant of neuroleptics' effects on behavior. Since any response requirement necessitates a motor process for its expression the interpretation that a neuroleptic is acting by attenuating some motor process can never be completely dismissed. Nevertheless, the lick model may prove a useful and sensitive tool in assessing the degree to which motoric effects may be separated from dissociative and anhedonic effects of neuroleptics.

36.16 EFFECTS OF PIMOZIDE ON APPETITIVE BEHAVIOR AND LOCOMOTOR ACTIVITY: A COMPLEX REWARD AND PERFORMANCE INTERACTION. Spivak,K*, Blair,R*, & Amit, Z. (Spon: P. Braun). Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd, Rm. H1013, Montreal, Que., Canada, H3G1M7.

Behavioral effects of pimozide, a dopamine receptor blocker were examined in a runway paradigm using food reward and in an open-field. Male rats were injected with one of 3 doses of pimozide (0.25 (P1), 0.5 (P2), 1.0mg/kg (P3)), vehicle (V1 or V2) or Ringer's (R) 4hr prior to testing in the runway (pretreatment). Two groups received pimozide (1.0mg/kg (PP)) or vehicle (PV) 1hr after the test trial in the home cage (postreatment). A no reward group (NR) received no food reward on test days. There was one test trial per day for 12 consecutive days. The running behavior of each animal was examined as a function of the individual measures comprising total running time performance (latency to leave start box, run time down the alley and latency to enter goalbox). There were no significant differences between groups P3 and NR in total running time performance and in run time down the alley nor between P3 and its respective control group. Groups P2 and P3 however, showed larger latencies to leave the start box in comparison to the NR and control groups. The behavioral effect was reversed at the entry to the goalbox. The NR group showed the largest latency to enter the goalbox across test days in comparison to P1, P2, P3, PP and control groups. The posttreated pimozide group (PP) showed reductions in saccharin-flavored food consumption over days indicative of a conditioned taste aversion (CTA). Group P1 demonstrated a similar pattern of decline in food consumption. The day following the runway testing phase, all animals were given their appropriate treatment condition and placed in open field chambers where activity levels were measured. Groups P1, P2, and P3 exhibited significantly lower activity counts than the NR and control groups. The results from this study using these paradigms indicate that the behavioral effects observed in pimozide treated rats are not equivalent to the behavioral effects demonstrated by rats receiving no reward.

36.17 ALTERATIONS OF APOMORPHINE INDUCED STEREOTYPED REARING BY HYPERBARIC AIR, J. P. Johnston and R. B. Philp* The Department of Pharmacology and Toxicology, The University of Western Ontario, London, Canada.

The effect of high partial pressures of nitrogen on neurochemical mechanisms is largely unknown. In order to study the effect of hyperbaric air on the function of dopaminergic systems, apomorphine induced rearing in mice, combined with HPLC analysis of its distribution was used. Male Swiss mice, acclimatized to the experimental conditions were injected subcutaneously with either apomorphine or saline. The mice were then subjected to either 1 ata, 4 ata, or 7 ata of air or an 80/20 Helium/Oxygen mixture. The behaviour of each animal was evaluated using a rating scale devised by Fray et al (1980). In a similiar experiment, mice were sacrificed at various time intervals and brain and plasma samples were analyzed by HPLC for apomorphine levels. Pressure had no significant effect on the onset of rearing. At the lowest dose (5.0 mg/kg), neither the onset nor the peak frequency of rearing was affected by pressure. There was, however, a significant increase in the duration of rearing activity at 4 ata air. On the other hand, high doses of apomorphine (50.0 mg/kg) showed no changes in the onset or duration of the rearing response, although the peak frequency at 7 ata was significantly less than at other pressures. No changes in the rearing response were observed with the He-O_2 gas mixture at all pressures tested. Analysis of brain and plasma samples showed that no change in the distribution of apomorphine occurs under pressure. At 60 minutes following injection of the high dose, apomorphine levels in plasma and brain were 210.99 ± 30.8 ng/ml and 2.20 ± 0.235 ug/g respectively. In animals receiving the same dose but subjected to 7 ata air, brain and plasma levels were 201.47 ± 36.27 and 2.08 ± 0.356 ug/g respectively. These results suggest that the observed changes in apomorphine induced stereotyped rearing under hyperbaric air are due to a central mechanism rather than to changes in central apomorphine distribution. Supported by The Defence and Civil Institute of Environmental Medicine, Downsview, Ontario, Canada.

P.J. Fray, B.J. Sahakian, T.W. Robbins, G.F. Koob, and S.D. Iversen, Psychopharmacology 69, 253-259 (1980)

36.18 DETERMINATION OF JERKER-MICE SPONTANEOUS ROTATION AND MEASUREMENT OF BEHAVIORAL EFFECTS OF NEUROLEPTICS. C. Hofer*, H. Utena* and M. Sakuma*. Dept. of Psychiatry, Wayne State University, 951 E. Lafayette, Detroit, MI 48207 (Spon: Robert Vertes, Ph.D.)

This study investigated rotation of jerker mice, a behavior controlled by a single gene (X11) and characterized by a strong compulsive circling locomotion of quite long duration. The specific stability of jerker-rotation makes this behavior amenable to instrumental measuring of variations. Spontaneous rotation appeared with greater frequency than the other stereotyped behaviors such as head tossing and was found to peak at midnight during a diurnal cycle (Experiment 1). The time-course change of jerker rotation indicated an increase of distance, duration and total amount of movement under conditions of amphetamine, chlorpromazine and perphenazine, whereas velocity decreased over time in the diazepam condition only, with the decreasing velocity peaking at 30 minutes following injection of diazepam. The log-effects of those drugs reveal the different clinical profiles of each drug. The stimulant (amphetamine) and the anti-psychotics (chlorpromazine, perphenazine) appear to produce jerker rotation, while diazepam appears to be disruptive (Experiment 2). To account for the data on amphetamine and diazepam it has been suggested that the dopinergic system may be involved in the spontaneous rotation, but contrariwise results of major tranquilizers suggest a specificity of jerker rotation as a phenotype. Since it is doubtful that the jerker mouse has a genotypic auditory disturbance, the discrepancy of the results may indicate that the sensory motor system is involved in some way in the interaction between serotonergic and dopinergic systems.

36.19 ASCORBIC ACID POTENTIATES THE BEHAVIORAL RESPONSE TO HALOPERIDOL BUT NOT TO CLOZAPINE. George V. Rebec, Laura K. White* and Kevin D. Alloway. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

We have previously shown that ascorbic acid (AA) crosses the blood-brain barrier and accelerates neuronal activity in the neostriatum of the rat (Ewing, A.G. et al., Brain Res., 261:101, 1983). Haloperidol, a dopamine (DA) receptor antagonist, produces comparable effects (Rebec, G.V. et al., Neuropharmacology, 19:281, 1980). In fact, an accumulating body of evidence suggests that AA may modulate DA transmission by interacting with the DA receptor (Heikkila, R.E. et al., Res. Comm. Chem. Pathol. Pharmacol., 34:409, 1981). If this is the case, then pretreatment with AA may potentiate the behavioral response to haloperidol and possibly other DA antagonists. To test this hypothesis, we examined the ability of AA to alter haloperidol-induced catalepsy, a presumed index of DA receptor blockade.

Adult, male rats received ip injections of either saline or 1000 mg/kg AA (pH adjusted to 7.0); 10 min later the animals received sc injections of either saline or 0.5 mg/kg haloperidol. Catalepsy was measured at 15 and 60 min after the last injection. Although AA alone produced no evidence of catalepsy, this compound significantly enhanced the cataleptic behavior produced by haloperidol at both the 15- and 60-min test times. It is conceivable, therefore, that AA interacts with the DA receptor to potentiate the action of haloperidol. This effect may not be generalizable to all DA antagonists, however, since AA failed to enhance the behavioral response to clozapine (10.0 mg/kg), a relatively weak cataleptogenic agent.

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36.20 AMPHETAMINE-INDUCED REGRESSION TO AKINESIA: CHANGES IN POSTURE & LOCOMOTION OPPOSITE TO THOSE IN RECOVERY FROM LATERAL HYPOTHALAMIC DAMAGE. S. Servidio, D. Alander*, T. Schallert, and P. Teitelbaum. Dept. Psychology, Ohio State Univ., Columbus, OH; Univ. Ill. Med. School, Rockford, IL; Dept. Psychology, Univ. Texas, Austin, TX; and Dept. Psychology, Univ. Ill., Urbana-Champaign, IL.

During recovery from lateral hypothalamic akinesia, movement first recovers in the lateral plane, followed by longitudinal movements. Verticle head scans and rearing are the last to recover. Recovery of locomotion following hypothalamic damage entails the gradual recruitment of more caudal body segments, as well as an increase in the amplitude of movements within a given movement dimension (lateral, verticle, eg.).

Rats injected with d-amphetamine sulfate (20 mg/kg, ip) regress through a sequence of motor behaviors opposite to what is seen during recovery from hypothalamic akinesia. This regression is characterized by a sequential reduction of movement along geometric dimensions, as well as by an overall decrease in the amplitude of movements within a given dimension. Thus, movements in the verticle plane (verticle head scans, rearing) are first seen, but are gradually replaced by longitudinal trajectories, consisting of forward locomotion with the head held in line with the body. Later, there is a decrease in movements in the longitudinal plane, as large lateral head scans emerge, often culminating in rotation and/or pivoting (pivoting involves lateral movement with no forward locomotion, while rotation entails both lateral and forward movement). Finally, as the amplitude of lateral movements decreases, the snout becomes fixed to the floor and the animal becomes virtually akinetic. Particular aspects of the environment (walls, corners) can redirect movements in any geometric plane.

In addition, rats given amphetamine drown when placed in a tank of water. This drowning can readily be attributed to the regression to akinesia. Indeed, the identical sequence of events observed in the open field is also observed in water; verticle movements, including raising the head and snout from the water, are replaced by longitudinal and finally by lateral movements (circling in water with the entire body held below the water level).

Amphetamine-induced motor behaviors have classically been described as "random", "non-directed", and "haphazard" stereotypies. The present analysis, however, indicates that changes in posture and movement induced by amphetamine reflect the systematic breakdown of movements in geometric planes, until finally, a state of akinesia is attained.

36.21 DOPAMINE RECEPTOR AGONIST PROPERTIES OF BROMOCRIPTINE. O.F.Jenkins* and D.M.Jackson. Dept. of Pharmacology, Univ. of Sydney, NSW 2006, Australia.

Bromocriptine (BRC) dose-dependently (5-20 mg/kg, IP) produced long lasting (up to 8 hr) locomotor stimulation in mice and stereotyped behaviour in rats. In mice, the locomotor stimulation was preceded by a period of depression lasting 60 min after injection. Low doses of apomorphine (<0.5 mg/kg, IP) selective for dopamine (DA) autoreceptors were able to enhance the BRC-induced depression of activity. The BRC-induced depression was not due to the blockade of postsynaptic DA receptors since, in mice pretreated with reserpine and alpha-methyl-p-tyrosine (AMPT), BRC had no effect on the locomotor stimulation induced by postsynaptic DA receptor active doses of apomorphine (>0.5 mg/kg, IP) at a time when the depressant effect of BRC was maximal (15-75 min post-BRC). Pretreatment with AMPT alone was without effect on the BRC-induced depression. The stimulant effect of BRC on locomotor activity in mice and stereotyped behaviour in rats was abolished by prior treatment with reserpine and AMPT. This is direct evidence that BRC does not exert its behavioural effects by direct stimulation of postsynaptic DA receptors. However, BRC was able to dose-dependently enhance the stimulant effect of apomorphine in both mice and rats after reserpine and AMPT treatment, at a time when the stimulant effect of BRC was maximal in naive animals (3-5h). (BRC also enhanced the effect of apomorphine in non-pretreated rats). Thus, while BRC appears to have some intrinsic activity on the postsynaptic DA receptors, concomitant stimulation by another agonist may be required for production of behavioural effects. Blockade of DA synthesis by pretreatment with AMPT alone also completely blocked BRC-induced locomotor stimulation in mice. This could be reinstated by administration of a low (behaviourally inactive) dose of L-DOPA (50 mg/kg, IP) which has been shown to restore normal intraneuronal DA stores (Ahlenius, S., Anden, N.-E., Engel, J., Brain Res., 62:189, 1973). This suggests that BRC may, at least partly, induce or require the release of DA from nerve terminals. Thus it appears that BRC may act by a combination of pre- and postsynaptic mechanisms which have yet to be fully elucidated. In any case, BRC cannot be considered a DA receptor agonist in the generally accepted sense of an apomorphine-like compound.

37.1 MECHANISMS INVOLVED IN THE SEX DIFFERENCE OBSERVED IN THE SEROTONIN BEHAVIORAL SYNDROME. C.T. Fischette, K. Renner* and B.S. McEwen. The Rockefeller University, New York, NY 10021.

The hormonal environment determines the response of male and female rats to a standard dose of pargyline (50mg/kg) and tryptophan (50mg/kg) which produces the "serotonin behavioral syndrome" (Fischette et al., Soc. of Neurosci. 1982). The sex difference observed in the frequency of animals exhibiting this syndrome could be due to differences in: (a) serotonin receptors (number or affinity within a particular region); (b) the ability of each sex to synthesize serotonin; (c) uptake of precursor or release, reuptake or catabolism of serotonin; or (d) drug metabolism. We have begun a series of experiments to differentiate between these possibilities. One approach is to examine the response of male and female rats to a direct serotonin receptor agonist (5 methoxy N,N dimethyltryptamine), thus bypassing the synthetic and metabolic pathways that are necessary steps in producing the syndrome using pargyline and tryptophan. We report that a sex difference in the frequency of animals exhibiting this syndrome occurs at the threshold dose (1 mg/kg). However, at all doses tested (0.5mg/kg - 5.0mg/kg), females displayed a greater intensity of each symptom than males. This suggests that there is a sex difference at the receptor level, although differences in drug availability and metabolism remain a possibility. Another approach is to examine the elevation in brain and spinal cord amine levels in males and females using pargyline and tryptophan. In spinal lumbar regions, for example, serotonin levels are similarly elevated in both sexes after drug treatment; norepinephrine and dopamine levels are also somewhat elevated. Thus, the role of other amines besides serotonin in the behavioral syndrome must also be investigated, even though a sex difference in sensitivity to serotonin appears to remain a central factor.

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37.2 A COMPARISON OF MIANSERIN AND METHYSERGIDE AS ANTAGONISTS OF THE EFFECT OF LYSERGIC ACID DIETHYLAMIDE ON FIXED RATIO BEHAVIOR IN RATS. L. P. Dwoskin* and S. B. Sparber*. (SPON: J. H. O'Brien). Department of Pharmacology, Medical School, University of Minnesota, Minneapolis, MN 55455.

In a previous study low doses of mianserin (0.1-1.0 mg/kg, ip), which did not alter fixed ratio (FR) behavior in rats, were found to completely block the behavioral effect of a low dose of lysergic acid diethylamide (LSD; 50 μg/kg, ip), a serotonin (5-HT) agonist (JPET 225, 77, 1983). The behavioral effect of higher doses of LSD (100 or 200 μg/kg) was antagonized, but not completely blocked by the behaviorally inactive doses of mianserin tested. The next higher dose (10 mg/kg) of mianserin was behaviorally active and produced a lesser antagonism of the behavioral effect of LSD.

In the present study, the capacity of mianserin (1.0-5.0 mg/kg) to more effectively block both response suppression and/or disruption produced by 100 μg of LSD/kg was examined, and compared to the antagonism produced by methysergide (0.1-35 mg/kg), a so-called classical 5-HT antagonist. Behaviorally inactive doses of mianserin (1.0-2.5 mg/kg) completely antagonized the behavioral effect of 100 μg of LSD/kg, such that responding was indistinguishable from control. The results support the hypothesis that mianserin, at doses which are behaviorally inactive, acts as a pharmacologic antagonist at 5-HT receptors stimulated by LSD. Low doses of methysergide (1.0-2.5 mg/kg) alone were found to be behavorally active; the rats earned a greater number of reinforcers (120% of control). A higher dose of methysergide (35 mg/kg) produced a suppression of responding and a decrease in the number of reinforcers earned. Behaviorally inactive and active doses of methysergide were able to only partially antagonize the behavioral effect of LSD. The dose of methysergide (35 mg/kg), which alone produced a significant behavioral suppression, antagonized the LSD-induced disruption of responding without antagonizing the LSD-induced suppression of responding. Therefore, the results indicate that mianserin is a much more effective antagonist of the behavioral effect produced by LSD than is methysergide, since mianserin was able to completely antagonize both effects of this dose of LSD on operant behavior. Additionally, the data suggest that there may be at least 2 separate mechanisms whereby LSD affects schedule-controlled responding. Methysergide, in some selective manner, may block the disruptive effect of LSD while producing a minimal or physiological antagonism of the LSD-induced rate-suppression.

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37.3 TOLERANCE AND REVERSE TOLERANCE TO LYSERGIC ACID DIETHYLAMIDE. P.B. Silverman. Neuropsychopharmacology Section, Texas Research Institute of Mental Sciences, Houston, Texas 77030.

Rats lesioned unilaterally along the nigrostriatal pathway exhibit rotational (circling) behavior in response to administration of dopamine agonists. In this paradigm, lysergic acid diethylamide (LSD) acts like a direct dopamine receptor agonist, inducing rotation contralateral to the lesioned side (Pieri et al, Nature 252, 586, 1974). While tolerance to LSD has been reported in a wide variety of behavioral tests, and tolerance to the LSD hallucinatory experience is rapid and widely reported, rotational behavior in response to LSD administration was reportedly unchanged by daily administration. We previously reported that a single small dose of apomorphine or LSD induced a permanent, environment specific, behavioral effect in 6-hydroxydopamine lesioned rats (Silverman and Ho, Nature 294, 475, 1981). Perhaps, then, unchanged response to daily LSD treatment was a result of two phenomena, the usual rapid tolerance to LSD and a contradictory long-term sensitization resulting from prior LSD administration. This possibility was tested by varying the interval between successive drug administrations.

Rats were anesthetized with pentobarbital and lesioned along the nigrostriatal pathway by stereotaxic microinjection of 6-hydroxydopamine HBr. At least two weeks passed prior to behavioral testing. Rotational behavior was tested in clear plastic hemispherical bowls and was counted by an observer.

When observed in the bowls after daily administration of LSD, rotation was found to be highly variable with no clear tolerance development. When tested in the bowls before and after two LSD treatments in the home cage, significant tolerance was found. Rats tested with LSD at two week intervals exhibited a remarkable increase in response to successive treatments. Rotations doubled on the second treatment compared with the first. When given a third treatment, explosively rapid rotation, hyperthermia, and death resulted. Controls showed that the increased response to LSD was not a result of the passage of time from lesion to test.

The results show that response to repeated LSD in a "dopaminergic" behavioral paradigm is a function of context and timing.

37.4 BLOCKADE OF 5-HTP INDUCED ANIMAL MODEL OF DEPRESSION WITH A POTENT AND SELECTIVE 5-HT_2 RECEPTOR ANTAGONIST (LY53857). J.N. Hingtgen*, Ray W. Fuller[1] and M.H. Aprison, Inst. of Psychiat. Research and Depts. of Psychiat. and Biochem., Indiana Univ. Sch. of Med., Indianapolis, IN 46223 and Lilly Research Laboratories,[1] Eli Lilly and Company, Indianapolis, IN 46285.

Some types of human depression may be related to an increased release of free serotonin (5-HT) acting at hypersensitive postsynaptic receptors (Aprison et al., In: Neuropharmacology and Behavior, Plenum, 1978; Aprison et al., In: New Vistas in Depression, Pergamon, 1982; Hingtgen and Aprison, Behav. Brain Sci. 5:108, 1982). Support for this hypothesis comes from our previous data indicating that acute pretreatment with clinical doses of the antidepressants, mianserin, amitriptyline, imipramine and iprindole resulted in varying degrees of blockade of D,L-5-hydroxytryptophan (5-HTP; 50 mg/kg I.P.) induced depression in rats working on an operant schedule for milk reinforcement. Additional studies with acute and chronic administration of amitriptyline, mianserin and trazodone have shown similar blockade of 5-HTP induced depression. To distinguish between presynaptic and postsynaptic events, these drug effects were compared to those of fluoxetine, a known specific uptake blocker of serotonin (5-HT), which potentiates a lower dose of 5-HTP and to methysergide, a postsynaptic blocker of 5-HT, which almost completely abolished the depressive effect of 5-HTP. Using the same animal model of depression, we studied the effects of a potent and selective 5-HT_2 receptor antagonist, LY53857 [4-isopropyl-7-methyl-9(2-hydroxy-1-methyl-propoxycarbonyl)4,6,6A,7,8,9,10,10A-oct-ahydroindolo(4,3-FG)quinoline maleate; Fuller and Snoddy, Endocrin. 105:923, 1979]. In doses as high as 5.0 mg/kg, LY53857 had no effect on the baseline performance of rats working on a VI 1 schedule. When LY53857 was given 60 min prior to 50 mg/kg D,L-5-HTP it blocked (90%) 5-HTP depression in doses as low as 0.1 mg/kg (I.P.). At a dose of 0.025 mg/kg, pretreatment with LY53857 resulted in less than a 50% blockade of 5-HTP induced depression. These results suggest that the 5-HTP depression is mediated by serotonergic mechanisms involving 5-HT_2 receptors, since LY53857 is a selective antagonist of these receptors. These data also support the suggestion that some antidepressants are acting on 5-HT_2 receptors in our model of depression. Recent data from two other research groups (Takahashi et al. and Segawa et al. In: New Vistas in Depression, Pergamon, 1982) strengthen this hypothesis. These latter studies as well as our data support our hypersensitive postsynaptic serotonergic receptor theory of depression and suggest that LY53857 could be of interest as a possible antidepressant agent of the general type proposed by Aprison and Hingtgen (In: Serotonin, Plenum, 1981).

37.5 **DIFFERENTIAL ACTIONS OF SEROTONIN RECEPTOR ANTAGONISTS ON THE HEAD SHAKE RESPONSE AND THE SEROTONIN SYNDROME IN RATS.** I. Lucki, M. Nobler* and A. Frazer. Depts. of Psychiatry and Pharmacology, University of Pennsylvania, & Veterans Administration Hospital, Phila., PA 19104.

Ligand binding studies have identified certain serotonin (5HT) receptor antagonists with selective affinity for 5HT-2 receptors, such as ketanserin or pipamperone, and other 5HT receptor antagonists with affinity for both 5HT-1 and 5HT-2 receptor sites, such as metergoline or methysergide (Leysen et al., Life Sci., 1981, 28:1015). This study compared the actions of these four 5HT receptor antagonists on two different behavioral responses in rats that are produced by 5HT receptor activation: 1) the head shake response; and 2) the serotonin syndrome.

The head shake response was produced by the administration of carbidopa (102 uMoles/kg, i.p.) followed 30 min later by 5-hydroxy-L-tryptophan (5HTP; 681 uMoles/kg, s.c.). Rats were observed for a 5 min period starting 90 min after the 5HTP injection. Pretreatment with all four 5HT receptor antagonists administered intraperitoneally 60 min prior to observation blocked the head shake response. The ID50s calculated for each antagonist were: metergoline (.10 uMoles/kg), ketanserin (.77), pipamperone (1.14), and methysergide (1.81). All four 5HT receptor antagonists also blocked the head shake response produced by the 5HT agonists, quipazine (12 uMoles/kg, i.p.).

All of the symptoms of the serotonin syndrome were produced by 5-methoxy-N,N-dimethyltryptamine (13.7 uMoles/kg, i.p.). Pretreatment with metergoline (2.5 uMoles/kg) and methysergide (18.9 uMoles/kg) blocked the appearance of the syndrome. In contrast, pretreatment with ketanserin and pipamperone (51.4 and 53.3 uMoles/kg, respectively) failed to block any of the symptoms of the serotonin syndrome.

The differential actions of 5HT receptor antagonists on the head shake response and the serotonin syndrome suggest that different 5HT receptors are involved in these two behavioral responses. The order of potency for the 5HT antagonists to block the head shake response agrees with the order of their potency to displace 3H-spiroperidol binding in rat frontal cortex, a measure of affinity for the 5HT-2 receptor (Leysen et al., 1981). In contrast, the ability of 5HT receptor antagonists that potently displace 3H-serotonin binding to block the serotonin syndrome, combined with the inability of selective 5HT-2 receptor antagonists to block the syndrome, suggests that the serotonin syndrome involves the activation of 5HT-1 receptors.

This research was supported by USPHS grants MH-36262, MH-29094, and funds from the Veterans Administration.

37.6 THE EFFECT OF METERGOLINE ON HABENULAR SELF-STIMULATION. Shinshu Nakajima. Dept. of Psychology, Dalhousie Univ., Halifax, Nova Scotia, Canada, B3H 4J1.

The habenular nuclei along with the stria medullaris and fasciculus retroflexus constitute an epithalamic pathway connecting the limbic forebrain with the limbic midbrain. This pathway is similar in many respects to the hypothalamic pathway, the medial forebrain bundle. As in the hypothalamus, electrical stimulation of the structures in the epithalamus gives rise to a rewarding effect. Since lessions in the median raphe suppresses habenular self-stimulation, a question was asked whether the rewarding effect of habenular stimulation depends on serotonergic transmission.

Rats were implanted with bipolar electrodes into the habenular complex or the lateral hypothalamic area, or both, and then trained to respond by making a contact with an empty drinking spout. Habenular self-stimulation was completely suppressed by metergoline (5 mg/kg, i.p.) for a period of 10-30 min in all animals, but hypothalamic self-stimulation was only a little influenced by the same dosage of the drug. The differential effect was particularly clear in those animals that had both habenular and hypothalamic electrodes: the same animals making the same response demonstrated different degrees of suppression depending on which electrode was connected to a stimulator. The difference was statistically significant. Chlorpromazine (2 mg/kg, i.p.) suppressed habenular and hypothalamic self-stimulation in an equal fashion; physiological saline had no effect. The suppression of habenular self-stimulation by metergoline cannot be attributed to perceptual or motoric disturbance, or to a change in arousal level.

The results suggest that habenular stimulation becomes no longer rewarding when serotonin receptors are blocked by metergoline. One possibility is that habenular stimulation produces a rewarding effect by exciting the serotonergic neurons originating from the raphe and projecting to the hypothalamus where catecholamines make important contributions. Another possibility is that there is a separate system of structures outside the medial forebrain bundle that is capable of generating a rewarding effect, and that serotonin plays a more important role in that system than catecholamines.

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37.7 ESTRADIOL AND PROGESTERONE INFLUENCE L-5HYDROXYTRYPTOPHAN-INDUCED MYOCLONUS IN GUINEA PIGS: SEX DIFFERENCES IN SEROTONIN-STEROID INTERACTIONS. L.H. O'Connor*and H.H. Feder*(SPON: I. Zucker). Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

L-5-Hydroxytryptophan (L-5HTP) induces a myoclonic behavior syndrome in guinea pigs characterized by rhythmic jerks of the head and body. This behavior is caused by intense central serotonergic activity. We evaluated effects of estradiol and progesterone on L-5HTP-induced myoclonus in male and female guinea pigs. Myoclonic behavior was assessed by measures of total number of myoclonic jumps over all observations and maximum number of myoclonic jumps per min. Effects of estradiol and progesterone on L-5HTP-induced myoclonus were examined using the same relatively low doses of the steroids used for activation of species-typical female sexual behavior. All animals were gonadectomized. In the absence of steroids, myoclonus was higher in males than females. Estradiol alone and sequential treatment with estradiol and progesterone altered myoclonus in both sexes.

Estradiol. When 3.5ug estradiol benzoate was injected 46 hours prior to L-5HTP (100mg/kg), myoclonus was enhanced in females but not in males. However, a higher dose of estradiol (10ug) injected 46 hours prior to a lower dose of L-5HTP (80mg/kg) enhanced myoclonus in males. Therefore, estradiol has similar effects on myoclonus in males and females although there are sex differences in sensitivity to L-5HTP and perhaps also to estradiol.

Progesterone. We found sex differences in effects of progesterone on myoclonus. In females, effects of progesterone depended on time since progesterone injection and pretreatment with estradiol. When progesterone (0.5mg) was given 6 hours before L-5HTP (100 or 125 mg/kg) in females, the facilitative effects of estradiol (3.5ug) were reversed. Progesterone given 6 hours before L-5HTP did not alter myoclonus unless females were primed with estradiol. The inhibitory effects of progesterone on myoclonus in estradiol-primed females followed the same time course as the facilitative effects of progesterone on sexual receptivity. When L-5HTP was given just prior to or after the period of sexual receptivity induced by progesterone in estradiol-primed females, there was no inhibitory effect of progesterone on myoclonus. But when L-5HTP was given during the period of sexual receptivity, then myoclonus was suppressed compared to females treated with estradiol alone. This temporal correlation suggests the progestin receptor mechanisms proposed to mediate progesterone's effects on sexual behavior are also involved in the inhibitory effects of progesterone on myoclonus in females. In contrast to females, progesterone (0.5mg) given 6 hours before L-5HTP (80mg/kg) enhanced myoclonus in estradiol-primed males. Therefore, progesterone has opposite effects on myoclonus in estradiol-primed males and females.

37.8 INSULIN INTENSIFIES THE DISCRIMINATIVE STIMULUS EFFECTS OF LSD AND SOME RELATED SUBSTANCES. J. M. Hanlin* and J. B. Appel (SPON: J. R. Coleman). Behav. Pharmacol. Lab., Dept. of Psychol., Univ. of South Carolina, Columbia, SC 29208.

In rats trained to discriminate LSD (0.08 mg/kg i.p., 15 min before session) from saline in a two-lever operant chamber, insulin (5.0-6.0 U/kg i.p., 15-25 min before session) more than doubled the percentage of LSD-appropriate responding in tests with low doses of LSD (0.02 mg/kg), mescaline (8.0 mg/kg), and psilocybin (0.25 mg/kg). In addition, insulin induced smaller increases in drug-appropriate responding following low doses of the nonhallucinogenic LSD congener lisuride and two tetrahydro-ß-carbolines (THBC's); it did not, however, alter the effects of either psilocin or 5-methoxy-N,N-dimethyltryptamine, both of which are known to be highly lipid soluble (P. K. Gessner et al., Life Sci., 7: 267-277, 1968).

Insulin can increase brain levels of certain large neutral amino acids, primarily by lowering plasma levels of other large neutral amino acids that share and compete for the leucine-preferring brain transport system (J. D. Fernstrom and R. J. Wurtman, Science, 174:1023-1025, 1971; D. C. Markowitz and J. D. Fernstrom, Science, 197:1014-1015, 1977). In the present study, valine (100.0 mg/kg i.p., 30 min before session) prevented the insulin-induced potentiation of 0.02 mg/kg LSD. In rats trained to discriminate d-amphetamine (1.0 mg/kg i.p., 15 min before session) from saline, insulin (5.0 U/kg i.p., 15 min before session) increased amphetamine-appropriate responding following lisuride but did not increase the effects of d-amphetamine itself, which enters the brain through an active transport system that is not competitively inhibited by large neutral amino acids (W. M. Pardridge and J. D. Connor, Experientia, 29:302-304, 1973). It was tentatively concluded that the potentiating effects of insulin were due to its indirect effect on the leucine-preferring brain transport system, and that LSD and several related substances to some extent depend upon this system for passage across the blood-brain barrier.

Although insulin-induced augmentation of the effects of THBC's was relatively small, this interaction may be of clinical significance, since THBC's occur endogenously in mammals in both brain and periphery (adrenal gland) (S. A. Barker et al., Biochem. Pharmacol., 30:9-17, 1981). Moreover, these compounds have potentially important behavioral effects in rats when injected intracerebroventricularly in nanogram quantities, e.g., they increase voluntary ethanol consumption (R. D. Myers and C. L. Melchior, Pharmacol. Biochem. Behav., 7:381-392, 1977; R. D. Myers and M. M. Oblinger, Drug Alc. Depend., 2:469-481, 1977; L. Tuomisto et al., Pharmacol. Biochem. Behav., 17:831-836, 1982).

37.9 5-HYDROXYTRYPTOPHAN AS A DISCRIMINATIVE STIMULUS: SUBSTITUTION BY LSD. P. M. Callahan*, K. A. Cunningham and J. B. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, S. C. 29208.

Recent (unpublished) research has indicated that the serotonin (5-HT) precursor l-5-hydroxytryptophan (5-HTP), when given in combination with a decarboxylase inhibitor (Ro 4-4602), substitutes partially (50%) for the hallucinogen lysergic acid diethylamide (LSD) in rats trained to discriminate LSD (0.08 mg/kg) from saline. In an effort to more fully characterize both the LSD and 5-HT cues, experimentally-naive male albino rats (N = 6) were trained to discriminate 5-HTP (50 mg/kg) from saline in a two-lever, water-reinforced drug discrimination procedure; Ro 4-4602 (50 mg/kg) was always administered 1 hr prior to 5-HTP to block the direct, peripherally-mediated disruptive effects of 5-HTP. LSD (0.04-0.16 mg/kg) produced a dose-dependent substitution for 5-HTP; maximum drug-lever responding was 83% after 0.08 mg/kg of LSD. The 5-HT_2 receptor antagonist ketanserin (1.0 mg/kg) partially antagonized the 5-HTP cue and completely antagonized the substitution of LSD (0.08 mg/kg) for 5-HTP. These data suggest that the similarity between the 5-HTP and LSD cues may be related to common interactions with central 5-HT_2 receptors, although further research utilizing various monoaminergic agonists and antagonists is required before definitive conclusions can be drawn.

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BEHAVIORAL PHARMACOLOGY I

38.1 DRUG-INDUCED REVERSIBLE HEMIPLEGIA: A RAT MODEL. S. Brailowsky, R.T. Knight* and K. Blood*. Dept. of Neurology, Univ. of Calif., Davis; V.A. Med. Ctr., Martinez, CA. 94553

In an attempt to develop a reversible animal model of sensorimotor deficits we administered epicortical GABA by means of osmotic minipumps, to the sensorimotor region of rats trained previously to run on a narrow beam. The chronic delivery of the drug (5, 50 and 500 ug/ul/hr. for 7 days) produced a motor deficit in a dose-dependent manner: the animals progressed from a complete inability to walk on the beam to different degrees of contralateral hemiparesis. The high dose group was totally unable to perform the test while in the low and medium dose groups slips and misplacing of the rear paw were seen in the first 3 days. These changes disappear in most animals by the 10th day, with a gradient in the seriousness of the deficits from the starting point to recovery. In the rats in which saline was the delivered solution or in those in which the minipump did not empty its content, the recovery was present by the third day post-surgery. The histology showed lesions at the cannula implantation site variable in size from 8 to 15 mm^2. There was no difference in size between the lesions of the control and low and medium dose animals. However, high dose rats had larger lesions. This data suggests that locally applied GABA can reliably produce a reversible sensorimotor deficit in rats.

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38.2 INDUCTION OF SELF-INJURIOUS BEHAVIOR IN RATS BY MICROINJECTION OF MUSCIMOL INTO THE SUBSTANTIA NIGRA. A. Baumeister* & G. Frye, Biol. Sci. Res. Ctr., Sch. of Med., Univ. N. Carolina, Chapel Hill, 27514.

Severe self-injurious behavior (SIB) is frequently exhibited by persons belonging to several clinical populations. Studies with humans and animals suggest that such behavior is related to perturbations in dopaminergic systems in the basal ganglia. While studying seizure susceptibility in rats following ethanol withdrawal we found that animals given microinjections of the GABA agonist muscimol into the substantia nigra frequently bit themselves. A review of the literature revealed other anecdotal reports of muscimol-induced SIB (e.g., Taha et al., Psychpharm., 1982, 77, 272). This finding was of particular interest because other dopamine-related behaviors (i.e., stereotypies) are also mediated through the striatalnigral GABAergic pathway. In the persent study we investigated further the role of GABA in the production of SIB. Muscimol (10, 30, 100 or 300ng) in 0.5ul saline was injected bilaterally (0.5ul/min) into the caudal substantia nigra (zona reticulata) of ether anesthetized male Sprague Dawley rats. Immediately afterwards, rats were placed in clear plastic cages with wire grid floors. Behavior was observed during one minute periods every ten minutes for three hours. Muscimol (30, 100 & 300ng) produced self-biting and tissue damage in 26, 46, and 10% of the rats, respectively. Among the animals that exhibited SIB, 67% bit their tail, 16% bit their paws, and 50% had multiple lesion sites. The animals showed no evidence that self-biting was painful, but bites by other animals did evoke squealing. Muscimol also produced stereotyped sniffing, head nodding, and gnawing on the wire floor. No SIB was observed after microinjection of saline. When the opportunity to gnaw was limited by testing the animals in cages with a smooth plastic floor the frequency of SIB increased and the dose-response curve was shifted to the left. SIB occurred most frequently at 30ng, the dose that produced the most intense stereotyped gnawing on the wire floor. The percentage of animals that exhibited SIB when gnawing was prevented was 42%, 69%, and 36%, in the 10, 30, and 100ng groups, respectively. Latency to onset of SIB was longest on the plastic floor but increased with dose on both floor types. Another GABA agonist, THIP, at 100 and 300ng produced SIB in 25% and 12% of the animals respectively. However, simultaneously administered bicuculline methiodide (300ng) did not block muscimol-induced SIB. (Supported by PHS grants AA-05713, HD03110, HD07201).

38.3 LIBRIUM PREVENTS THE ANALGESIA AND SHUTTLEBOX ESCAPE DEFICIT TYPICALLY OBSERVED FOLLOWING INESCAPABLE SHOCK. Robert C. Drugan*, Susan M. Ryan, Thomas R. Minor and Steven F. Maier*. Department of Psychology, Box 345, University of Colorado, Boulder, CO 80309 SPON: M. Laudenslager.

It has been shown that GABA is an important neurotransmitter involved in the production of the learned helplessness effect. Gavish and Snyder (1980) demonstrated that GABA receptors have benzodiazepine recognition sites. From this data, they proposed that these two receptor systems are functionally interrelated. Due to the potential interactions between GABA and benzodiazepines it seemed reasonable to investigate the impact of a benzodiazepine on the learned helplessness effect using both behavioral and analgesic indices. Rats received 10 mg/kg i.p. chlordiazepoxide (CDP) daily for four days in order to tolerate out any sedative effects. On Day 5, animals received 5 mg/kg CDP or equivolume vehicle 30 minutes prior to 80 five-second, 1-mA inescapable tail shocks. On Day 6, subjects were also injected with either CDP or vehicle 30 minutes prior to test. In Experiment 1 testing, animals then received 5 five-second 1-mA footshocks in a shuttlebox. Tail-flick analgesia was measured both prior to and following the shocks. In Experiment 2 testing, animals received 5 FR-1 and 25 FR-2 signalled 1-mA escape trials for which latency to escape was measured. CDP administered prior to the inescapable shock session completely abolished both the analgesia and the shuttlebox escape deficit typically observed 24 hours following inescapable shock. Control groups determined that neither blocking analgesia nor abolishing the escape deficit was a state dependent effect. Finally, subjects given CDP immediately preceding shuttlebox tests showed facilitated learning. However, this was demonstrated not to be a general learning facilitation since nonshock controls given CDP prior to test showed equivalent performance to nonshock vehicle controls; instead, facilitation by CDP suggests that shuttlebox impairment may result partially from fear and anxiety. Overall, these data suggest that fear, anxiety, and the benzodiazepine receptor complex may participate in the production of several behavioral indices of learned helplessness.

38.4 CNS AND BEHAVIORAL CONSEQUENCES OF EXPOSURE TO PHENOBARBITAL EARLY IN THE BRAIN GROWTH SPURT. C.R. Stamper, E.J. Moore* and J. Diaz, Dept. of Psych., Univ. of Wash., Seattle, WA,98195.

The brain growth spurt marks a time of enhanced vulnerability to insults. We have demonstrated that exposure to phenobarbital during the entire brain growth spurt results in a 10% brain growth deficit. This study describes the effects of phenobarbital exposure early in the brain growth spurt.

Four day old female Long-Evans rats were matched by weight and assigned to one of 7 groups. Drug group animals were given daily subcutaneous injections of 60mg/kg of phenobarbital from days 5-8, while vehicle group animals were given daily subcutaneous injections of the vehicle from days 5-8. The groups were as follows: 1) artificially reared (AR) animals, given phenobarbital and sacrificed on day 9 (n=12); 2) AR animals, given the vehicle and sacrificed on day 9 (n=10); 3) normally reared (NR) siblings of groups 1 and 2 sacrificed on day 9 (n=18); 4) AR animals, given phenobarbital, returned to their mothers on day 9 and sacrificed on day 18, (n=7); 5) AR animals, given vehicle, returned to their mothers on day 9 and sacrificed on day 18, (n=7); 6) animals that were given phenobarbital and artificially reared from days 4-18, (n=8); 7) animals that were normally reared from days 4-18 in litters of 10, (n=10).

A battery of reflex tests, including negative geotaxis, free fall righting and cliff avoidance were conducted each day on animals from groups 4-7. The occurrence of eye opening and incisor eruption was noted. At the time of sacrifice, each animal's brain, liver, kidney and spleen were removed and weighed.

Chi Square tests revealed that incisor eruption and eye opening occurred earlier in AR animals than in NR animals ($p<.05$). The phenobarbital animals reached the criteria for free fall righting and cliff avoidance later than the vehicle animals ($p<.05$). This table summarizes mean weights (grams):

	BARB-9	VEH-9	NR-9	BARB-NR	VEH-NR	BARB-AR	NR
BODY WT	16.7	15.6	16.9	28.3	28.2	30.7	32
BRAIN WT	.693	.739	.841*	1.22	1.22	1.14	1.35*
LIVER WT	.747	.574	.454*	1.01	.961	1.33	.996*

The 7% brain weight deficit after 4 days of phenobarbital suggests that the early part of the brain growth spurt may be especially vulnerable to disruption. Behavioral deficits from the phenobarbital were observed days after the administration of the drug. The recovery of brain weight in animals normally reared after the drug exposure, which was not seen in animals that remained artificially reared, further suggests that environmental factors following a drug insult may be crucial in recovery. The course of recovery from the behavioral deficits remains to be determined. (* $p<.05$)

38.5 ANXIOGENIC PROPERTIES OF CONVULSIVE AGENTS. L. Prado de Carvalho,* P. Venault *, J. Rossier and G. Chapouthier * (SPON : M.L. Simon), Depts I and II, Laboratoire de Physiologie Nerveuse, CNRS, 91190 Gif-sur-Yvette, France.

In a previous paper, we have demonstrated that Methyl β-Carboline-3-Carboxylate (β-CCM), a convulsant benzodiazepine (BZ)-antagonist, has anxiogenic properties. (Prado de Carvalho, L. et al., Nature 301: 64-66, 1983). In that study, β-CCM in a subconvulsive dose (1mg/kg s.c.) had clear conflict enhancing effects. Here we use the same mouse-conflict-model to study the effects of other convulsant agents. Pentylenetetrazol (PTZ) and Picrotoxin were of interest because both these drugs have been variously implicated with the BZ-GABA-Cl^- ionophore complex. In the conflict model used, mice previously trained to press a lever for food were trained on 15 min conflict/non-conflict sessions divided as follows : two 5 min periods during which each lever press resulted in a food pellet ; one 5 min period (conflict) where each lever press was similarly rewarded but also concomitantly punished with a 50-100 μA electric foot-shock. Pressing rates were always reduced during conflict periods as compared to non-conflict periods. At higher shock intensities,conflict lever pressing levels were low and anxiolytic drugs had a lever pressing enhancing effect. Conversely, at lower shock intensities, high pressing levels were obtained during conflict and anxiogenic effects could thus be studied. The duration of lever presses were always shorter during conflict than during non-conflict periods, and comparable under all shock intensities. Mice were trained until stable day-to-day performances were obtained. Base line pressing rates were established for each 5 min period. The effect of a drug was evaluated by comparing performances (5 min periods) of each mouse under the effect of the drug to previous day performances. Anxiety was assessed by a decrease in the number of lever presses during the period of conflict. In control experiments mice were trained in comparable 15 min food rewarded sessions but not submitted to conflict training. Drugs were administered subcutaneously, in subconvulsive doses, at appropriate times before the beginning of the test : 5 min for PTZ (25 mg/kg) and 30 min for Picrotoxin (0.75 mg /kg). Our results show that under PTZ or Picrotoxin, the number of lever presses during conflict were significantly reduced as compared to previous day levels. In control mice, no reductions were seen following PTZ or Picrotoxin, thus the observed effect seems to be conflict related. The reduction of lever presses during conflict was interpreted as a demonstration of the anxiogenic effects of PTZ and Picrotoxin.

38.6 EFFECTS OF PROPRANOLOL AND MIDAZOLAM ON PENTYLENETETRAZOL DISCRIMINATION AND LEVER PRESSING BEHAVIOR. Douglas M. Wilkison*. (SPON: Liang-Fu Tseng). Dept. of Pharmacology, Med. Col. of Wisconsin, Milwaukee, WI 53226.

The two-lever pentylenetetrazol (PTZ)-discrimination paradigm has been proposed as a model for the interoceptive cues of anxiety based on the ability of antianxiety agents, esp. benzodiazepines, to block the PTZ-cue and the anxiogenic properties of PTZ in humans.

Propranolol, on the other hand, has been proposed as a clinically useful antianxiety agent, which exhibits little anticonflict action in the Geller-Seifter conflict test. To further characterize the antianxiety effects of propranolol and the utility of the PTZ-discrimination model for anxiety the effects of propranolol (2-10 mg/kg) were determined on PTZ-discrimination and lever pressing in trained rats. Likewise the effects of midazolam (0.03-0.6 mg/kg), a benzodiazepine, and combined treatment were determined.

Rats were shaped to lever-press for food reinforcement (60% sweetened condensed milk) to an FR10, then trained to discriminate 20 mg/kg PTZ from saline. Midazolam administered ip 15 minutes before PTZ blocked the PTZ-discrimination (ED50 = 0.11 mg/kg, n=7). Propranolol (2-10 mg/kg) was without effect on PTZ-discrimination. However, 10 mg/kg propranolol shifted the dose-response curve for midazolam to the right. ED50 = 0.39 mg/kg, n=7 for midazolam with 10 mg/kg propranolol. Operant behavior was measured by the number of reinforcements earned in 15 minutes and the latency to the first reinforcement. PTZ depressed reinforcements 50% and increased latency 113% compared to saline pretreatment during discrimination.

Midazolam pretreatment of PTZ treated rats produced a dose-dependent increase in reinforcements and decrease in latency. Behavior approached that during saline discrimination at 0.3125 mg/kg midzolam. Propranolol pretreatment (10 mg/kg) significantly depressed reinforcement rate and increased latency but did not significantly change the normalizing action of midazolam on these parameters. As with diazepam, midazolam is a potent antagonist of the interoceptive cue of PTZ. However, propranolol up to doses which depress operant behavior does not antagonize PTZ discrimination. Evidence suggests that propranolol either augments PTZ or antagonizes midazolam. This work is supported, in part, by Pharmaceutical Manufacturers Association Foundation.

38.7 STIMULUS PROPERTIES OF OXAZEPAM: A 1,4-BENZODIAZEPINE. W. J. Millard, P. Riley* and J. Szostak*. Laboratory of Behavioral Medicine and Pharmacology, Mount Holyoke College, South Hadley, MA 01075-1462 USA.

The stimulus properties of oxazepam were evaluated in a drug discrimination procedure in which the administration of the drug (5.0 mg/kg, i.p.) and vehicle were correlated with fixed-ratio schedules of food reinforcement. Acquisition of the discrimination by the rat subjects was followed by generalization tests in which the dose of oxazepam was varied (1.0-24.0 mg/kg), and substitution tests in which varying doses of benzodiazepine anxiolytics other than oxazepam where administered (e.g., chlordiazepoxide). Stimulus generalization to the oxazepam stimulus was found, and varied in a dose-dependent manner. Further tests were completed with compounds having a high affinity for the benzodiazepine recognition site (e.g., Ro 15-1788, CL 218,872), and compounds possessing anxiolytic properties but not benzodiazepine ligands (e.g., ethanol, pentobarbital, buspirone, clonidine). The degree of drug generalization was highly correlated with anxiolytic potency.

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38.8 PROCONVULSANT EFFECTS OF THE BENZODIAZEPINE AGONIST, CL 218,872. C.L. Melchior,[1] K. Garrett[1]* and B. Tabakoff.[1,2] [1]Alcohol and Drug Abuse Research and Training Program, Department of Physiology and Biophysics, University of Illinois at Chicago, Health Sciences Center, Chicago, IL 60612.

Both the triazolopyridazine, CL 218,872, and methyl beta-carboline carboxylate (MBCC) interact with the Type 1 benzodiazepine receptor. CL 218,872 is regarded as a specific agonist, displaying anxiolytic, sedative and anticonvulsant properties. MBCC has been classified as an antagonist or inverse agonist and has been shown to cause convulsions. In this study, we examined the interactions of these two compounds in C57Bl mice.

As measured on a Stoelting Activity Monitor, motor activity was decreased by an intraperitoneal injection of 10.0 mg/kg CL 218,-872. Both 0.25-1.0 mg/kg I.P. of MBCC and 0.5-2.0 mg/ kg of another benzodiazepine receptor antagonist, RO 15-1788 were equally effective in blocking CL 218,872 depression of motor activity. Given alone, the above-stated doses of RO 15-1788 had no effect on motor activity, while MBCC, in doses of 1.0 mg/kg or higher, decreased motor activity.

In comparison, RO 15-1788 was more effective than MBCC in blocking the depression in motor activity caused by 2.0 mg/kg diazepam. MBCC blocked diazepam induced depression only at doses which, by themselves, produce convulsions.

A dose of 5.0 mg/kg of MBCC caused convulsions in 100% of the treated animals. The MBCC-induced convulsions could be blocked by prior injection of 2.0 mg/kg of diazepam, 1.0 mg/kg of RO 15-1788, or 20.0 mg/kg of CL 218,872. Doses of MBCC of 1.0 or 2.0 mg/kg produce little or no convulsant activity by themselves. Interestingly, convulsant activity was significantly increased when these doses of MBCC were given together with low doses of CL 218,872 (0.5-10.0 mg/kg). In contrast, low doses of diazepam, given with 1.0 or 2.0 mg/kg of MBCC, did not result in convulsions. When 3.0 mg/kg of bicuculline was used in place of MBCC to produce convulsions, 0.5 mg/kg of CL 218,872 again increased the severity of the convulsions. CL 218,872 alone did not cause any convulsions.

These results indicate that high doses of CL 218,872 are sedative and anticonvulsant, but low doses are proconvulsant. Proconvulsant activity gives CL 218,872 a unique profile among compounds classified as anxiolytic benzodiazepine agonists.

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38.9 ANXIOGENIC-LIKE PROPERTIES OF BENZODIAZEPINE ANTAGONISTS. D.K. Hoffman* and D.R. Britton. Department of Physiology, University of New Mexico School of Medicine, Albuquerque, N.M. 87131.

Benzodiazepine (BDZ) antagonists have been identified on the basis of their ability to block the effects of anxiolytic and anticonvulsant BDZs such as diazepam (DZP). Two of these compounds, the convulsant Ro 5-3663 and the so-called pure antagonist Ro 15-1788, were studied for their ability to block the behavioral effects of DZP. These effects were assessed by testing animals in an open field paradigm (Britton and Britton, 1981) and by challenging the ability of DZP to stimulate food consumption in a familiar environment.

Male, Sprague-Dawley albino rats were treated with an i.p. injection 30 min. prior to testing with either DZP, Ro 15-1788, Ro 5-3663 or a combination of DZP and an antagonist. Control animals received an injection of the drug carrier. Both antagonists reversed the hyperphagic actions of DZP when animals were tested in a familiar environment. In the open field paradigm, animals were fasted for twenty-four hours then introduced into a well illuminated open field where they had access to a food pellet secured to the center of the 40 cm diameter enclosure. As has been previously reported, DZP alone attenuated the disruptive effects of novelty in this setting by increasing the amount of food eaten and the mean amount eaten per approach. DZP also decreases grooming and rearing. Both of the antagonists effectively reversed these effects of DZP. Ro 15-1788 at a dose of 0.5 mg/Kg completely reversed the effects of 2.0 mg/Kg DZP. In addition, RO 15-1788 given alone at higher doses (up to 8.0 mg/Kg) accentuated the animals' responsiveness to novelty. Although Ro 15-1788 has been shown not to have significant convulsant or pro-convulsant activity, these data show that it is not pharmacologically inert. Rather, it appears to interact with a population of BDZ receptors in a manner which potentiates the animals response to "anxiogenic" stimuli.

38.10 DIAMINE INHIBITION OF GLYCINE AND GABA UPTAKE. George M. Strain and Wayne Flory. Vet. Physiol., Pharmacol., and Toxicol., Louisiana St. Univ. Sch. Vet. Med., Baton Rouge, LA 70803.

Several diamines have been reported to block the uptake of amino acid transmitters: diaminopropionic acid (glutamate, GABA), β-N-oxalyl-L-α, β-diaminopropionic acid (glutamate), and ethylenediamine (GABA). When simple aliphatic diamines are administered in the brain ventricles they produce effects ranging from purely depressant (ethylenediamine) to purely excitatory (1,3-diaminopropane) to a mixture of both effects (1,6-diaminohexane) [Soc. Neurosci. Abstr. 6:149, 1980]. We therefore examined the effects of the simple aliphatic diamines on synaptosomal uptake of the amino acids glutamate, aspartate, GABA and glycine as an explanation for the in vivo neurotoxic effects.

Crude synaptosomes were prepared by a modification of the method of Gray and Whittaker [J. Anat. 96:79, 1962]. Whole brain was used for glycine transport; otherwise the forebrain was dissected free. Amino acid concentration was 10 μM, as a mixture of tritiated and cold. Synaptosomes (P_2 pellet) were preincubated for 5 min at 25°C. Transmitter was added and transport was terminated after 10 min by a rinse of cold buffer and vacuum filtration. Controls were transported in sodium-free buffer. Diamines studied for inhibitory effects were the homologous series of ethylenediamine to 1,7-diaminoheptane. DL-2,4-diaminobutyric acid (DABA) was also included for comparative purposes. Results (average of three experiments performed in triplicate) were expressed as a percentage of control.

The diamines were most inhibitory to the uptake of glycine and GABA. Weaker effects were seen on glutamate, while aspartate was unaffected. The pattern of inhibition for glycine and GABA was similar, and effects were dose-dependent. 1,2-diaminopropane was most inhibitory, followed by ethylenediamine and 1,7-diaminoheptane. The reported inhibitory effect of DABA on GABA and glutamate uptake was confirmed. Comparable effects were seen on glycine and aspartate uptake, but GABA effects were by far the most potent. Diamine inhibitory effects were approximately 100x less potent than DABA.

The observed effects provide an explanation for the in vivo depressant effects of the diamines, but not the excitatory effects. The relatively weak inhibitory effects preclude the use of diamines as efficient pharmacologic agents, but the structure-activity results will provide information for the development of more effective agents.

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38.11 THE INTERACTION OF CNS DEPRESSANTS AND GABAERGIC DRUGS IN MICE SELECTIVELY-BRED FOR THE NARCOTIC EFFECTS OF ETHANOL. T.D. McIntyre, H.P. Alpern. Department of Psychology, University of Colorado, Boulder, CO 80309.

Recent evidence strongly suggests a common mechanism linking the effects of ethanol (ETOH), barbiturates, and benzodiazepines (J.N. Nestoros, Prog. Neuro-Psychopharmacol. 5:591, 1981). This premise was investigated in two lines of mice (short sleep, SS and long sleep, LS) which have been genetically selected for differential narcotic sensitivity to ETOH. Earlier reports indicated either no differential sleep-time response to barbiturates (Erwin et al. Pharmacol., Biochem. and Behav. 4:679, 1976) or a greater sensitivity to sodium pentobarbital in the SS line (O'Connor et al. Pharmacol., Biochem. and Behav. 17:245, 1982). This report demonstrates that if the dose-response relationship is examined thoroughly the mice more sensitive to the narcotic effects of ETOH (LS) are also significantly more sensitive to the narcotic effects of pentobarbital and barbital as well as the benzodiazepine, chlordiazepoxide (CDX). Additionally, two doses (10 & 20 mg/kg) of the γ-aminobutyric acid (GABA) agonist amino-oxyacetic acid (AOAA) were employed in conjunction with these central depressants in order to demonstrate a common substrate of action in these mice. Results indicate that (AOAA) differentially affects sleep-time in a dose-dependent manner in these lines of mice. Therefore, we conclude that these results support the notion that the differential narcotic response of SS and LS mice to intraperitoneal injections of ETOH, barbital and CDX is mediated, at least in part, by the GABA system.

38.12 DISCRIMINATIVE STIMULUS PROPERTIES OF L-PHENYLISOPROPYLADENOSINE. Ronald G. Browne, Pfizer Inc., Central Research, Groton, CT 06340.

The recent discovery of metabolically stable analogs of adenosine has contributed to the elucidation of adenosine receptors in brain. The present investigation examined the ability of adenosine analogs to bind to adenosine A1 receptors, and their ability to mimic the behavioral effects of L-PIA in a drug discrimination paradigm. Adenosine receptor binding was performed using the method of Bruns et. al. (PNAS 77: 5547-51, 1980) and the displacement by various compounds of 1 nM [3H-CHA] binding to adenosine deaminase treated rat membranes was determined. Non-specific binding was defined by 10 μM L-PIA, and IC50's were calculated by linear regression analysis. Male Sprague-Dawley rats were trained to discriminate 0.1 mg/kg of L-PIA from vehicle in a two-lever operant paradigm. Discrimination accuracy was established following about 40 training sessions as evidenced by most animals emitting their first FR-10 responses on the appropriate lever in 9 out of 10 consecutive sessions. The discriminative stimulus properties of L-PIA could be completely blocked with the methylxanthines IBMX, caffeine, theophylline and partially antagonized by 8-para-sulfophenyltheophylline. Generalization testing indicated that a number of adenosine analogs were capable of mimicking the discriminative effects of L-PIA; the D-isomer of PIA was found to be about 30 times weaker than the training drug. By contrast, the A2 agonist NECA and adenosine 5'-cyclopropyl carboxamide (5'-CPC) were more potent than L-PIA in eliciting the L-PIA cue. In general, there was a good correlation between the potency whereby A1 agonists displaced [3H-CHA] and induced generalization in the discrimination test. However, the A2 agonists NECA and 5'-CPC were more potent in cueing than would be predicted by their ability to bind to A1 sites. Furthermore, we found that both clonidine (0.1 mg/kg) and diazepam (3.2 mg/kg) substituted completely in the discriminative stimulus assay, but were relatively weak in binding to A1 sites. These results indicate the rats trained to discriminate L-PIA are probably cueing on the profound sedative effects produced by the moderately high training dose of L-PIA (0.1 mg/kg). Alternatively, the discriminative stimulus properties of L-PIA may be related to the possible anxiolytic activity of these compounds.

38.13 CENTRAL EFFECTS OF ADENOSINE AND ITS ANALOGS ON SPONTANEOUS LOCOMOTOR ACTIVITY. V.L. Coffin,* J. W. Phillis, H.J. Altman, and R.A. Barraco. Wayne State University, School of Medicine, Detroit, Mich. 48201.

Adenosine and its analogs depress neuronal firing at many levels of the neural axis. The case for adenosine being a synaptic modulator in the CNS is supported by demonstrations of its release from a variety of in vivo and in vitro preparations, by its effects on adenylate cyclase activity, and by its inhibitory effects on the presynaptic release of several different types of neurotransmitters (Phillis, J.W. and P.H. Wu, Prog. in Neurobiol., 16: 187, 1981). Adenosine and its analogs are also known to have marked behavioral effects, including sedative and anticonvulsant activity. L-N^6-phenylisopropyl-adenosine (L-PIA), a metabolically stable analog of adenosine, has been shown to produce decreases in spontaneous locomotor activity (Snyder, et al., Proc. Natl. Acad Sci., 78: 3260, 1981) and schedule-controlled operant responding (Coffin, V.L. and J.M. Carney, In: Physiology and Pharmacology of Adenosine Derivatives, 1983) following parenteral injections.

Mice were implanted with chronic indwelling cannulas and injected in the lateral cerebral ventricle (ICVT) with adenosine, adenosine analogs, and an adenosine uptake inhibitor. The effects on spontaneous locomotor activity were assessed. Adenosine, as well as its analogs, produced dose-related decreases in locomotor activity. The relative order of potency for locomotor depression was: 5'ethylcarboxamide adenosine (NECA) >> L-PIA > 2-chloroadenosine > D-PIA > adenosine. Caffeine blocked adenosine's effects, as well as the effects of an analog (NECA) on spontaneous locomotor activity. Certain dose combinations of adenosine and caffeine produced stimulation, whereas this effect was not observed with NECA. Papverine, a specific adenosine uptake inhibitor, was also studied in this paradigm. The dose-effect curve of papaverine had the same slope and relative potency as adenosine. Caffeine had the same effect on papaverine as with adenosine, producing an antagonism at some doses and stimulation at others. Combinations of adenosine and papaverine had additive effects in depressing spontaneous locomotory activity. In summary, the data show that adenosine and its analogs can potently depress spontaneous locomotor activity in mice and these depressant effects are mediated by central mechanisms. Furthermore, caffeine can antagonize these central depressant effects.

38.14 DEVELOPMENT OF TOLERANCE TO THE HYPOTHERMIC ACTIONS OF CAFFEINE IN THE RAT. A.J. Schlosberg. Neurosciences Program, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Groups of adult, male rats received caffeine (100 mg/kg, i.p.) or vehicle for 1, 2, 4 or 7 days. Rectal temperatures were recorded immediately prior to and 2 hr after drug administration. Caffeine-induced hypothermia (-2.0 to -2.5°C) was only observed after the first injection of the drug. Within 24 hr, an apparent tolerance to caffeine was displayed by all rats which received additional injections of the drug: The hypothermic response was significantly attenuated or absent in rats given 2 or 4 and 7 daily drug treatments, respectively. In order to assess possible changes in brain amine levels and endocrine responsivity following repeated exposure to this xanthine, rats in each test group were killed 2 hr after their last caffeine injection. Caffeine significantly increased the brain content of tryptophan, serotonin and 5-hydroxyindoleacetic acid, and these effects were relatively constant over days. Acute and chronic caffeine failed to systematically alter the brain levels of norepinephrine and dopamine. Caffeine significantly elevated the serum levels of non-esterified fatty acids (NEFA) irrespective of the number of injections; the rise in serum NEFA was, however, greater with subsequent administrations of the drug (days 4 and 7), but a drug x days interaction was not found. In contrast to the above effects, caffeine-induced changes in the serum levels of glucose and insulin followed the same time-course described for the emergence of tolerance to the hypothermic actions of the drug. Increases in serum glucose (+55%) and insulin (+93%) levels were only observed in rats given a single injection of caffeine. After the second through seventh injection, glucose and insulin levels did not vary significantly from control levels in vehicle-treated rats. That chronically caffeinated rats were capable of exhibiting glucose and insulin responses to other agents was confirmed in rats given daily injections of caffeine over a 7 day period. On the eighth day, rats previously exposed to 100 mg/kg of caffeine displayed an increase in the level of serum insulin 1 hr after an oral glucose load (2.8 M, 10 ml/kg) which was comparable to that found in caffeine-free rats. In similarly caffeinated rats, serum glucose levels were significantly increased 2 hr after the injection of epinephrine (.250 mg/kg, s.c.), but the effect was not as great as was found in caffeine-free rats. Recently, brain serotonin was shown to play a modulatory role in the dose-dependent hyper- and hypothermic effects of acute caffeine. To what extent changes in energy substrate mobilization and utilization may participate in the acute and, perhaps more importantly, the chronic thermoregulatory effects of caffeine remains to be determined. (Supported by UAB Faculty Research Grant)

38.15 BEHAVIOURAL STIMULANT EFFECT OF A SMALL DOSE OF β-PHENYLETHYLAMINE FOLLOWING SPECIFIC INHIBITION OF TYPE B MONOAMINE OXIDASE. C.T. Dourish, Psychiatric Research Division, CMR Building, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0.

The endogenous amine β-phenylethylamine (PEA) is structurally similar to the psychomotor stimulant amphetamine (AMPH) and has been proposed to mediate the actions of the latter compound. The behavioural effects of AMPH in rodents are well documented. Doses of 0.5 to 2.0 mg/kg of AMPH increase co-ordinated locomotion and exploration whereas doses of 5 mg/kg and above induce progressively more severe forms of stereotyped sniffing, rearing, gnawing and body movements. A major weakness in the argument that PEA may be an endogenous AMPH is the inability of PEA to elicit a behavioural stimulant effect comparable to that induced by low doses of AMPH. Recent studies in this laboratory have demonstrated that the administration of increasing doses of PEA to rodents induces "AMPH-like" stereotypy but not locomotor stimulation. PEA is a specific substrate for type B monoamine oxidase (MAO) and, therefore, is metabolized extremely rapidly in tissue. The present study examined the behavioural effects of low doses of PEA following pretreatment with deprenyl, a specific inhibitor of type B MAO. Male Wistar rats were injected with PEA at doses of 0, 1, 2, 4, 8 or 16 mg/kg 4h after an injection of 4 mg/kg l-deprenyl or saline. Testing was conducted in individual plexiglass cages positioned in automatic activity recording devices (Opto Varimex Minor) controlled by a microprocessor/microcomputer system. The system generated scores for horizontal and vertical activity and ambulation. Behavioural observation supplemented the automatic recording during a 60 min test. PEA or deprenyl alone had no significant effect on behaviour. However, ANOVA revealed a significant interaction of the two drug treatments. In deprenyl-pretreated rats PEA (4 mg/kg) elicited an "AMPH-like" increase in co-ordinated locomotor activity and exploration which was devoid of stereotypy. This behavioural pattern was never observed in rats treated with PEA alone. Higher doses of PEA (8 or 16 mg/kg) in deprenyl-pretreated rats induced hyperactivity and stereotyped sniffing accompanied by head and body movements, such as have been previously described after large doses of PEA alone. These data suggest that pretreatment with the specific type B MAO inhibitor deprenyl can unmask the behavioural stimulant effect of PEA. The present results do not support the hypothesis that MAO inhibition simply produces a shift in the PEA dose response curve. Supported by Dept. Health, Province of Saskatchewan.

38.16 THE EFFECTS OF AMPHETAMINE AND APOMORPHINE ON FRONTAL CORTEX SELF-STIMULATION. Nancy J. Leith, Dept. Pharmacol., Vanderbilt Med. Sch., Nashville, TN 37232.

Although investigators (Mora, Life Sci. 22, 1978; Mora and Myers, Science 197, 1977) have concluded that dopamine (DA) is the important neurochemical substrate for frontal cortex (FC) self-stimulation (SS) behavior, pharmacological manipulations of DA function have yielded data inconsistent with such a straightforward relationship. Most notably, d-amphetamine (d-AMPH) did not significantly facilitate FC SS (Goodall and Carey, JCPP, 89, 1975) and apomorphine (APO) decreased responding over a wide range of doses (Mora, Life Sci. 22, 1978). The latter effect was interpreted as indicative that the direct activation of DA receptors by APO disrupts the contingency relationship between the rat's behavior and the functioning of the DA system. However, recent work (Leith, Brain Res., 1983, in press) has demonstrated that the effects of APO on medial forebrain bundle (MFB) SS are a complex function of drug dose and current intensity and previous work (Leith and Barrett, Psychopharmacologia 46, 1976) indicated that AMPH facilitation of MFB SS is also a function of current intensity. The present study was undertaken to pharmacologically characterize FC SS using a procedure that generates a response rate-current intensity function for each animal which allows assessment of drug effects at both supra and subthreshold intensities.

Twenty-four rats were implanted with bipolar electrodes in the medial FC and trained to bar press to receive 0.5 sec trains of 60 Hz ac current. Daily sessions consisted of repeated sampling of the rat's responding at 14 current intensities. The reinforcement threshold was defined as the current intensity at which responding decreased to 50% of the maximum rate. The average baseline threshold value for these animals was 15 μA.

Using this procedure, d-AMPH (.3 mg/kg) significantly lowered the threshold, with no effect on response rates that were above threshold. The l isomer was equipotent and produced identical changes. Apomorphine (.1-.2 mg/kg) lowered responding at suprathreshold intensities and increased responding at subthreshold values, identical to that obtained with MFB electrodes. However, low doses of APO (.02 mg/kg) had no effect on SS responding whereas such doses elevate the threshold for MFB SS. This effect on MFB SS is thought to be related to a selective interaction of low doses of APO with presynaptic autoreceptors and the lack of effect on FC SS is consistent with the absence of autoreceptors on FC dopamine neurons (Bannon et al., Molec. Pharmacol. 19, 1981).

Thus, the present work demonstrates that the effects of DA agonists on FC SS are nearly identical to the effects of those same drugs on MFB SS. Previously reported differences are most likely the result of sampling the responding only at suprathreshold values. (Supported by MH29217).

38.17 DOPAMINERGIC FACILITATION OF ACOUSTIC STARTLE IS DUE TO ENHANCED SENSORY RATHER THAN MOTOR EFFECTS. R.L. Commissaris*, S. Yang*, L. Dember* and M. Davis. Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508 (SPON: M. Bowers).

Previous work has suggested that the primary acoustic startle circuit in the rat is: auditory nerve, ventral cochlear nucleus (VCN), nuclei of the lateral lemniscus, nucleus reticularis pontis caudalis (RPC), spinal interneuron, lower motor neuron, muscles (Davis et al., 1983). Using electrical stimulation at various nuclei along the acoustic startle circuit, it has been possible to identify the locus within the startle circuit for the expression of a number of modulatory influences on this behavior, including the potentiated startle response (Berg and Davis, 1983) and response habituation and sensitization (Davis et al., 1982). This technique has also been used to localize the site of ultimate action of some systemically administered drugs (Commissaris and Davis, 1982). In these studies, 'startle' is elicited progressively along the pathway from the VCN to the RPC. The site farthest along the circuit where a treatment still affects electrically-elicited 'startle' is considered to be the point where that modulatory influence is inserted into the startle pathway.

The present study employed this electrically-elicited 'startle' response to examine the neural loci for the facilitation of startle by 1) decreased glycinergic transmission produced by strychnine and 2) increased dopaminergic transmission produced d-amphetamine and apomorphine. Male rats (350-400g) received bilateral, single-pulse stimulations of RPC or VCN alternating with acoustic noise bursts. Both acoustic and electrically-elicited 'startle' responses were elicited for a 14-min baseline period before i.p. injection of various doses of strychnine (0.25-1 mg/kg), d-amphetamine (2-8 mg/kg), apomorphine (1.0,3.0 mg/kg) or vehicle. Acoustic and electrically-elicited 'startle' responses were then measured for 20 min post-injection.

As expected, all three agents markedly increased acoustic startle amplitude. Strychnine increased the 'startle' elicited electrically from either the VCN or the RPC; these data are consistent with its facilitatory effects on acoustic startle when administered spinally. In contrast, both d-amphetamine and apomorphine failed to enhance 'startle' responses elicited from either the VCN or RPC, suggesting that dopaminergic modulation of acoustic startle is expressed at or before the VCN (i.e., VIII nerve or cochlea). Taken together, the data suggest that glycinergic modulation of the startle response occurs in the motor portion of the reflex arc, while the dopaminergic modulation of startle occurs on the sensory side.

38.18 CHRONIC ADMINISTRATION OF ATYPICAL NEUROLEPTICS DIFFERENTIALLY ALTERS BEHAVIORAL SUPERSENSITIVITY MEDIATED BY TWO DISTINCT BRAIN REGIONS. R. Halperin, V. Haroutunian*, P. Szynkowicz*, J. J. Guerin, Jr.* and K. L. Davis*. Psychiatry Service, V.A. Medical Center, Bronx, NY 10468, and Mt. Sinai Medical School, N.Y.NY 10029

In the rat, direct injection of dopamine (DA) into the caudate putamen (CP) or nucleus accumbens (AC) elicits stereotypy and locomotor responses, respectively. The magnitude of these behavioral responses is believed to index DA receptor sensitivity at the injection site. We have shown that chronic pre-treatment with the atypical neuroleptic fluotracen can selectively enhance the locomotor response to intra-accumbens injection of DA, leaving the stereotypy response to striatal DA injection unaltered (Halperin, R., Guerin, J.J. & Davis, K.L. Life Sci. In press, 1983). We now report the effects of pre-treatment with the atypical neuroleptics metoclopramide (Met), sulpiride (Sul), and clozapine (Cloz) as compared to haloperidol (Hal) or vehicle (Veh) in terms of their ability to enhance the stereotypy and locomotor responses to intracerebral DA injection.

Each rat was chronically implanted with drug-injection cannulas aimed bilaterally at either the CP or AC. In Experiment 1, 46 rats were given sweetened drinking water containing either Met (100 - 400 mg/l), Hal (10 - 40 mg/l) or sucrose alone. Drug concentrations increased progressively over a period of 21 consecutive days. In Experiment 2, 61 rats were injected once daily (i.p.) with either Cloz(30mg/kg), Sul (50 - 100 mg/kg), Hal(0.5 mg/kg) or saline for 21 consecutive days. In both experiments each rat was centrally injected with 10 μg of DA bilaterally five days after the termination of drug treatment. Stereotypy in CP-cannulated rats, or locomotion in AC-cannulated rats was measured immediately after the central DA injection.

Pre-treatment with Met, as compared to sucrose alone, enhanced the stereotypy response to DA injection to the CP, but did not alter the locomotor response to DA injection to the AC. Pre-treatment with both Cloz and Sul, as compared to Veh, enhanced the locomotor response to DA injection to the AC, but did not alter the stereotypy response to DA injection to the CP. Pre-treatment with Hal enhanced both behavioral responses to central DA injection.

Understanding the mechanisms through which these systemically administered drugs exert their selective effects on behaviors mediated by different brain regions has important implications for dissociating the antipsychotic and dyskinetic effects of chronic neuroleptic treatment.

(Supported by the Schizophrenia Biological Research Center of the Veterans Administration)

38.19 ATTENUATION OF APOMORPHINE INDUCED STEREOTYPY BY CAPTOPRIL PRETREATMENT. A. Sudilovsky, B. A. Turnbull,* L. H. Miller,* and L. J. Traficante.* Squibb Institute for Medical Research, Princeton, N. J. and Department of Biobehavioral Sciences, Boston University Medical Center, Boston, Mass.

Captopril, an antihypertensive agent that inhibits the angiotensin converting enzyme (ACE) has also been found to significantly increase serum prolactin levels in hypertensive patients (Aberg, H.E. et al, IRCS Medical Science, 8: 217, 1980). This increase suggests a possible captopril-dopamine system interaction and led us to examine the effect of captopril on apomorphine induced behavioral stereotypy.

In the first series of trials 10 adult Sprague-Dawley male rats were injected intraperitoneally with apomorphine (2 mg/kg) or with apomorphine (2 mg/kg) plus captopril (5, 10, 20, or 50 mg/kg) following a fully crossed design. Treatment groups were randomly assigned and, at least, seven days passed between treatment administrations. Stereotyped behavior was rated using a 0-3 point scoring method (Tarsy, D. and Baldessarini, R.J., Neuropharmacol., 13: 927, 1974) for segments of 10 seconds every 15 minutes over a period of 80 minutes starting immediately after injection. Although a trend toward attenuation of the stereotyped behavior induced by apomorphine was observed during the 60 and 75 minute scoring segments, ANOVA indicated that the concurrent administration of captopril did not significantly alter stereotypy.

In the second series of trials, rats (N=10) received captopril (20 mg/kg, i.p.) at 60, 90, or 120 minutes prior to the administration of apomorphine (2 mg/kg, i.p.). Scoring and all other procedures were as noted above. The data obtained demonstrated a significant attenuation of apomorphine induced stereotypy, ($p = 0.0026$). Post-hoc analyses utilizing the Newman-Keuls method determined that whereas no significant differences existed between animals pretreated with captopril, all three pretreated groups were significantly different from that receiving apomorphine alone ($p < 0.05$).

Radio-receptor assay measuring labelled spiroperidol displacement (Creese, I. and Snyder, S.H., Nature, 270: 180, 1977) demonstrated that captopril does not competitively bind to the dopamine receptor at concentrations ranging from 0.01 to 10 µg/ml. Thus the attenuation of the apomorphine induced stereotypy in rats pretreated with captopril does not appear to be due to a displacement of apomorphine from the dopamine receptor site by captopril. Alternatively, the observed results may be explained by captopril-induced alterations in endogenous modulators or antagonists of the dopamine receptor.

38.20 DISCRIMINATIVE STIMULUS PROPERTIES OF INTRACRANIAL SELF-STIMULATION: EFFECTS OF D-AMPHETAMINE, MORPHINE, NICOTINE AND PHENCYCLIDINE. Gerald J. Schaefer and Richard P. Michael. Depts. of Psychiatry and Pharmacology, School of Medicine, Emory University and The Georgia Mental Health Institute, Atlanta, GA 30306.

Rats were implanted with single stimulating electrodes in the medial forebrain bundle-lateral hypothalamus (MFB-LH) and trained to discriminate the presence or absence of intracranial self-stimulation (ICSS) in a two-choice discrete trial task. For each trial, the animal was required first to press an "initiating" lever which gave either suprathreshold ICSS or no ICSS. If the animal received ICSS, it was required to go to the right "choice" lever for further ICSS. If it received no ICSS on the initiating lever, the animal was required to go to the left choice lever for ICSS. A wrong choice terminated the trial without reward. Half of the animals were required to press the right choice lever when the initiating lever produced a suprathreshold stimulus and the left choice lever when the initiating lever produced no stimulus to obtain the reinforcing stimulus. The opposite conditions were in effect for the other half of the animals. After establishing the discrimination, test sessions with vehicle and drug solutions were interposed between training sessions. During test sessions, the initiating lever produced current intensities that were either 0-10-20-40-60-80-100% of that produced by the choice lever. In addition, during test sessions, in contrast to training sessions, a response on either choice lever produced the reinforcing stimulus and terminated the trial. The dependent measure was the number of trials completed on the ICSS-appropriate choice lever as a function of the current intensity of the initiating lever. Animals were tested with d-amphetamine (0.1 - 1.0 mg/kg), morphine (0.1 - 1.0 mg/kg), nicotine (0.1 - 0.56 mg/kg), and phencyclidine (0.3 - 3.0 mg/kg). For each dose of drug a function relating the selection of choice lever to the current intensity on the initiating lever was determined and this was compared to the function produced by the vehicle using the Litchfield and Wilcoxon (1949) procedure. None of the doses of these drugs altered the discriminative stimulus properties of ICSS as evidenced by an absence of changes in the detection of the discriminative stimulus. While ICSS produces reliable discriminative stimulus properties in the MFB-LH of rats, the effects are not altered by drugs that have been demonstrated in other tasks to produce discriminative stimulus effects and to alter the reinforcement threshold for ICSS. These data suggest that the effects of drugs on the ICSS reinforcement threshold can be dissociated from the effects on the detection threshold. (Work supported by Georgia Department of Human Resources.)

38.21 DISCRIMINATIVE STIMULUS PROPERTIES OF TWO ERGOTS, ERGONOVINE AND LERGOTRILE. K. A. Cunningham, P. M. Callahan* and J. B. Appel. Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, S. C., 29208.

Ergot derivatives exhibit diverse central and peripheral pharmacological properties, possibly because of structural similarities between the ergoline ring and monoaminergic neurotransmitters. Clinically, these compounds are useful in the treatment of disorders ranging from migraine headache and post-partum hemorrhage to Parkinsonism and senile dementia; several ergots may also be hallucinogenic. Since drug discrimination (DD) has been useful in reliably differentiating between the effects of LSD and lisuride in vivo and in delineating their underlying neuronal mechanisms (White and Appel, JPET, 221: 421, 1982), we have been using this method recently to investigate several other ergots.

Thus, male albino rats were trained to discriminate ergonovine (2.0 mg/kg, i.p.) (N = 7) or lergotrile (N = 7) from saline in a two-lever, water-reinforced task. Within 20-30 sessions, both groups had reached a criterion of >85% correct. During extinction test sessions, dose-response tests indicated that both the ergonovine and lergotrile cues were dose-dependent. LSD (0.04-0.24 mg/kg) and lisuride (0.02-0.08 mg/kg) were shown to substitute completely for the training drug cues in both groups. Administration of the direct dopamine (DA) agonist apomorphine (0.01-0.25 mg/kg) was followed by drug-appropriate responding in the lergotrile-trained group but saline-appropriate responding in the ergonovine group; at the doses tested (1.0-2.0 mg/kg), the direct serotonin (5-HT) agonist quipazine did not mimic either cue. Attempts to antagonize the discriminable effects of ergots suggest that the DA antagonist haloperidol (0.0625 mg/kg) attenuates the lergotrile cue (partially), but has little or no effect on the ergonovine cue.

While these preliminary results might support our earlier hypothesis (Holohean et al., Eur. J. Pharmacol., 81: 595, 1982) that DA plays a prominent role in mediating the effects of lergotrile, the mechanism(s) underlying the ergonovine cue remain obscure. More extensive research, in which several DA, 5-HT and noradrenergic agonists and antagonists are being administered alone or in combination with the training drug, is in progress.

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38.22 LONG-LASTING ANTICONVULSANT EFFECTS OF DIAZEPAM ARE STRAIN DEPENDENT. Sandra E. File, Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London WC1N 1AX, England.

Paul et al (Nature 281:688,1979) reported a high correlation between receptor occupancy by diazepam and protection against pentylenetetrazole-induced seizures. A full anticonvulsant effect was achieved as long as 15 h after diazepam injection, at a time when brain concentrations of the parent compound and its metabolite would be very low. Such long-lasting protective effects of diazepam have both theoretical and clinical importance; we therefore tried to replicate the results, using several mouse strains and more than one convulsant agent.

We tested two inbred strains (C3H/HE from Olac, and NIH from Hacking & Churchill) and two outbred strains (Tuck No. 1 strain, and CFLP from Hacking & Churchill). Male mice (n=8/group) were injected with 4 mg/kg diazepam and 1.5, 3, 6, 12, 18 or 24 h later they were challenged with a convulsant dose of pentylenetetrazole (80 mg/kg) or of picrotoxin (8 mg/kg). The latencies to show myoclonic jerks and full tonic-clonic convulsions were scored. Six hours after diazepam injection, 90% of the C3H/HE mice were protected against pentylenetetrazole seizures, but only 60% of the CFLP mice, 25% of the NIH and 12.5% of the Tuck mice. By 12 h even the C3H/HE mice showed 0% protection. The protective effects of diazepam against picrotoxin-induced seizures lasted no longer.

Thus we have been unable to show a full anticonvulsant effect any longer than 6 h after diazepam injection, and for this length of time only in one strain. The clear strain differences in the duration of diazepam's effects are likely to be pharmacokinetic in nature (Schweri,WCBR Jan. 1983). It seems that the long-lasting protection reported by Paul et al may apply only to the strain of mice used in that study. We used the same doses of diazepam and pentylenetetrazole and our longest-lasting protection was found in a strain closely related to that used in the original study.

38.23 CONDITIONED EMOTIONAL RESPONDING: EFFECTS OF SAFETY SIGNALS ARE BLOCKED BY A BENZODIAZEPINE ANTAGONIST. K. L. Hadskis and H. A. Robertson. Depts. of Psychology and Pharmacology, Dalhousie University, Halifax, N.S., Canada, B3H 4H7

Since the discovery of a specific receptor for benzodiazepines in 1977, it has been postulated than an endogenous benzodiazepine-like compound might exist. However, there has as yet been no conclusive proof for the existance of such a ligand. In order to provide proof for the existance of such a compound, we have studied the effects of a benzodiazepine agonist and antagonist on conditioned emotional responding in rats. A benzodiazepine agonist with anxiolytic but no anticonvulsant properties (PK 9084), and a benzodiazepine receptor antagonist (CGS 8216) which appears to lack any of the partial agonist effects seen with Ro15-1788 (Robertson & Riives, Brain Res., in press) were studied. The phenol quinoline PK 9084 effectively displaces ^{3}H-benzodiazepines from receptor sites in vitro and in our experiments significantly increased responding in rats experiencing chronic fear. It was without effect on animals given a safety signal (and consequently not experiencing chronic fear). CGS 8216 significantly decreased responding in the animals receiving the safety signal but had no effect on the chronic fear group. It also antagonized the anxiolytic effects of PK 9084.

These results support the hypothesis that safety signals produce their effects by causing a release of an endogenous benzodiazepine-like compound. The reversal of the effects of PK 9084 by CGS 8216 provides evidence that this anxiolytic agent produces its effects through a benzodiazepine receptor, perhaps a receptor which mediates anxiolytic effects uniquely. (Supported by the MRC).

38.24 INTERACTION OF BENZODIAZEPINE ANTAGONISTS AND NEUROTRANSMITTER ANTAGONISTS WITH CHLORDIAZEPOXIDE UNDER A DIFFERENTIAL REINFORCEMENT OF LOW RATE (DRL) SCHEDULE. John M. Carney* and Mitsutaka Nakamura* (SPON: R. Blair). Dept. of Pharmacology, Univ. Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Male Sprague Dawley (SD) rats were food deprived to 80% of ad libitum feeding weight and trained to respond under a differential reinforcement of low rate (DRL) 15 sec schedule of food reinforcement. Under control conditions approximately 60% of the total DRL responses resulted in food reinforcement. Relatively low doses of chlordiazepoxide produced a dose related increase in the non-reinforced DRL responses and a decrease in the percentage of food reinforced responses. A dose of 32 mg/kg chlordiazepoxide produced decreases in total DRL responses and resulted in a roughly equal number of reinforced and non-reinforced responses. Both CGS8216 and RO15-1788 blocked the effects 10 mg/kg chlordiazepoxide, which produced substantial increases in non-reinforced responding. Pretreatment with 10 mg/kg methysergide had no direct effect on DRL behavior and failed to alter the effects of 10 mg/kg chlordiazepoxide Bicuculine (3 mg/kg) and Picrotoxin (3 mg/kg) also failed to modify the effects of 10 mg/kg chlordiazepoxide. In contrast to the interaction of these compounds with the relatively low chlordiazepoxide dose, antagonism of the behavioral suppressant dose of chlordiazepoxide (32 mg/kg) was obtained with RO15-1788, CGS8216 and bicuculine. A bicuculine dose of 3 mg/kg reversed the disruption produced by 32 mg/kg chlordiazepoxide and returned the DRL responding to its normal IRT pattern. The data to date are consistent with a benzodiazepine/GABA receptor mediated mechanism for suppression behavior, but do not support a simple GABA mediated mechanism for the response rate increasing and possibly the anxiolytic effects.

38.25 NORADRENERGIC INVOLVEMENT IN THE YOHIMBINE-INDUCED EXCITATION OF ACOUSTIC STARTLE: EFFECTS OF 6-OHDA AND DSP4. John H. Kehne* and Michael Davis. Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508 (SPONSOR: J. Tallman).

The acoustic startle reflex in rats has been shown to be augmented by IP administration of the α_2-adrenergic antagonist yohimbine (Davis & Astrachan, Psychopharmacol., 75: 219-225, 1981). The purpose of the present study was to determine whether peripheral and/or central noradrenergic neurons are necessary for the expression of the excitatory effect of yohimbine on acoustic startle.

The neurotoxins 6-hydroxydopamine (6-OHDA) or N-(2-chloroethyl)- N- ethyl-2-bromobenzylamine (DSP4) were used to produce depletions of norepinephrine (NE). The ability of yohimbine (1.25 mg/kg, IP) to increase startle was tested in neurotoxin-pretreated rats or their appropriate controls.

Depletion of both central and peripheral NE with DSP4 (50 mg/kg, IP; 1-2 days prior to testing) completely blocked the excitatory effect of yohimbine on acoustic startle. The yohimbine-induced excitation was still observed in adrenalectomized rats, indicating that release of adrenal catecholamines is not involved. Furthermore, pretreatment with intravenous 6-OHDA (20 mg/kg, 1-3 days prior to testing) failed to block the yohimbine excitatory effect, indicating that peripheral noradrenergic neurons are not involved. In contrast, when central noradrenergic denervation was produced by direct infusion of 6-OHDA into the lateral ventricle, a significant attenuation of the yohimbine-induced excitation was seen. Since there is evidence that activation of spinal noradrenergic neurons facilitates the startle reflex (Astrachan & Davis, Brain Res. 206: 223-228, 1981), the effect of yohimbine was assessed in rats specifically depleted of spinal NE. Intrathecal administration of 6-OHDA into the lumbar spinal cord, which causes a potent and selective reduction in spinal NE content, blocked the excitatory effect of yohimbine on startle.

In summary, these data indicate that central, and in particular, spinal NE-containing neurons are necessary for the expression of yohimbine-induced excitation of acoustic startle.

We would like to thank Dr. G. Jonsson, Dr. S. Ross, and Astra-Lakemedel AB for generously supplying the DSP4. Supported by NSF Grant BNS-8120476 and the State of CT.

39.1 AN ANIMAL MODEL FOR EVALUATING THE BEHAVIORAL EFFECTS OF THE INTERACTION OF EXERCISE AND CNS DRUGS. S.B. McMaster* and J.M. Carney* (SPON: H.D. Christensen). Dept. of Psychiatry and Behavioral Sciences and Dept. of Pharmacology, College of Medicine, Univ. of Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Previous work has shown that exercise in the form of treadmill running results in enhanced sensitivity to the behavioral effects of a variety of compounds (Carney, J.M.; Nakamura, M. and Christensen, H.D. Pharmacologist 24:130, 1982). Rats tested on an operant task exhibit greater performance disruption in response to drugs administered following an exercise session than to either drug or exercise alone. This effect has been demonstrated with muscarinic antagonists and psychomotor stimulants following fixed speed and duration running.

The present study was undertaken to examine various parameters of the exercise treatment. Male Sprague-Dawley rats (275-300g) were food deprived to 80% of their ad lib feeding weight and trained to respond under a multi-component fixed ratio (FR) 30 time out (TO) 10 min. schedule. Each daily session consisted of four alternating FR and TO components. Under this schedule, relatively little responding occurred during the TO components; response rates during the FR components were between one and two responses per sec with little intra-subject variability. Exercise conditions tested included four treadmill speeds and four session durations.

These conditions resulted in a graded disruption of operant behavior. The degree of impairment was directly related to the level of exercise. Exercise-induced changes in body temperature were not predictive of behavioral disruption. For example, running at a speed of 1 mph for 30 and 60 min resulted in equivalent temperature increases. In contrast, 30 min of treadmill exercise resulted in no significant disruption of performance while 60 min of exercise produced significant decreases in operant behavior. The impact of exercise speed and duration on the behavioral potency of muscarinic antagonists and the suitability of this paradigm as a model of the CNS effects of exercise will be discussed. (Supported in part by USAMRDC DAMD 17-81-C-1246.)

39.2 MORPHINE PLACE-PREFERENCE: EXAMINATION OF THE PARADIGM AND ITS ASSOCIATIVE PROPERTIES. A. Rackham, A. Blander*, & Z. Amit. Center for Studies in Behavioral Neurobiology, Concordia University, 1455 de Maisonneuve Blvd. W., H1013, Montreal, Quebec, Canada, H3G 1M7.

Previous morphine place-preference (P.P) studies have shown that rats will spend from 29-66% of the time in an environment where they experienced the effects of morphine. To examine the possibility that the low magnitude of morphine P.P. is a function of weak associations formed between the drug and the environment, the following studies sought to determine whether there is any difference in the magnitude of P.P. for a previously established non preferred side between rats that experienced the effects of morphine (10mg/kg., ip.) and rats that experienced the effects of morphine in the home cage. Expt.1 included 4 habituation days followed by 3 repeated trials ((4 Drug-Treatment (D.T.) days and 1 non-drug Test day)). Following habituation days, rats were divided into 8 equal groups (n=6) which determined the place (non preferred side or home cage) and sequence of D.T. (established by a latin square design) over the 12 D.T. days. Results showed no significant (sig.) difference in time spent on the non preferred side of the box as a change from habituation days, between groups, over Test days 1,2 and 3. Thus rats that never experienced the effects of morphine in the non preferred side of the box during D.T. days, spent the same amount of time on that side over Test days as rats that were conditioned with morphine in that side over D.T. days. To determine whether the time spent on the non preferred side was in fact opiate receptor mediated, all rats in expt.2 were pretreated with naloxone (7mg/kg.ip.) prior to morphine injections. The procedure was identical to that used in expt.1. Results showed no sig. difference between groups. Within groups analysis showed that the shift in time spent on the non preferred side of the box was blocked by naloxone when D.T. took place in the non preferred side of the box, however, not when D.T. took place in the home cage. These results suggest that the low magnitude of preference observed in morphine P.P. studies may be attributed to the inability of rats to form strong associations between the effects of morphine and the environmental cues in the box.

39.3 RELIABILITY AND VALIDITY OF THE CUMULATIVE DOSING PROCEDURE FOR BEHAVIORAL PHARMACOLOGY, M.F. Jarvis§ E.A. Walker§ E.L. Rameriz§ G.C. Wagner. Dept. Psychology, Rutgers Univ. New Brunswick, NJ.

The cumulative dosing procedure has recently been applied to schedule-controled and drug discrimination paradigms. The main advantage of the procedure is that an entire dose-response curve may be determined in one day. To date,however, there has been no systematic attempt to establish its reliability or validity.

11 male Long Evans rats were trained to respond for water delivery on an FI 90 sec schedule. When responding had stabilized, rats were advanced to the cumulative dosing schedule of five 10 min sessions separated by 5 min time out periods. Amphetamine (AMPH) was administered i.p. in doses of 0.5, 0.5, 1.0, 2.0, & 4.0 mg/kg starting 5 min before the first session and then repeated at the start of each time out period. This procedure was repeated following 2 baseline days. Thereafter, single daily sessions of 30 min were conducted. When responding had restabilized, AMPH (.5, 1.0, 2.0, 4.0, 8.0) was administered every third day 15 min before the session (traditional dose response curve).

Under baseline (saline) conditions, the response rates for the 5 daily (cumulative) sessions were 12.1, 12.4, 13.1, 13.3, 13.6 ($\bar{x}$=12.9) resp/min and for the single (traditional) session was 11.6 resp/min. There baseline response rates were not significantly different. Across rats the correlation for the mean cumulative and traditional response rates was .62 ($p<.05$). Also under baseline conditions, the quater life values were .67, .67, .69, .67, .69 ($\bar{x}$=.68) for the cumulative and .72 for the traditional procedures. These baseline quater life values were significantly different but correlated across all rats ($r=.81; p<.01$).

Amph disrupted responding in a dose-dependent manner (albeit differentially) in the cumulative (ED50=4.7mg/kg) and traditional (ED50=3.4mg/kg) procedures. Although significantly different, these ED50 values were highly correlated ($r=.73; p<.01$). The ED50 obtained in the first and second cumulative determinations were 5.7 and 3.3mg/kg. These values were significantly different but correlated across rats ($r=.95; p<.01$). Finally,AMPH exerted rate dependent effects, thereby, producing a decrease in quater life values. For the five doses of AMPH the quater life values were .63, .55, .44, .27, .11. (cumulative) and .67, .58, .32, .35, & .08 (traditional). Except at the lowest AMPH dose, these quater life values did not correlate.

These results indicate that the rate but not pattern of responding in multiple sessions is similar to that obtained in a single session and that the cumulative dosing procedure is reliable and valid. However, differences in the pattern of responding may interact with the rate dependent effects of a drug.

39.4 ANALGESIA IN DEFEATED MICE: EVIDENCE FOR MEDIATION VIA CENTRAL RATHER THAN PITUITARY OR ADRENAL ENDOGENOUS OPIOIDS. Michael L. Thompson* and Klaus A. Miczek (SPON: L. Shuster). Dept. of Psychology, Biochemistry and Pharmacology, Tufts Univ., Medford and Boston, MA 02155.

It has been suggested that endogenous opioids released from the pituitary and/or adrenal glands are critical for the analgesia produced by stress. Recently, we have demonstrated that mice subjected to defeat in a confrontation with another mouse exhibit pronounced analgesia which is mediated by endogenous opioids, as evidenced by naloxone reversibility and morphine cross-tolerance. The present experiments pursued two different approaches in order to determine the relative contributions of endogenous opioid sources in the brain vs. those from the pituitary and adrenal glands.

In order to investigate the contribution of the adrenals, separate groups of B6AF mice were (1) adrenalectomized one week prior to defeat, (2) pretreated with reserpine (2 mg/kg IP) for two days prior to defeat, or (3) injected with corticosterone (1 mg/kg) 30 min prior to defeat. Neither elimination nor facilitation of adrenal activity affected the analgesic response observed following defeat. These data suggest that adrenal activation during defeat is not crucial for this type of analgesic response. Since we have previously demonstrated that reduction of beta-endorphin from the pituitary by dexamethasone or hypertonic saline pretreatment did not alter the analgesic response, we conclude that endogenous opioids from the CNS and not from peripheral sources mediate this analgesic response.

In further experiments we determined possible sites in the brain at which endogenous opioids may mediate the analgesic response in defeated mice. B6AF mice were implanted with a guide cannula; 2.5-10 µg naloxone or morphine were infused in 2.5-5 µl through a 33-ga. injector cannula into the periaqueductal gray or the arcuate nucleus. After the sites were determined to produce morphine analgesia, naloxone was infused immediately prior to the defeat test. When infused into periaqueductal gray or arcuate n., but not into surrounding regions, naloxone abolished the analgesic response. These results indicate that analgesia from defeat is produced by action of endogenous opioids originating from CNS sources on classic pain pathways.

39.5 THE SALINE EFFECT: BEHAVIORAL RECOVERY FROM BRAIN DAMAGE FOLLOWING INTRACRANIAL INJECIONS OF AN ISOTONIC SUBSTANCE. R. Labbe*, D. G. Stein and B. A. Sabel (SPON: D. Chad). Brain Res. Lab., Dept. Psychology, Clark Univ., Worcester, MA 01610.

We have recently shown (Sabel and Stein, Physiol. Behav., 28:1017, 1982) that isotonic saline, a widely used control substance, prevented behavioral deficits, neuronal death and reactive gliosis when injected intracerebrally following caudate nucleus (CN) damage. With the present experiment we attempted to replicate our previous findings and to determine whether sparing or recovery had taken place.

Thirty male, Long-Evans rats (170 days old, 360-470 g) were randomly assigned to the five surgical groups (n=6): group C received sham operations, whereas all other groups received bilateral intracerebral injections of 5 ul of isotonic saline immediately following surgery 1.5 mm posterior to the lesion site. The animals survived either 7 days (groups 7S and 7L) or 31 days (31L and 31S) after surgery. In the 31-day survival group, secondary lesions were made in the CN on day 25 to prepare the brains for subsequent Fink-Heimer analysis. The animals' behavior was tested from postoperative day 2-6 (7L and 7S) or days 10-25 and 27-31 (31L and 31S) on a 2-choice footshock learning maze without undergoing spatial reversals.

Confirming our earlier results, saline-treated animals (31S) showed (1) less refusals to run, (2) shorter latencies to reach the goal box and (3) more avoidances on significantly more days than rats of group 31L. No differences were found between groups 7S and 7L. It is interesting to note that various behavioral and lesion measures correlated significantly in group 31S but not in group 31L. These findings may be taken to suggest that recovery of function rather than sparing had taken place. Presently, using the Fink-Heimer technique, we are studying whether saline may have prevented degeneration secondary to brain trauma. It should be emphasized again that the assumption that saline is a "neutral" substance with no effects on morphology and behavior may have to be reconsidered.

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39.6 TOLERANCE TO AND WITHDRAWAL FROM THE EFFECTS OF CHRONIC DEFEAT. L. Shuster, M.L. Thompson*, K. A. Miczek and J.T. Winslow*. (SPON:Herbert Barry, III). Depts. of Biochemistry, Pharmacology, and Psychology, Tufts Univ., Boston and Medford, MA 02111.

Mice subjected to repeated attack by another mouse display an analgesic response that is fully blocked by naltrexone or naloxone, and shows complete cross tolerance with morphine. The aim of the present series of experiments was (1) to characterize the tolerance phenomenon in greater detail and (2) to determine whether or not physical dependence develops to the endogenously released opioids.

B6AF mice were exposed to daily defeat for 5 to 6 days. The development of tolerance to the effects of defeat was assessed by the tail flick method. Immediately following defeat on the 6th day, mice were observed closely for 2 hours for standard signs of spontaneous withdrawal. On the following day, the same mice were defeated and then observed for withdrawal signs for 20 mins. following the injection of 10 mg/kg naloxone, IP. Results for these mice were compared to a group of mice subjected to a single defeat test immediately before the withdrawal test.

Tail flick latency following defeat was significantly reduced by the third day, and continued to decline until by the 6th day the response was similar to baseline. Mice showed very few signs of spontaneous withdrawal. However, chronically defeated mice displayed significant amounts of withdrawal jumping following naloxone injection on the 7th day. These results suggest that a dependence-like phenomenon develops to physiologically released endogenous opioids following chronic defeat.

Defeat tolerant mice showed cross tolerance to morphine and also to the putative kappa receptor agonist ketocyclazocine. Defeat tolerant mice showed reduced sleep time to barbiturates, but not to alcohol.

In additional experiments, acute defeat experience disrupted operant fixed-ratio performance. Within 3-14 days of chronic defeat, baseline operant performance was recovered. However, the dose effect function for morphine on fixed-ratio performance was the same before and after defeat. The results suggest that the effects of chronic defeat are not limited to pain perception but extend to other behavioral processes.

39.7 BEHAVIORAL EFFECTS OF SARIN IN RATS. M.R. Landauer*, J.A. Romano, R.M. Alvarez*, and J.H. McDonough (SPON: M.J. Kallman). US Army Chemical Systems Laboratory and US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

The effects of the organophosphate sarin (isopropyl methyl phosphonofluoridate) on the establishment of a conditioned taste aversion (CTA), rotorod performance (RTR) and spontaneous motor activity (SMA), as well as the LD50 were evaluated in 171 adult male rats. In Exp 1, rats were acclimated to a daily 30 min period of water availability. When consumption had stabilized, they were given a 30 min access period to 0.2% saccharin (SAC) solution which was immediately followed by a sc injection of 61, 71, 84, 98 µg/kg sarin or the saline vehicle. Three days later, the animals (N=6-7/group) were given a two-bottle choice test (SAC vs water) and the percent SAC consumed was calculated. The ED50 was determined by graphical interpolation from the dose effect curve and was calculated to be 71 µg/kg sarin. In Exp 2, motor coordination was evaluated by placing rats on an accelerating rotorod (0-45 rpm in 90 sec). Time spent on the rotorod in sec was recorded as the dependent variable. Rats received four training trials and 30 min prior to the 90 sec test trial they received a sc injection of 61, 71, 84, 98 or 115 µg/kg sarin or saline (N=7-8/group). Doses of 98 and 115 µg/kg produced performance decrements that differed significantly from saline control values. The ED50 was 95 µg/kg sarin. In Exp 3, locomotor activity was measured by placing an animal into an open-field (41 X 43 cm) and determining the number of photobeam interruptions in a 30 min test. All animals received three 30 min acclimation trials and were administered 61, 71, 84, 98, 115 µg/kg sarin or saline vehicle 30 min prior to the fourth (test) trial (N=6/group). Motor activity was significantly decreased from control values for the two highest doses and the ED50 was determined to be 91 µg/kg sarin. In Exp 4, the 24 hr LD50 was calculated using seven doses of sarin (N=8/group) and found to be 165 µg/kg. The ED50/LD50 ratio for the behavioral measures were thus 0.43, 0.58 and 0.55 for CTA, RTR and SMA, respectively. Of the three tests evaluated, the CTA procedure appears to be the most sensitive in detecting the behavioral toxicity of the organophosphorous compound, sarin.

39.8 HYPERTHERMIC RESPONSES TO THYROTROPIN RELEASING HORMONE (TRH) AND CHOLERA ENTEROTOXIN (CE) ARE MEDIATED BY DIFFERENT MECHANISMS. M. Cohn, D. Taube*, D. J. Wooten*, and M. L. Cohn. Dept of Anesthesiology Research, C.R. Drew Med Sch, Los Angeles, CA 90059

Anti-inflammatory steroids (AIS) and nonsteroidal anti-inflammatory drugs (NSAID) have been shown to inhibit prostaglandin (PR) synthesis. While a strong correlation has been established between anti-inflammatory properties of both groups of drugs and their potencies as inhibitors of PR synthesis, AIS have been found to inhibit phospholipase activity and NSAID to directly inhibit PR synthetase complex. Data indicating that thermic response to TRH is blocked by NSAID (Cohn et al, 1980) combined with reports that antipyretic properties of NSAID are also associated with PR inhibition suggested that TRH produces hyperthermia by stimulating PR synthesis and release. More recent observations that AIS fail to suppress thermic response to TRH (Cohn et al, 1983) suggest that antipyretic activities of AIS and NSAID are regulated by different mechanisms. To test further this hypothesis, we compared here the antipyretic effects of AIS and NSAID on thermic responses to TRH and CE. Male Sprague-Dawley rats (250g) were implanted with cannulae secured 2 mm above preoptic anterior hypothalamic (PO/AH) nuclei. After 10-day recovery period, rats were treated with either lysine acetylsalicylate (.32 µM), tolmetin (.40 µM), acetaminophen (.10 µM), hydrocortisone sodium succinate (HSS - .03 - .7 µM) or the vehicle phosphate buffer injected into PO/AH 5 min prior to injection of either TRH (.003 - .14 µM) or CE (.2 ug) into same locus. Test substances were freshly prepared, Millipore filtered and injected over 60 sec period in constant volume of .5 ul into PO/AH through injection needles 2 mm longer than guide cannulae. Equipment was sterilized prior to use. Temperature (T^{o}) was recorded every 30 min for 3 h prior to treatment and every 15 min thereafter with thermistor probe inserted 6 cm into colon. Groups consisted of at least 6 rats for each drug and dose. Each rat was used once; sites of injections were histologically confirmed upon completion of experiment. Injected into PO/AH, TRH increased T^{o} up to 3^{o} C for 6-8 h while TRH-induced behavioral alterations rarely exceeded 20-30 min. Soluble aspirin, tolmetin and acetaminophen blocked thermic response to TRH but failed to antagonize the behavioral manifestations. Like phosphate buffer, HSS was ineffective in blocking TRH-induced hyperthermia and behavioral manifestations. Microinjected into PO/AH, CE increased T^{o} for well over 3 h. Prior injection of either NSAID or HSS into PO/AH failed to suppress febrile response to CE. Our data suggest that, 1) NSAID suppress hyperthermia through central mechanisms while antipyretic activity of AIS is regulated by peripheral mechanisms; and 2) TRH and CE elicit hyperthermia through different mechanisms.

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39.9 ABSTINENCE SYNDROME FOLLOWING CONTINUOUS INFUSION OF CLONIDINE. R.J. Exley*, D.H. Malin, A.G. Hempel* and T.H. Schauweker*. Univ. of Houston-Clear Lake, Houston, Tx. 77058.

The alpha-2 adrenergic agonist, clonidine, has a number of opiate-like actions. These include sedative, anxiolytic, analgesic and respiratory-depressing effects. In addition, like the opiates, clonidine can potently reverse many symptoms produced by opiate abstinence and continuous naloxone infusion. In view of these similarities, it was hypothesized that continuous clonidine infusion might produce a state of dependence similar to opiate dependence.

Four male rats were implanted s.c. under either anesthesia with Alzet model 2001 osmotic minipumps filled with 4 mg/ml clonidine in normal saline. These rats received .033 mg/kg/hr clonidine on a continuous basis. Four other rats were implanted with osmotic minipumps filled with saline alone. After 120 hrs. of infusion, all pumps were removed. Twenty four hours after removal, all animals were observed for 15 min. for standard behavioral signs seen in opiate withdrawal. The clonidine abstinent group displayed 23.0 ± 2.3 symptoms (mean ± SEM), while the control group displayed 1.2 ± .9 symptoms. This difference was significant, $p < .001$. The clonidine abstinent group had highly elevated numbers of wet-dog shakes, head shakes, abdominal writhes, scratches and teeth chattering.

At 25 hours post pump removal, all animals received a low subcutaneous dose of morphine sulphate (4 mg/kg). Fifteen minutes later, all rats were observed again. As in opiate abstinence syndrome, morphine virtually reversed the symptoms. The clonidine-abstinent group and the control group had 2.5 ± 1.8 and 0 ± 0 symptoms, respectively. By the 39 hours post pump removal, the clonidine abstinent group again had significantly more overall symptoms than the control group: 18.8 ± 3.9 vs 3.8 ± 2.4, respectively. At 40 hrs. post pump removal, all rats were injected with .033 mg/kg clonidine s.c. Fifteen minutes post injection, all rats were observed again. Once more, the symptoms were virtually reversed. The clonidine abstinent group and the control group had 0.5 ± 0.3 and 0 ± 0 overall symptoms, respectively.

The present findings may be consistent with several reports in the clinical literature of patient distress following abrupt termination of clonidine treatment. The present findings may also have implications regarding mechanisms of opiate dependence. One of the many effects of opiates is suppression of brain adrenergic systems. The data presented here suggest that prolonged suppression of adrenergic systems alone may be sufficient to produce a state of dependence, and account for certain behavioral symptoms of opiate abstinence syndrome.

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39.10 NORADRENERGIC MEDIATION OF ETHANOL INDUCED DEPRESSION OF LOCOMOTOR ACTIVITY. M. Abitbol*, C.M.G. Aragon*, Z.W. Brown, and Z. Amit. Center for Studies in Behavioural Neurobiology, Concordia University, 1455 DeMaisonneuve Blvd., Montreal, Que. Canada, H3G1M7.

The effect of FLA-57 on ethanol induced excitation and depression of locomotor activity was tested in the open field. Male Long Evans rats were pretreated with the dopamine-beta-hydroxylase inhibitor, FLA-57 (30mg/kg), or saline for five days. On day six rats were intraperitoneally injected with either one of three doses of ethanol (0.5, 0.8 or 2.0gm/kg), or saline and were then immediately placed in the open field. Locomotor activity was measured at 5 minute intervals for a 30 minute period. Immediately after the 30-minute period animals were sacrificed by decapitation. Trunk blood was collected and gas chromatographic analysis was used to determine blood ethanol levels.

The brains were rapidly extracted and then assayed by High Pressure Liquid Chromatography (HPLC) with electrochemical detection. No variation in the levels of ethanol were found in animals pretreated with FLA-57 or saline. HPLC norepinephrine determinations revealed that whole brain NE levels were reduced by 25% in comparison to controls.

The results show that treatment in all three doses of ethanol produces a dose dependent depression of locomotor activity and this depression seems to be attenuated with animals pretreated with FLA-57. In addition, it is interesting to note that significant positive correlations were found between activity and NE levels in saline-saline animals. Data also indicate a significant negative correlation between activity and ethanol levels in saline-ethanol animals with all three doses. No significant correlations between activity and NE levels or activity and ethanol levels were found with groups receiving FLA-57 pretreatment. The results are discussed in terms of the possible role of the central noradrenergic system in the effects of ethanol on locomotor activity.

39.11 INTRACEREBRAL INFUSIONS OF CARBACHOL AFFECT MALE SEX BEHAVIOR. E. M. Hull, D. Bitran*, E. A. Pehek*, and L. G. Clemens. Dept. of Psychology, State Univ. of New York at Buffalo, Amherst, NY 14226; and Dept. of Zoology, Michigan State Univ., East Lansing, MI 48824.

Feminine sexual behavior is facilitated by intracerebral infusions of cholinergic agonists in male and female rats. This facilitation is blocked by peripheral injections of the muscarinic antagonist atropine. Peripheral cholinergic manipulations in male rats have yielded contradictory results, suggesting variously that cholinergic mechanisms facilitate, impair, or fail to affect masculine sex behavior. The effects of central cholinergic manipulations on masculine sex behavior have not been explored previously.

The medial preoptic area (MPOA) is an important regulatory area for the expression of masculine sex behavior. Preoptic lesions disrupt copulation, while electrical stimulation of the area facilitates it. Furthermore, testosterone implanted in the MPOA restores copulation in castrated males. The present study investigated the effects on masculine sex behavior of the cholinergic agonist carbachol, infused into the MPOA and the lateral ventricles (LV).

Cannulae were implanted bilaterally in the MPOA or the LV of male Long-Evans rats. Carbachol (.10 µg or .25 µg) or the artificial cerebrospinal fluid vehicle was slowly infused in .5 µl volumes into each cannula. Tests with a receptive female began immediately and lasted until 30 min after the first intromission.

When compared to vehicle, infusion of carbachol into the LV significantly delayed the onset of masculine sex behavior. Infusions of carbachol into the MPOA also increased the latency to begin copulation, but then decreased the number of intromissions required for an ejaculation. Other measures of sex behavior, including the time interval between intromissions, were not significantly altered by carbachol infusions.

These data may suggest a cholinergic inhibition of sexual arousal in male rats, and a more localized cholinergic enhancement, within the MPOA, of a copulatory mechanism.

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39.12 EFFECTS OF PYRIDOSTIGMINE AND SOMAN ON OPERANT BEHAVIOR IN THE RAT H.E. Modrow* and J.H. McDonough. (SPON: B. Hackley). U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Anticholinesterase compounds typically depress behavioral outputs in a variety of behavioral test situations. This depression of behavior affords a distinct end-point in the study of the toxicology of these compounds. Recovery of normal behavior provides another objective measure of the impact of toxic compounds on behavioral function. The purpose of the present study was to compare the effects of two anticholinesterase compounds, the peripherally acting reversible carbamate, pyridostigmine, and the centrally active irreversible organophosphate, soman, on steady-state variable interval (VI) operant performance in rats.

Twenty-three rats were trained to lever press for sweetened milk reinforcement on a VI-60 schedule. Session length was 60 min with responses printed out in five min blocks. A performance stability criterion of $\leq \pm$ 10% variation from the mean of three consecutive days performance was imposed on each animal for each drug test. Pyridostigmine Br (vehicle, 0.01, .032, .10, .32, 1.0 mg/kg) was administered i.m. according to a Latin square design. Testing began immediately following injection, thus allowing observation of both the onset and decline of drug effect. Following completion of the pyridostigmine dose effect curve, the rats were tested with one of five doses of soman, 45.6, 50.0, 54.8, 60.1, or 65.9 µg/kg. Although the test was conducted identically to the pyridostigmine tests, each animal was tested at only one dose of soman due to its permanent bond with the cholinesterase molecule.

Low doses of pyridostigmine did not appear to have a effect on performance. Only 1.0 mg/kg pyridostigmine produced a significant reduction in responding, a decline to approximately 67% of baseline levels. Soman-induced effects were markedly dissimilar. Total cessation of operant responding was seen in nearly all animals. The time to cessation of responding was found to be dose dependent.

Operant response rates returned to baseline levels within two days after every dose of pyridostigmine. This was not the case with soman. Toxicity was seen after the three highest doses. The surviving animals at the dose of 54.8 µg/kg soman rapidly reattained baseline levels of performance. However, surviving animals tested at 60.1 and 65.9 µg/kg soman required in excess of nine days to return to baseline levels. This effect may be due to tolerance, as cholinesterase levels were still very low, or simply to recovery from the acute debilitating effects of soman exposure.

39.13 BEHAVIORAL EVIDENCE THAT NICOTINE ADMINISTRATION HAS ANXIOLYTIC ACTIONS IN RATS. C.A. Sorenson and L.O. Wilkinson*. Neuroscience Program, Amherst College, Amherst, MA 01002.

A series of experiments was designed to examine possible anxiolytic actions of nicotine using the acoustic startle response (ASR), a skeletal reflex elicited by the presentation of a loud tone, and the potentiated startle effect (PSE), an increase in the magnitude of the reflex which occurs if the tone is presented during a light stimulus previously paired with footshock. Since all known anxiolytic drugs depress or block the PSE, it appears to be a valid model of anxiety.

The first experiment tested the effect of nicotine administration on the PSE. Rats were trained over a two day period to associate a dim light with footshock. On the following day, they were presented with 80 115 db tones, of which half were accompanied by the light. Half of these animals were pre-injected with .4 mg/kg nicotine, s.c., and the others received the saline vehicle. Nicotine treated rats showed a significant attenuation of the PSE with no change in startle baseline. Thus, nicotine appears to have anxiolytic properties by this test.

The second experiment tested the effect of nicotine withdrawal on baseline ASR. It was predicted that if nicotine has anxiolytic effects, animals withdrawn from chronic treatment should show a rebound anxiogenic effect. Two groups of rats were administered a low (approximately 3 mg/kg) and a high (approximately 15 mg/kg) dose of nicotine in their drinking water. Rats withdrawn after 10 days from the high dose and tested daily for 4 days with 40 tones showed a significant increase in ASR on day 3 of withdrawal compared to rats maintained on the high dose. Rats withdrawn after 15 days from the low dose showed no change in ASR amplitude compared to controls on each of the 4 daily tests following withdrawal.

The third experiment examined the possible locus of action of nicotine by comparing the effects of nicotine and diazepam administration on the increase in ASR produced by treatment with 5.0 mg/kg of the anxiogenic drug, yohimbine, which is known to increase locus coeruleus unit activity. Yohimbine-enhanced startle appeared to be partially depressed by the administration of 1.25 mg/kg diazepam, i.p., whereas the administration of .4 mg/kg nicotine, s.c., had no effect on yohimbine-enhanced startle. These results suggest that the anxiolytic effects of diazepam and nicotine are mediated at least in part by different neural mechanisms.

39.14 DOES NICOTINE STIMULATE LOCOMOTOR ACTIVITY BY ACTING CENTRALLY? P.B.S. Clarke* and R. Kumar* (SPON: R. J. Dooling) Dept. of Psychiatry, Institute of Psychiatry, London SE5 8AF, U.K.

We have previously reported that in non-tolerant rats, (-)-nicotine depressed and then increased locomotor activity. With repeated drug testing, the initial depressant action was replaced by a marked stimulation which was prevented by systemic injection of the secondary amine mecamylamine, but not by systemic injection of the quaternary ganglion blocker hexamethonium. Since neither nicotinic antagonist altered locomotor activity when given alone, we suggested that nicotine may increase locomotor activity by stimulating central receptors (Br. J. Pharmac. 78, 329-37). However, hexamethonium has surprisingly low peripheral potency in rats (Romano, C. J. Pharm. Exp. Ther. 217, 828-33, 1981), and so we have reexamined this conclusion using another quaternary nicotinic antagonist, chlorisondamine. As previously described, rats were tested for gross locomotor activity in photocell cages, for 80 minutes starting immediately after sc injection of (-)-nicotine bitartrate (neutralized, dose as base) or saline (control).

The first experiment investigated whether the development of tolerance depended on repeated exposure to the photocell cages. Male rats were randomly allocated to 4 groups, each of 8 subjects. Groups 1 and 2 were tested daily for 5 consecutive days with saline and nicotine (0.4 mg/kg), respectively. The remaining two groups received equivalent injections of saline or nicotine in their home cages and were not tested. On days 6 and 7, all 32 subjects were tested once with nicotine (0.4 mg/kg) and once with saline, counterbalanced within each group. Tolerance to the locomotor depressant action occurred only in rats pretreated with nicotine ($p < 0.0001$) and was unaffected by apparatus familiarity.

Subsequent experiments employed nicotine-tolerant rats. Locomotor activity was increased in a stereospecific way; (-)-nicotine (0.1-0.4 mg/kg) was about ten times more potent than (+)-nicotine (0.4-1.6 mg/kg). Mecamylamine (1.0 mg/kg), given sc either 20 minutes before or 20 minutes after nicotine (0.4 mg/kg), blocked the drug's stimulant action. Pretreatment with chlorisondamine (0.01, 0.1 mg/kg sc) had little or no such effect, whereas 2 µg (base) given intraventricularly completely prevented the nicotine-induced locomotor stimulation for at least three weeks. Neither antagonist altered locomotor activity in saline test sessions.

Thus a few injections of nicotine, rather than apparatus familiarity, unmasked a marked locomotor stimulant action which may reflect a tonic stimulation of brain nicotine receptors. Intraventricular injection of chlorisondamine produced an extremely long-lasting blockade of this central action.

PEPTIDES: BIOCHEMICAL CHARACTERIZATION

40.1 THE DISTRIBUTION, CHROMATOGRAPHIC CHARACTERIZATION, AND RECEPTOR INTERACTION OF A PHI-LIKE PEPTIDE IN RAT AND PORCINE BRAIN. M. C. Beinfeld*, D. M. Korchak*, B. L. Roth* and T. L. O'Donohue (SPON: C. L. McLaughlin). Dept. Pharm., St. Louis Univ. Med. Sch. 1402 S. Grand Blvd., St. Louis, MO 63104 and NINCDS, Bethesda, MD 20205.

PHI (acronym for a peptide with a C-terminal histidine and a N-terminal isoleucine amide) is a recently discovered porcine intestinal peptide with strong sequence homology with VIP, secretin, glucagon, GIP (gastric inhibitory peptide), and hGRF (human growth hormone releasing factor). PHI shares many biological actions of VIP, though is frequently less potent than VIP.

Preliminary studies by Christofides et al. indicate that brain contains a PHI-like peptide with a distribution identical to VIP. This observation was confirmed by the isolation of PHI from porcine brain by Tatemoto et al.

A PHI-specific antiserum with weak cross-reactivity with secretin (0.65%) and glucagon (0.46%), but none with VIP, GIP, hGRF, or motilin was developed and used to characterize and measure PHI peptides in rat and porcine brain.

In agreement with Christofides et al., the distribution of PHI in the rat brain closely follows that of VIP. In most of the areas the PHI and VIP concentrations were the same within experimental error. Highest PHI/VIP levels were in cerebral cortex, amygdala, hippocampus, anterior hypothalamus, and striatum. The cerebellum and pineal were very low in both VIP and PHI. In porcine brain the VIP content is much lower than rat brain and the PHI levels are correspondingly lower. This high correlation between the distribution of VIP and PHI agrees with the suggestion of Christofides et al. that PHI and VIP may arise from the same precursor.

C18 HPLC analysis of porcine brain extracts reveals a single peak of PHI-like immunoreactivity which co-elutes with synthetic PHI but which separates from secretin, motilin, and VIP. Rat brain extracts, run on either C18 HPLC or phenyl HPLC chromatographic systems, contain a single PHI-like peptide which separates from porcine PHI, eluting earlier by several minutes. This rat PHI peptide also separates from secretin, VIP, and motilin. This data suggests that rat brain contains a PHI-like peptide with similar but not identical amino acid composition to porcine PHI, which is slightly less hydrophobic. We also determined that PHI had high affinity for inhibition of (^{125}I)-VIP binding in rat brain. In general, PHI had an IC_{50} of 20-30 nM in rat forebrain and approximately 300 nM in rat cerebellum. These data indicated that PHI could serve as an endogenous modulator of VIP binding in rat brain.

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40.2 PHE-MET-ARG-PHE-NH_2-LIKE IMMUNOREACTIVITY IN RAT SPINAL CORD: DISTRIBUTION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY. H.-Y. T. Yang, J. Tang* and E. Costa, Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Previously, we have determined that Try-Gly-Gly-Phe-Met-Arg-Phe (YGGFMRF) is unevenly distributed in rat spinal cords. During the course of this study, Phe-Met-Arg-Phe-NH_2 (FMRF-NH_2)-like immunoreactivity in rat central nervous system including spinal cord was described by immunohistochemical technique (Weber et al., Science 214:1248, 1981). Because of the structural similarity between these two neuropeptides, we have decided also to study, in rat spinal cord, the distribution, biochemical characterization and biological activity of FMRF-NH_2-like peptide. Antiserum to FMRF-NH_2 was prepared by immunizing rabbits with FMRF-NH_2-hemocyanin conjugate. The antiserum shows no significant affinity for CCK 8, CCK 4, substance P, met-enkephalin but cross-reacts with YGGFMRF very slightly (0.1%). Rostro-caudally, FMRF-NH-like immunoreactivity is evenly distributed ranging from 0.21 ± 0.01 to 0.29 ± 0.02 for cervical to sacral region. With respect to dorso-ventral distribution, FMRF-NH_2-like immunoreactivity is higher in dorsal half (0.46 ± 0.02) than in ventral half (0.18 ± 0.01). The values are calculated as pmol FMRF-NH_2 equivalent per mg protein. Biochemical analysis of the immunoreactive material by gel filtration and reverse phase chromotography revealed that the main endogenous FMRF-NH_2-like peptide is larger than the authentic FMRF-NH_2 in molecular weight and is not reactive to YGGFMRF antiserum. The immunoreactivity of this FMRF-NH_2-like material, similarly to authentic FMRF-NH_2, is reduced by CNBr, almost totally abolished by trypsin, but not affected by carboxypeptidase A treatment. The results indicate that the endogenous FMRF-NH_2-like material is similar to but not identical to authentic FMRF-NH_2. Because of this, endogenous FMRF-NH_2 immunoreactive material was partially purified by antibody-sepharose affinity column chromatography from bovine medulla oblongata and its analgesic activity tested. When injected intrathecally, it was found not be analgesic but reduced the analgesia elicited by YGGFMRF.

40.3 HETEROGENEITY OF LUTEINIZING HORMONE RELEASING HORMONE-IMMUNOREACTIVITY STUDIED USING A MONOCLONAL ANTIBODY. Richard J. Knapp* and Ludwig A. Sternberger* (SPON: Shailesh P. Banerjee). Center for Brain Research, Univ. of Rochester Sch. of Med., Rochester, N.Y. 14642.

Different studies have presented evidence for the presence of extended forms of luteinizing hormone releasing hormone (LHRH) in the hypothalamus and elsewhere. These studies have depended on low resolution gel filtration chromatography and antisera-based RIA for the characterization of these immunoreactive substances. Using a high affinity (Kd = 2.2 x 10^{-10}M) monoclonal antibody against LHRH, we were able to determine the anatomical distribution and concentration of hypothalamic immunoreactivity and, in addition, isolate high molecular weight material by combined dialysis and immunoaffinity chromatography.

In the isolation procedure, hypothalami dissected from male Sprague-Dawley rats were extracted with 2N acetic acid and the insoluble material removed by centrifugation. LHRH was removed from the extract by dialysis against 2N acetic acid using dialysis tubing having a molecular weight cutoff of 2,000. The acetic acid was replaced by 1mM H_3PO_4 in a second dialysis step. Insoluble material was removed after four washes in buffer by centrifugation and neutralized with NaOH. Monoclonal antibody immobilized on CH Sepharose 4B was then added to the combined soluble material. Following incubation, the gel was packed into a syringe column, washed with high ionic strength buffer, rinsed with saline and eluted with 10mM H_3PO_4.

The distribution of the immunoreactivity recognized by the monoclonal antibody in Zamboni-fixed, vibratome-sectioned brain, includes the staining of neuronal perikarya in the hypothalamic preoptic area as well as fibers in the organum vasculosum laminae terminalis and median eminence. Total hypothalamic immunoreactivity determined by RIA using the antibody is between 4.0 - 4.5 ng/hypothalamus. The separation procedure reduces the concentration of LHRH to 0.0084% of these values. The impermeable immunoreactivity comprises about 14% of the total immunoreactivity on a molar basis. Between 0.6 - 0.3 pmole/hypothalamus of the high molecular weight immunoreactive material is isolated by this procedure. The bulk of this material has a molecular weight of over 10,000. Taken together, these results show that there are at least two forms of LHRH-like immunoreactivity, distinguished by differences in molecular weight, in the rat hypothalamus that are recognized by the monoclonal antibody used.

40.4 DELTA-SLEEPING-INDUCING PEPTIDE (DSIP): OCCURRENCE IN DIFFERENT FORMS. Markus Graf* and Abba J. Kastin. VA Medical Center and Tulane University School of Medicine, New Orleans, LA. 70146.

DSIP has been isolated from the blood of sleeping rabbits and, in 1977, characterized as a nonapeptide. Measurement by RIA showed this peptide to occur in brain and plasma mainly in a large molecular form. We have now assayed DSIP levels in peripheral organs of the rat by RIA after extraction of the tissue with water, a procedure yielding a 10x higher level of immunoreactive DSIP than after acid extraction. The measurable amount of DSIP-like material decreased in frozen organs to about 50% over 5 months of storage. Although no marked differences due to the temperature (-20°C or -80°C) of storage were found, stability seemed to be better at -80°C, at least for the first month. DSIP-like material was found in all rat organs tested. The levels (per mg wet weight tissue ± SEM) were: stomach 849 ± 41 pg, kidney 799 ± 59 pg, jejunum 730 ± 36 pg, duodenum 627 ± 63 pg, pancreas 539 ± 83 pg, spleen 521 ± 18 pg, thymus 484 ± 23 pg, ileum 455 ± 40 pg, colon 331 ± 26 pg, adrenal 270 ± 22 pg, liver 268 ± 32 pg, and muscle 86 ± 7 pg. The low value in muscle is still 10x higher than the amount of immunoreactive DSIP in blood. The rank order of the organs according to their content of DSIP-material was similar whether levels were based on mg wet weight or mg protein. Based on protein, the parts of the intestine showed increased levels compared to the other organs; e.g. in jejunum and duodenum these levels were significantly ($p<0.01$) higher than in stomach. Gel-chromatography on Sephadex G-15 and G-25 of organ extracts (spleen, liver and jejunum) revealed DSIP-like material mostly larger in size than DSIP. The amount of small DSIP-like material was determined by preadsorption to charcoal-dextran. At tissue concentrations of 2mg/ml, charcoal-dextran did not remove much DSIP-like immunoreactivity. At higher concentrations of the tissues (>2mg/ml), even more DSIP-like structures were found after treatment with charcoal. Addition of synthetic DSIP resulted in a recovery of around 80% in stomach, jejunum, spleen, and thymus apparently independent from the concentrations of the homogenates. At a liver concentration of 4mg per ml, however, no increase of DSIP was found after addition of synthetic peptide to the homogenate. Yet with decreasing concentrations of the homogenate to less than 1mg/ml, the recovery of the added peptide increased to reach about 80%. These results suggest a binding process with masking properties at higher concentrations. We find α-globulin to be responsible for at least part of this binding effect. Albumin and iron ions (1 mM) also appear to be able to bind or aggregate DSIP. We conclude that in the periphery, DSIP is present in a bound form with only minor amounts freely available at more physiological conditions.

40.5 PICROTOXININ AND PMA STIMULATE THE SECRETION OF MULTIPLE FORMS OF SOMATOSTATIN FROM CULTURED RAT BRAIN CELLS. R. A. Peterfreund and W. W. Vale. Peptide Biology Laboratory, The Salk Institute, La Jolla, California 92037.

Picrotoxinin (PTX) and Phorbol 12-myristate, 13-acetate (PMA) are two effective stimulators for secretion of somatostatin like immunoactivity (SSLI) from dispersed cells of fetal rat brain maintained in long term primary culture. Previous work in vivo using electrical stimulation and our own investigations in vitro with potassium depolarization have suggested that secreted SSLI is composed of at least two molecular forms, authentic somatostatin 14 (SS14) and a 28-residue, amino terminally extended form of somatostatin,SS28. The present experiments were intended to identify the molecular forms of SSLI secreted by cultured hypothalamus cells following stimulation by PMA or PTX. Hypothalamus tissue from fetal day-18 Sprague Dawley rats was enzymatically dispersed with collagenase and plated in a serum supplemented medium on poly-D-lysine coated dishes. On day 10 in culture, cells were washed with Krebs Ringer solution, allowed to equilibrate and then treated with either PMA (100 nM), PTX (5 μM) or high potassium Krebs Ringer solution (59 mM, isotonic). Medium was collected into an equal volume of 2N acetic acid containing protease inhibitors, heated, cooled and applied to Bond Elut C18 reverse phase cartridges for concentration, desalting and deproteinizing. SSLI was eluted with a solution of CH_3CN/TEAF, collected into glass tubes and lyophilized. The dried material was resuspended in TEAF and applied to a Waters μBond Pak C18 reverse phase HPLC column eluted with a gradient of CH_3CN. Three main peaks were recovered from stimulation buffer of cells treated with PMA, PTX or 59 mM K+ as determined by RIA with antiserum S201, which detects the central portions of SS14. Peak 1 comigrated with synthetic SS28 and represented 10-15% of total recovered SSLI. Peak 2 coeluted with synthetic SS14 and represented 70-80% of total recovered SSLI. Peak 3, representing 10% of SSLI eluted after SS14 (more hydrophobic). Only Peak 2 was detected by antiserum S39 which is directed towards the amino terminus of SS14. All three peaks were also detected in acetic acid extracts of cultured hypothalamus cells. Together the data suggest that PTX and PMA, as well as 59 mM K+, stimulate the release of at least two forms of somatostatin from brain cells in vitro. The major form appears to be SS14. However, significant quantities of an SS28-like form of somatostatin are recovered. Since PMA and PTX may exert their action through receptors or receptor linked ionophores, the present findings are consistent with a possible receptor mediated release of SS28 in the brain.

40.6 THE EFFECTS OF MORPHINE ON GLUCOSE METABOLISM (C^{14}-2-DEOXY-GLUCOSE) IN RAT BRAIN AS MEASURED BY QUANTITATIVE AUTO-RADIOGRAPHY. E.M. Hiesiger*, R.M. Voorhies*, L. Lipschutz*, G. Basler*, W.R. Shapiro and G.W. Pasternak. Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, Departments of Neurology, Neurosurgery and Pharmacology, Cornell University Medical College, New York, N.Y. 10021 USA.

The effect of opiates on cerebral ischaemia has become a timely issue in clinical and laboratory investigations. The two major parameters affecting ischaemia are tissue metabolism and blood flow. Using C^{14}-2-deoxyglucose (2DG) and quantitative autoradiographic technique (QAR), we measured the effect of acute morphine administration on local cerebral glucose utilization (LCGU) in awake adult rats. Five mg/kg of morphine was given intravenously as a bolus, ten minutes prior to LCGU determinations, lowering mean systolic pressure 7 mm Hg, P_aO_2 7.37 torr and pH .08. Mean P_aCO_2 increased 10.13 torr and mean bicarbonate ion concetration rose by 1.7 mg %. During the course of the experiment, hemoglobin fell by 1.9 gms. Temperature was maintained at a physiologic level. During the experiment, blood pressure was maintained through volume expansion. Eleven gray and three white matter structures were examined. LCGU was depressed in a number of gray matter structures: caudate (-28%), medial thalamus (-31%), hypothalamus (-29%), cortex (-20%), pyriform cortex (-19%), medial geniculate (-30%), habenula (-32%), inferior colliculus (-25%), periventricular gray (-19%), amygdala (-16%), and hippocampus (-11%) showed no significant decrease in LCGU when compared to controls. However, statistical significance was achieved only in medial thalamus ($p<0.05$) and habenula ($p<0.03$), areas very rich in opiate receptors. Reductions in LCGU were also seen in some white matter regions: corpus callosum (-30%) and internal capsule (-46%), and anterior commissure (-4%). Although difficult to dissect from the effects of hypercapnia, morphine administered acutely to the rat at 5 mg/kg i.v., does decrease LCGU. Further work is needed to define better the relationship between the opiate system and cerebral metabolism.

40.7 MOTILIN-LIKE PEPTIDES IN RAT AND PORCINE BRAIN: FURTHER CHROMATOGRAPHIC, IMMUNOLOGIC, AND DEVELOPMENTAL STUDIES. D.M. Korchak*, G. Nilaver, T.L. O'Donohue and M.C. Beinfeld* (SPON: P.A. Young). Dept. of Pharmacol., St. Louis Univ. Med. Sch., 1402 S. Grand Blvd., St. Louis, MO 63104, Dept. of Neurology, Columbia Univ. College of Physicians & Surgeons, New York, NY 10032 and NINCDS, Bethesda, MD 20205.

We have previously reported the presence of motilin-like peptides detected by radioimmunoassay (RIA) and immunocytochemistry in rat and human brain. These early studies demonstrated that this motilin-like substance differs both immunologically and chromatographically from porcine intestinal motilin (PIM). To eliminate the possibility that these differences are due to rat vs. pig species differences, we have examined motilin-like peptides in porcine brain extracts.

Our preliminary results indicate that porcine brain, like rat brain, contains motilin-like peptides which also differ immunologically and chromatographically from PIM. Our conclusion from these results is that both porcine and rat brain contain motilin-like peptides which differ substantially from PIM and may represent a class of novel neuropeptides which share some immunologic determinants with PIM.

We have examined the development of this motilin-like immunoreactivity from 4 days prior to birth to greater than 180 days after in the forebrain, hindbrain, cerebellum, and pituitary of rats in comparison with VIP and CCK. In all regions examined, the motilin concentration was highest 4 days before birth and decreased after that time, with different time courses in different tissues. The motilin content in forebrain and hindbrain was about 50% of the maximum content 4 days before birth, was maximal at about 10-30 days, and decreased after that. In the cerebellum, the motilin content was about 10% of the maximum 4 days before birth, was maximal from about 5 to 40 days after birth and declined sharply afterward. In the pituitary, the motilin content was about 15% of the maximum 4 days before birth and rose gradually between 20 and 180 days. In contrast, the development of CCK and VIP was entirely postnatal. In both the forebrain and hindbrain both VIP and CCK rose slowly from day 1 and peaked between day 28 and 60. In forebrain and hindbrain the concentration of motilin, CCK and VIP was clearly decreased in the older animals.

The presence of substantial motilin immunoreactivity in the brain before birth is suggestive of a possible role for motilin in the regulation of development.

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40.8 THE CEREBELLINS: ISOLATION OF TWO RELATED CEREBELLUM-SPECIFIC PEPTIDES. J. R. Slemmon* J. Hempstead* and J. Morgan* (SPON: R. Chizzonite). Dept. of Physiol. Chem. and Pharmacol., Roche Institute of Molecular Biology, Nutley, N.J. 07110

Peptides could provide a useful tool for identifying specific cell types and metabolic specialities present in anatomically defined areas. In order to detect such peptides, tissue from various brain regions were analyzed on high performance liquid chromatography(HPLC). The work described here centers on two such region-specific peptides, isolated from rat cerebellum, that share almost identical primary structure. The cerebelli were dissected from young rats (6-8 weeks) and immersed in liquid nitrogen. This material was held in a liquid nitrogen freezer until use. Frozen cerebelli (24 gms) were homogenized in 6M guanidine HCl using a Brinkman polytron, after which an equal volume of 1M pyridine acetate, pH 4, was added. The homogenate was centrifuged at 20,000g for 1 hr, and the supernate was passed through reversed phase preparative cartridges(Waters Assoc., Sep Pak C18, 20ml per cartridge). The two cerebellum-specific peptides were eluted from the cartridges in 0.1% TFA, 30% acetonitrile and taken to dryness. HPLC purification included two chromatographic systems. Initially the dried fractions were resuspended in 0.1% TFA amd separated by reversed-phase chromatography (Altex Ultrasphere ODS column, 1 x 25 cm) using a linear acetonitrile gradient. The peptides were eluted at 18% acetonitrile. The peptide-containing fractions were pooled, dried and resuspended in 0.1% TFA containing 5mM SDS. This material was chromatographed in an ion-pairing system (Altex IP column, 0.46 x 25cm) employing 5mM SDS as the pairing reagent in all buffers. The peptides were eluted at 56% acetonitrile and showed baseline resolution. Amino acid analysis indicated the peptides differed by only one extra serine residue. Gas phase sequencing demonstrated one of the peptides contained an extra serine residue on the amino terminus. The carboxyl terminus proved resistant to exopeptidase treatment and preliminary evidence suggests it to be a modified glutamyl or glutaminyl residue.

The developmental time-course for these peptides indicated they appear about six days after birth and reach a maximum by approximately day twenty, and in general parallel the formation of the internal granular cell layer of the albino rat cerebellum.

40.9 DISTRIBUTION AND CHARACTERIZATION OF FMRFamide-LIKE PEPTIDES IN RAT BRAIN AND DIGESTIVE SYSTEM. J.F. Bishop* (SPON: R.P. White), W.W. Watson, J. Groome* and T.L. O'Donohue. Experimental Therapeutics Branch, NINCDS, Bethesda, MD 20205, and Dept. of Zoology, Univ. of New Hampshire, Durham, NH 03824.

The molluscan cardioexcitatory peptide Phe-Met-Arg-Phe-NH_2 (FMRFamide) has recently been found in rat brain and may therefore serve a neuromodulatory or neurotransmitter role in mammals. Studies conducted in this lab have utilized a FMRFamide specific radioimmunoassay (RIA) to investigate: 1) the distribution of FMRFamide immunoreactivity in the rat CNS and digestive system, and 2) the high pressure liquid chromatographic (HPLC) elution profile of immunoreactive FMRFamide in extracts of rat brain, pancreas, and duodenum. Preliminary experiments indicated that one of three FMRFamide-directed antisera generated in this lab was highly specific for the C terminal of FMRFamide, and the RIA consistently registered linear increases in FMRFamide equivalent immunoreactivity with increasing aliquots of tissue extract. This antiserum was used for all subsequent experiments.

FMRFamide immunoreactivity was assessed for 14 rat brain regions, as well as for rat pancreas and duodenum. Highest concentrations were found in pancreas and duodenum, and within the brain, highest concentrations were found in septum, hypothalamus, and midbrain. Lowest concentrations were found in striatum and cerebellum.

In addition to tissue distribution, HPLC elution characteristics of the FMRFamide-like substance were examined. Results of reverse phase HPLC of brain, pancreas, and duodenum indicated that three separate immunoreactive peaks were present in each tissue. All three peaks eluted significantly later than standard FMRFamide. As the pancreas had the highest concentration of FMRFamide-like material, the presence of FMRFamide bioactivity in this region was examined using the Busycon radula protractor muscle bioassay. Significant levels of FMRFamide bioactivity were detected in extracts of this tissue.

Although the precise structure of the FMRFamide-like peptides remains to be determined, their widespread distribution indicates possible central and peripheral roles.

40.10 CHARACTERIZATION OF PANCREATIC POLYPEPTIDE IMMUNOREACTIVITY IN RAT BRAIN USING A C-TERMINAL HEXAPEPTIDE (CTH) ANTISERUM. D.A. DiMaggio*, J.A. Olschowka, D.M. Jacobowitz, K.Buchanan* and T.L. O'Donohue (SPON: B. Turner). Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke, and Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20205 and The Queen's University of Belfast, Belfast Ireland.

In previous immunocytochemical studies (Olschowka et al. Peptide 2:309-331, 1981) an extensive system of bovine pancreatic polypeptide-like immunoreactive neurons was identified in rat brain. However, when using the antibody employed in these studies for radioimmunoassay of brain tissue, essentially no immunoreactive material could be measured. As there is interspecies variation in the structure of pancreatic polypeptide (PP), it was hypothesized that the radioimmunoassay for bovine PP may not recognize rat PP. Since the C-terminal hexapeptide (CTH) of PP is relatively conserved between species, an antibody was generated in rabbits against CTH-amide conjugated to succinylated thyroglobulin. Using the C-terminally directed antisera, PP-like immunoreactivity was detected in rat brain and pancreas by radioimmunoassay. Antibody specificity was assessed with various structurally related peptides. No cross reactivity of CTH free acid was found in the RIA at doses up to 1 uM, but some cross reactivity was evident with neuropeptide Y (NPY, 0.02%).

When used immunocytochemically, the CTH-directed antiserum stained the same neuronal system in rat brain as did the BPP antiserum. Preabsorption of each antisera with the respective hapten (1 uM) eliminated staining. In addition, a decrease in staining was apparent following preabsorption of both antisera with NPY (1 uM).

Gel filtration chromatography and radioimmunoassay of pancreas tissue produced a single peak of immunoreactivity eluting considerably later than BPP and NPY, suggesting the peptide has a lower molecular weight than these peptides.

Fractionation of brain and pancreas tissue by reverse phase high performance liquid chromatography resulted in a major CTH-immunoreactive peak at 28% acetonitrile as well as an occasional peak eluting earlier. The CTH-immunoreactive material eluted prior to both NPY and BPP.

Consistent with the HPLC and gel filtration data suggesting that rat brain and pancreas immunoreactive peptide is distinct from NPY or BPP is data obtained from a preliminary amino acid analysis of HPLC purified immunoreactive peptide from pancreas. Further studies are now in progress to identify the structure of brain and pancreas CTH-immunoreactive material.

41.1 SPECIFIC, CALCIUM-DEPENDENT ACTIVATION OF CYCLIC AMP ACCUMULATION BY VASOACTIVE INTESTINAL PEPTIDE (VIP) IN RAT HIPPOCAMPAL SLICES. Anne M. Etgen and Edward T. Browning. Dept. Biol. Sci., Rutgers Univ., New Brunswick, NJ 08903 and Dept. Pharmacol., Rutgers Med. School, Piscataway, NJ 08854.

The intraneuronal localization of VIP and its release from synaptosomes and brain slices by depolarizing agents suggest that this peptide may serve as a neurotransmitter or neuromodulator. Because the greatest endogenous levels of VIP are found in higher brain centers (e.g., cerebral cortex and limbic system, including the hippocampus) and VIP is a stimulator of adenylate cyclase, we examined its effects on cAMP accumulation in rat hippocampal slices. Transverse slices (350 μm) from ovariectomized adult female rats were equilibrated for 1 hr, incubated with VIP under various conditions, and cAMP extracted and quantified using a competitive protein binding assay. VIP (10 μM) produced rapid (16-fold in 1 min) and striking elevations of hippocampal slice cAMP content (approximately 140-fold in 30 min). In contrast, other peptides (e.g., glucagon, somatostatin, enkephalins, LHRH, ACTH analog, arginine-vasopressin) produced little or no change in cellular cAMP levels. Two-fold elevations in cAMP were seen with VIP concentrations as low as 20 nM; the cAMP response was half maximal a 1 μM VIP and maximal between 10 and 20 μM. At maximally effective concentrations, VIP was 86% as effective in increasing cAMP as maximal concentrations of forskolin, a compound which activates adenylate cyclase in most cell types. Further experiments demonstrated that removal of calicum from the bathing medium severely attenuated the ability of VIP to stimulate cAMP accumulation in hippocampal slices; the addition of EGTA to the bath decreased the cAMP response further. Moreover, VIP-induced cAMP elevations were not dependent on neural firing since coincubation of slices with VIP and 2 μM tetrodotoxin did not alter the cAMP response. Other brain regions known to contain high VIP levels (cerebral cortex) also responded to VIP with 30- 50-fold elevations in cellular cAMP while hypothalamus-preoptic area, midbrain and cerebellum, whose endogenous VIP levels decrease in that order, showed correspondingly reduced cAMP responses. These data suggest that VIP may be a physiological regulator of hippocampal cAMP, probably via direct interactions of the peptide with VIP-specific receptors, and support the notion that VIP may serve a neurotransmitter or neuromodulator function in higher brain centers.

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41.2 DEVELOPMENT OF VIP-STIMULATED ADENYLATE CYCLASE IN THE RABBIT RETINA. Jonathan A. Pachter* and Dominic Man-Kit Lam (SPON: Peter Kellaway). Program in Neuroscience and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

In the adult rabbit retina, vasoactive intestinal polypeptide (VIP) immunoreactivity has been detected and localized in sublaminas 1 and 5 of the inner plexiform layer (Tornqvist et al., Histochem. 76:137). Although the function of VIP in the retina is unknown, it has been shown to be a very potent stimulator of adenylate cyclase activity in the adult rabbit retina (Schorderet et al., Eur. J. Pharmacol. 71:131). In order to study the development of putative postsynaptic VIP receptor systems, we have investigated the maturation of VIP-stimulated adenylate cyclase activity in the developing rabbit retina. Several correlations were found between the time courses of development of VIP-stimulated adenylate cyclase activity and the previously described development of dopamine-stimulated cAMP formation in the rabbit retina (Pachter and Lam, ARVO '83).

VIP-stimulated activity is evident at birth and rises sharply at approximately postnatal day 6 (D6). This rise in the stimulatory potency of VIP is concurrent with a similar sharp increase in dopamine-stimulated adenylate cyclase activity. VIP-stimulated activity reaches adult levels approximately two weeks after birth, which is shortly after the rabbits first open their eyes. The maturation of VIP-stimulated adenylate cyclase activity to adult levels also correlates well with the timing of maturation of the dopamine-stimulated system. These results suggest that common factors may influence the development of VIP-stimulation and dopaminergic stimulation of adenylate cyclase activity. Alternatively, the development of one system may influence that of the other.

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41.3 ACTIVATION OF LOCUS COERULEUS NEURONS BY CORTICOTROPIN-RELEASING FACTOR (CRF). R.J. Valentino, S.L. Foote, G. Aston-Jones, and F.E. Bloom. Behavioral Neurobiology Laboratory, The Salk Institute, La Jolla 92037.

The recently characterized hypothalamic peptide, CRF, is thought to be important in the mediation of physiological and behavioral responses to stressful stimuli (Vale, W., Science 231:1494, 1981). We investigated the effects of CRF on spontaneous activity of noradrenergic neurons of the locus coeruleus (NE-LC) since CRF-immunoreactive fibers have been localized in this area (Bloom, F.E., Reg. Peptides, 4:43, 1982; Olschowka, J.A., Neuroendocrinology, 35:305, 1982; Swanson, L.W., Neuroendocrinology, 36:165, 1983) and because these neurons have been implicated in the mediation of stress responses. Spontaneous single unit activity of NE-LC neurons in halothane-anesthetized rats was recorded before and after intraventricular (i.v.t.) injections of CRF or its acidic derivative, CRF-OH. CRF (3 μg, i.v.t.) increased the spontaneous discharge rate of all 12 NE-LC neurons tested by an average of 65 + 17% (S.E.M.), with the largest increases occurring 7 min after injection. In contrast, CRF-OH, the less active derivative, did not alter the spontaneous activity of NE-LC neurons when administered in the same dose (maximum increase = 18 + 11%; n = 5 cells). Likewise, lower doses of CRF (1 μg, i.v.t.) were ineffective. While iontophoretic application of CRF to NE-LC cells did not consistently alter spontaneous discharge rate, the pressure application (4-40 psi) of CRF directly to these cells through a multibarrel micropipette resulted in increases in discharge rate in 9 of 14 neurons. Pressure application of CRF to cerebellar purkinje cells or to neurons of the mesencephalic nucleus of the trigeminus had no effect on the spontaneous activity of these cells. Preliminary studies in 3 unanethetized, sling-restrained rats support these findings. Intraventricular injection of CRF (1 μg) increased the spontaneous single unit and multiple unit activity of NE-LC cells in all 3 rats, and 0.3 μg was effective in 1 of 2 rats tested. The onset of the increase in activity varied from 1 to 6 min after injection. The increase produced by 1 μg CRF often preceded struggling by animals in the sling. In summary, CRF activation of NE-LC neurons exhibits the following characteristics: 1) dose dependency 2) structural restrictions for the agonist 3) responses exhibited only by certain neuronal classes, and 4) increased sensitivity of unanesthetized animals. These results suggest that low concentrations of CRF released into the ventricular system or from fibers innervating the LC can directly activate these neurons, providing a possible mechanism for the CNS mediation of stress responses by this hormone. Supported by U.S.P.H.S. Grants DA 05194, NS 16209, AA 03504 and Training Grant AA 07273.

41.4 CALCITONIN RECEPTORS IN THE PERIAQUEDUCTAL GRAY MATTER MEDIATE ITS ANALGESIC ACTIONS. A. Fabbri*, C.B. Pert, and A. Pert. Clinical Neuroscience Branch and Biological Psychiatry Branch, NIMH, Bethesda, MD 20205

Calcitonin (CT) evokes a potent and long lasting analgesic effect that is not opiate-mediated following intraventricular or intrathecal administration in rodents and man (Fraioli et al., Eur. J. Pharmacol. 78:381, 1982). Little, however, is known regarding the specific sites of action of this peptide in eliciting analgesia. In this study, we have visualized the calcitonin receptor distribution in the rat midbrain and hindbrain with autoradiographic procedures and then evaluated the analgesic effects of both human (hCT) and salmon (sCT) calcitonin following direct injections into the periaqueductal gray matter (PAG), a region of uniquely high CT binding.

For receptor visualization, serial 25-um sections were taken through the caudal half of the rat brain. These sections were exposed to ^{125}I-sCT and then processed for autoradiography using tritium-sensitive film. The autoradiography revealed exceptionally heavy binding of sCT in the ventral and ventrolateral segments of the PAG extending along the entire rostral-caudal axis. The mesencephalic reticular formation and the nucleus tractus spinalis nervi trigemini were also relatively high in sCT binding at this level of the brain.

In order to assess the analgesic effects of calcitonin and relate these effects to the receptor distribution, rats were implanted with chronically indwelling cannulae guides aimed for the PAG. Following recovery, the animals were injected intracerebrally with 0.3, 1, 3, and 10 nmoles of sCT, 10 nmoles hCT, or saline. Additional groups of rats were pretreated with either 5 mg/kg of naloxone or saline prior to intracerebral sCT. All animals were tested in the tail-flick and hot plate tests at 5, 15, 30, 60, 90, 120, and 150 min after intracerebral injections. sCT was found to induce a dose-dependent increase in hot-plate latencies but appeared to have little effect in the tail-flick test. At all doses tested, the effect was significant between 15 and 30 min, reaching a peak at 60 min and lasting throughout the duration of testing. hCT was much less effective than sCT in eliciting an analgesic effect and was also less potent in displacing ^{125}I-sCT bound to slide-mounted sections of rat PAG. Autoradiographic and histological analyses revealed that in rats in which CT elicited a signficant analgesic effect, the injection had been made into the area of the PAG containing high CT binding. Injections which were less effective or ineffective had been made outside of this region. These findings suggest that the ventral aspect of the PAG may be an important site of action of CT or a CT-like peptide in eliciting analgesia.

41.5 EFFECTS OF CALCITONIN ON SEROTONERGIC NEURONS IN RAT CNS: LACK OF CORRELATION TO PEPTIDE'S ANALGESIA. M.Parenti, F.Tirone°, A.Groppetti°, V.Sibilia°, A.Pecile° and V.R.Olgiati. Department of Pharmacology, University of Milan, Italy.

Several evidences suggest that calcitonin (CT) may act as a neuro transmitter in CNS. Immunoreactive CT-like substance has been detected in the nervous system of different animal species including man. Binding sites for the peptide have been demonstrated in rat and human brain. Analgesia, changes in prolactin release, and inhibition of feeding have also been reported after CT administration.

However, most of the information concerning the anatomical organization of CT neurons, their connections with other neuronal systems, and in general, their physiological role in CNS, are still lacking. Only very recently, evidence has been given suggesting functional interactions between CT and dopaminergic or gabaergic neurons.

In this context, using autoradiographic techniques, we have found that CT-binding sites are clustered in bulbar areas densely populated of serotonergic neurons. We have therefore investigated whether CT and serotonergic neurons are functionally connected in CNS.

Since serotonin (5-HT) has been often involved in noxious inhibitory control we have also evaluated whether these connections are relevant to the CT's antinociceptive activity.

Here we report that intraventricular administration of CT at doses ranging from 0.1 to 5.0 ug/rat, enhances 5-hydroxyindoleacetic acid (5-HIAA) concentrations in several CNS areas including striatum, hypothalamus, mesencephalon, pons, medulla oblungata, cerebellum and spinal cord.

However, no correlations have been found between the alteration of 5-HT metabolism and the analgesic effect of the peptide.

The increase in 5-HIIA levels 1) is longer-lasting, 2) is elicited by doses of CT by far lower than those necessary to induce analgesia and 3) is not restricted to the brain areas involved in pain control.

Moreover, neither inhibition of tryptophan hydroxylase by p-chloro-phenylalanine nor block of 5-HT receptors by metergoline reduces the analgesic effect of CT, suggesting that the integrity of serotonergic system is not relevant to the CT's antinociceptive activity.

It is concluded that 5-HT neuronal activity may be modulated by CT. This effect however does not seem to be related to the peptide-induced analgesia.

41.6 ELECTROPHYSIOLOGICAL ACTIONS OF NEUROTENSIN$_{(1-13)}$, NEUROTENSIN$_{(1-8)}$, AND NEUROTENSIN$_{(8-13)}$ ON SINGLE NEURONS IN HYPOTHALAMIC EXPLANTS. F. Baldino, Jr., L. G. Davis and B. Wolfson*, E.I. du Pont de Nemours & Co., Glenolden Laboratory, Glenolden, PA 19036

Neurotensin, a tridecapeptide, (NT) is differentially distributed throughout the CNS in a pattern which parallels the distribution of specific high-affinity binding sites for this peptide. This observation, coupled with the known pharmacological actions of NT, suggests a neurotransmitter-like role for this peptide in the CNS. The medial preoptic hypothalamus (MPO) contains dense concentrations of NT and NT binding sites. Furthermore, microinjection of NT into this nucleus produces a profound decrease in body temperature. Because the physiological actions of NT on individual neurons is unknown, we chose to study the effects of NT on single neurons in explants of the MPO in vitro. Standard in vitro electrophysiological techniques were employed. Cultures were prepared from newborn rats and maintained in roller tubes 3-4 weeks. NT was administered either through the superfusion fluid or via micropressure ejection (0.5-10 psi). On line peri-event histograms were used to quantitate neuronal responsiveness to NT.

Ejection of NT (1mM-50pM) produced a consistent, dose-related, excitatory effect on the majority of cells studied. This effect also varied linearly as a function of the pressure used to eject the peptide (0.7-10 psi). The remaining cells studied were either unresponsive to NT or inhibited by NT. The N & C terminal fragments of NT ($NT_{(1-8)}$; $NT_{(8-13)}$) did not influence the spontaneous rate of these neurons, suggesting that the active (excitatory) portion of NT may be contained in a mid-region of NT. Additionally, NT was tested for its ability to interact with other putative neurotransmitter systems that have been co-localized with NT in MPO. NT was ineffective in modifying the reductions in spontaneous rate produced by iontophoretically applied GABA (10 nA) and dopamine (100 nA). However, the excitatory effects of iontophoretically applied glutamate (10-30 nA) were markedly enhanced by NT applied at concentrations that did not alter spontaneous rate. $NT_{(8-13)}$ and $NT_{(1-8)}$ were without effect on the excitatory actions of glutamate.

These data indicate that NT, but not the $NT_{(1-8)}$ or $NT_{(8-13)}$ fragments, exerts a predominantly excitatory effect on single neurons in the MPO in vitro and at subthreshold (below excitatory) concentrations enhances the actions of excitatory amino acids.

41.7 NEUROTENSIN: A POSSIBLE FACTOR IN HIBERNATION IN THE WHITE-FOOTED MOUSE (PEROMYSCUS LEUCOPUS). J.E. Coughlin*, and W.H. Watson, III (SPON: E. Hagstrom, Dept. of Psychology). Dept. of Zoology, UNH, Durham, NH 03824.

A study is being conducted to investigate the role of the brain and gastrointestinal peptide, neurotensin (NT), in hibernation. The hypothesis that NT is involved in hibernation is based on four observations: (1) Injection of NT into the brains of a variety of species of mammals including the white-footed mouse (Peromyscus leucopus) causes hypothermia; (2) The area of the brain associated with temperature regulation (the preoptic-anterior hypothalamus, POAH) contains the greatest concentration of sites sensitive to NT's hypothermic effect; (3) The POAH contains the highest concentrations of NT as well as NT specific binding sites (for review see Uhl, Annals of N.Y. Acad. Sci., 400:132, 1982); (4) NT hypothermia, like hibernation, occurs via decreased metabolic rate rather than increased heat loss thru the skin (for review see Bissette, et. al., Annals of N.Y. Acad. Sci., 400:268, 1982).

To prove NT is involved in mammalian hibernation, it must be demonstrated that blocking the activity of NT alters hibernation. This is being accomplished by injecting NT specific antibodies into the brains of hibernating white-footed mice (antibodies generously supplied by Dr. Marvin Brown, Salk Inst., La Jolla, CA and Dr. Robert E. Carraway, Univ. of MA, Worcester, MA). Following ten weeks of short day photoperiod exposure (6L:18D) and an ambient temperature of 4°C, white-footed mice exhibited daily bouts of hibernation of 4-12 hours in length, during which body temperature dropped from 36°C to ~22°C. This system allows for the testing of inhibitors on bouts of hypothermia on a daily basis. This is not possible with other hibernators, such as the woodchuck (Marmota monax), which exhibits hypothermic bouts of 10-20 days in length, in the winter only.

Preliminary results have shown that intraventricular injections of NT antibodies (2 ul; 1 ul will bind with 1 p mole of NT) 12 hours before the onset of torpor caused a delay in the occurrence of hibernation, but did not alter the duration of the bout or the degree of temperature change from the control serum injections. A more elegant system using osmotic pumps to continuously infuse the inhibitor or the control vehicle is currently being employed. This will allow for the injection of the drugs during an entire bout of hypothermia, and avoid any artifacts due to handling of the animals.

41.8 PRIOR COLD EXPOSURE ANTAGONIZES NEUROTENSIN-INDUCED HYPOTHERMIA IN MICE. W.D. Merritt*, G. Bissette, D. Luttinger, A.J. Prange, Jr. and C.B. Nemeroff, Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514.

Neurotensin (NT), an endogenous tridecapeptide, produces hypothermia after injection into the cerebroventricular system of many mammals and has been postulated to play a role in mammalian thermoregulation. Previous studies have shown that central or peripheral administration of thyrotropin-releasing hormone antagonizes the hypothermia induced by intracisternal (IC) NT and this antagonism is dependent upon the synthesis of prostaglandins (PG) within the CNS (Regulatory Peptides 4:285-292, 1982). This study was designed to investigate the effect of prior cold exposure on NT-induced hypothermia in mice. Once defined, we determined whether the effect of prior cold exposure was dependent upon prostaglandin synthesis within the central nervous system.

Adult male Swiss-Webster mice were exposed to a 6°C environment in individual metal cages for 3 hours per day on 4 consecutive days. Controls were placed in identical cages at 26°C. On the fifth day, all animals received IC NT or saline vehicle alone. Colonic temperatures were measured using a digital display thermocouple probe 0, 30, 60, 120, and 180 minutes post-injection. After exposure of the mice to a 6°C environment for 4 days, the hypothermia produced by IC NT (1, 3, and 10 µg) was significantly attenuated. Interestingly, cold exposure and subsequent IC NT in a 26°C ambient temperature did not produce any alteration in the hypothermic response to IC NT (1, 10 µg). However, when mice were briefly exposed (30 min) to a 6°C environment immediately prior to IC injection and then placed in a 26°C environment, antagonism of NT (1 µg) induced hypothermia did occur. In contrast to the effects of 4 days of cold exposure, mice placed in a cold (6°C) environment for 3 hours per day for only one or two days prior to injection did not exhibit any antagonism of the hypothermic effects of NT (1 µg) when studied in a cold (6°C) environment. Four days of cold exposure also antagonized the hypothermia produced by bombesin (0.3, 1 µg), a neuropeptide which is thought to produce hypothermia by a different mechanism than NT (Regulatory Peptides 1:53-60, 1980). Pretreatment with indomethacin (15 mg/kg, SC), but not acetylsalicylic acid (100 mg/kg, SC), 30 min prior to IC NT completely prevented the antagonism of NT-induced hypothermia in the cold-exposed animals. Indomethacin inhibits PG synthesis in both the CNS and in the periphery whereas acetylsalicylic acid inhibits PG synthesis only in the periphery (but not in the CNS). These results suggest that blockade of PG synthesis in the CNS can prevent the antagonism of NT-induced hypothermia induced by previous cold exposure. (Supported by NIMH MH-32316, MH-33127, MH-34121, MH-22536 and NICHHD HD-03110).

41.9 TOPOGRAPHIC ORGANIZATION OF THE MESOLIMBIC DOPAMINE SYSTEM AS DEMONSTRATED FUNCTIONALLY BY MICROINJECTION OF NEUROTENSIN INTO SUBNUCLEI OF THE VENTRAL TEGMENTAL AREA. P. W. Kalivas* and J. S. Miller* (SPON: W. Macklin). Dept. Pharmacol., LSU Med. Ctr., New Orleans, 70112.

Microinjection of neurotensin (NT) into the ventral tegmental area (VTA) of the rat has been shown to produce both an increase in locomotor activity and a decrease in colonic temperature. In this study, male S. D. rats were implanted with injection cannulae into the VTA, and NT (2.5 µg) or saline infused in a volume of 0.25 µl/side. Careful histological analysis of cannulae placement revealed that hypothermia was most often produced following injection of NT into the caudal, midline VTA; corresponding to the nucleus linearis centralis. In contrast, increased motor activity occured after injection of NT into the lateral VTA (nucleus paranigralis) and the nucleus fascicularis. Both hypothermia and hyperactivity were seen simultaneously in rats injected into the nucleus linearis rostralis.

It is possible that both the hypothermia and hyperactivity produced by intra-VTA NT result from activiation of ascending dopamine (DA) neurons. To test this, a neuroleptic, fluphenazine (2.5 µg/side) was microinjected into various forebrain loci simultaneously with NT infusion into the VTA. Fluphenazine injection into the diagonal band of Broca (DBB) blocked NT hypothermia, while intra-nucleus accumbens injection blocked the hyperactivity. Thus, NT may activate DA neurons in the medial VTA which project to the DBB to produce hypothermia and neurons in the lateral VTA projecting to the nucleus accumbens to produce hyperactivity. This postulate was further substantiated by HPLC measurement of DA metabolites in various forebrain regions after NT injection into the VTA. Injection of NT into the nucleus linearis centralis produced a marked increase in DA metabolites in the DBB, while injection into the nucleus paranigralis produced a perferential rise in DA metabolites in the nucleus accumbens. Further supporting a VTA-diagonal band projection system was the observation that HRP injection into the DBB resulted in retrogradely labeled cells predominately in the midline VTA, of which 35% were immunohistochemically double labeled for tyrosine hydroxylase.

Thus, the mesolimbic DA system is topographically organized with respect to NT action in the VTA. NT acts in the nucleus linearis to activate a VTA-DBB DA system causing hypothermia, and in the nucleus paranigralis to activate a VTA-accumbens DA pathway producing hyperactivity.

41.10 STRUCTURE-ACTIVITY RELATIONSHIPS OF THE INHIBITORY ACTIONS OF SUBSTANCE P ON NICOTINIC CHOLINERGIC RECEPTORS. N.D. Boyd*, M.P. Anthony,* S.E. Leeman, Department of Physiology, University of Massachusetts Medical Center, Worcester, MA 01605

Substance P (SP) inhibits the cholinergic stimulated release of dopamine from PC_{12} cells, a sympathetic neuronal cell line. The concentration dependency and reversibility of this inhibition is similar to that previously reported for adrenal chromaffin cells. In order to characterize the site on PC_{12} cells through which SP exerts this action, we have examined the effect of SP, SP fragments and related peptides on the influx of $^{22}Na^+$ that is stimulated by nicotinic agonists. A concentration dependent inhibiton of carbamylcholine-induced $^{22}Na^+$ uptake was observed; the ID_{50}'s of the various peptides examined are:

	% Relative Potency
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	100
Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	25
Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	3
Gln-Phe-Phe-Gly-Leu-Met-NH_2	3
Phe-Phe-Gly-Leu-Met-NH_2	5
Phe-Gly-Leu-Met-NH_2	0.8
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-COOH	3
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-OMe	50
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-NH_2	38
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-NH_2	25
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-COOH	0.4
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-NH_2	6
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Leu-NH_2	63
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Tyr-Gly-Leu-Met-NH_2	83
Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Leu-Gly-Leu-Met-NH_2	0.3
Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2	1.0

These results indicate that both the N-terminal and the C-terminal sequences of SP are necessary for maximal inhibition. The low potency of eledoisin and physalemin which share a similar C-terminal sequence with SP but different N-terminal amino acids, reinforce the importance of the N-terminal sequence of the SP molecule in its inhibitory action.

These results are consistent with the existence of a SP receptor site on PC_{12} cells that modulate nicotinic cholinergic function and thus catecholamine release. The structure-activity relationships observed here suggest that these sites possess different structural requirements for activity when compared with those on guinea pig ileum smooth muscle, frog spinal motoneurons and rat salivary glands, where the biological activity has been viewed for the most part as residing only in the C-terminal portion of the SP molecule. (NIH Grant # AM29876)

41.11 EFFECT OF ENKEPHALINS AND SUBSTANCE P ON THE CONTRACTION OF VAS DEFERENS OF AGING RAT. J.T. Huang* and W.C. Ko* (Sponsor: D. Sutton). Virginia Mason Res. Ctr., Seattle, WA 98101.

The question of whether the vas deferens from an aging rat has a different response to enkephalin and substance P than that from a young rat was investigated in this communication. Vas deferens from Fisher 344 strain rats were isolated after the rats were decapitated. The vas deferens was then mounted in a 10 ml organ bath containing Krebs solution maintained at 37°C and bubbled with 95% O_2 and 5% CO_2. The electrical field stimulation was applied to the vas deferens at 60 v with 0.5 m sec. duration in every 10 seconds. After a stable twitching contraction was established, the effect of enkephalin and substance P was studied by adding these substances to the organ bath.

The concentration of D'Ala^2-D-Leu^2-enkephalin (DADLE), which inhibited 50% of the twitching contraction of the vas deferens, was 8×10^{-8}M for young rats (4 to 6 months) and 2.4×10^{-7}M for old rats (24 to 26 months). The concentration of D-Ala^2-Methionine-enkephalinamide (DAME) which inhibited 50% of the contraction was 10 times higher than that of DADLE. These results indicate that the vas deferens of the old rats were less sensitive to the enkephalin than that of the young rats. The difference between young and old vas deferens was also exhibited after the vas deferens were treated with trypsin for 30 min. The sensitivity of the young vas deferens to DADLE or DAME was significantly decreased after it was treated with trypsin but there was no significant change of the sensitivity in the old vas deferens. 2.5×10^{-7}M of substance P potentiated the contraction of the young vas deferens about 30% and the old vas deferens about 130%. After the vas deferens was treated with trypsin, the potentiation effect of substance P was increased to 70% and 240% in the young and old vas deferens, respectively. These results indicate that the old vas deferens was more sensitive to substance P. Since enkephalin may primarily affect the neural elements of the vas deferens and substance P may primarily affect the muscle elements of the vas deferens (Blackwell, et al., 1978), the effect of aging on the nerve and muscle of the vas deferens is different. (Supported by NIH RR05588)

41.12 NOVEL HEXAPEPTIDE ANALOGS OF SUBSTANCE P (SP) AS PUTATIVE SP ANTAGONISTS. E.R.Baizman*,D.A.Kiefer*,D.M.LoPresti*,N.J.Meo*,M.H.Perrone*,R.E.Diehl*,A.K.Pierson*,P.E.Hansen*,T.D.Gordon*, and B.A.Morgan*.(SPON: H. Tedeschi). Sterling-Winthrop Research Institute,Rensselaer N.Y. 12144.

The proposed physiological role of SP as a nociceptive sensory transmitter has prompted us and others to investigate SP antagonist analogs as a new class of potential analgesics (Baizman,et al.,Soc. Neurosci. 8:986 1982;Sandberg and Iversen,J.Med.Chem.25:1009,1982). Two hexapeptides, Win 51149 (A) and Win 49337 (B) were synthesized and compared with two undecapeptides previously described as being SP antagonists: D-Pro^2,D-$Trp^{7,9}$SP (C) and D-Pro^2,D-Phe^7,D-Trp^9SP (D) (Folkers,et al., Acta Physiol. Scand. 111:505,1981).

In our experiments, all 4 analogs showed parallel displacement curves for ^{3}H-SP binding in brain,with Ki values ranging from 0.7-12.9uM. In the atropinized guinea pig ileum, pA_2 values for 3 of the 4 analogs were approximately equal (pA_2: A= 6.4; B= 5.6; C=5.7;D= 4.6) and reflect competitive antagonism of SP. Both hexapeptides showed little or no agonist effect in this tissue. At concentrations of 1-30uM, each analog also produced reversal of SP-induced (10uM) increases in the electrically stimulated twitch height of the rat vas deferens, but none reversed contractions augmented by acetylcholine (ACh), bradykinin or histamine. Salivation induced in the rat by i.v. bolus injection of SP (0.7-2.2nmoles/kg) was significantly inhibited by 10-min. infusion of 0.7umole/kg of (C) and by 3.4umoles/kg of (A). Intrathecal (i.t.) pretreatment inhibited ACh-induced writhing behavior in the mouse in a dose-related manner(AD_{50} in nmoles/mouse: A= 4.7; B= 1.8; C= 3; D= 2.4). In agreement with others (Piercey, et al., Science 214:1361, 1981) who have reported hindlimb flaccidity after intraspinal injections of SP antagonists, we find that analog (C) showed an AD_{50} for antagonism of writhing (3nmoles i.t.) that was similar to its ED_{50} for production of motor deficit in a rotarod performance test (5.5nmoles i.t.). In contrast, i.t. injections of the hexapeptide (A) showed a 14-fold separation between inhibition of writhing (AD_{50} = 4.7nmoles/mouse) and impairment of rotarod performance (ED_{50}= 66nmoles/mouse). Thus, results in vivo suggest that intraspinal administration of the hexapeptides results in antinociceptive effects similar to those of the undecapeptides but at doses below those producing motor dysfunction.

41.13 EXCESSIVE GROOMING INDUCED BY SUBSTANCE P, BOMBESIN, AND RELATED PEPTIDES IN MICE. W. H. Simmons* and G. Neisenberg. Dept. of Biochemistry and Biophysics, Loyola University Medical School, Maywood, IL, 60153.

After intracerebroventricular injection in mice, substance P (20 µg), physalaemin (1 µg), and eledoisin (40 ng, 200 ng, and 1 µg) induced excessive grooming behavior, but no sustained scratching. In contrast, bombesin (50 ng, 250 ng, and 1.25 µg) induced both grooming and scratching behavior. The effect of substance P (20 µg) completely disappeared after 17 minutes. The duration of action of eledoisin (1 µg) was somewhat longer while bombesin (100 ng) was still strongly active after 53 minutes. [pGlu^5, MePhe^8, Sar^9] (substance P 5-11), a metabolically stable analog of substance P, induced enhanced locomotion and grooming at doses of 1 and 5 µg with a duration of action of about 30 minutes (1 µg). Treatment with twice daily injections of 10 µg [pGlu^5, MePhe^8, Sar^9] (substance P 5-11) or 1 µg bombesin did not induce significant tolerance to the effects of substance P, eledoisin, or bombesin. The observations described here suggest that the grooming/scratching behavior induced by bombesin is phenotypically different from that induced by substance P, physalaemin, and eledoisin. The very short duration of action of substance P suggests that this peptide is rapidly inactivated. The virtual lack of tolerance formation is compatible with the view that substance P and bombesin may be involved in the mediation of nociceptive stimuli.

41.14 Effects of Hypoxia and Denervations on Carotid Body Peptides. G.R. Hanson, L. Jones and S. Fidone, Depts. Biochem. Pharmacol. & Toxicol. and Physiol., Univ. of Utah, Salt Lake City, UT. 84112.

The carotid body is the chief peripheral organ of arterial chemoreception in mammals and plays an important role in the regulation of respiratory and cardiovascular systems. Regulatory neuropeptides, such as substance P and metenkephalin, have been localized in the carotid body and are thought to affect the chemosensory activity of this organ; however their precise function is unknown. We have conducted studies to determine if these peptide systems might assist in mediating the response of the carotid body to natural stimuli, such as hypoxia. Thus, using RIA techniques (Hanson, et al., J. Neurochem., 35 [1980] 1370-1374) concentrations of both substance P- and metenkephalin-like immunoreactivity were determined in the carotid bodies of rabbits placed in clear Lucite chambers and exposed (two 30 min. periods with 20 min. interim) to either an hypoxic gas mixture (5% O_2 in N_2) or room air. The carotid body contents of substance P and metenkephalin were significantly decreased by 28% and 33%, respectively, in animals exposed to hypoxic conditions.

Innervation of the carotid body is provided by the carotid sinus nerve (CSN) and both preganglionic and postganglionic sympathetic fibers from the superior cervical ganglion. Experiments were also conducted to determine if these innervations influence the response of the neuropeptide systems to hypoxia. Rabbits were surgically treated by removing either a 5 mm section of the CSN or the superior cervical ganglia. Rabbits were exposed to air or hypoxic conditions 12-15 days following surgery. CSN denervation blocked the hypoxia-induced changes in the carotid body concentrations of both substance P and metenkephalin while sympathectomy only prevented the hypoxia-related changes in metenkephalin levels.

Although additional studies are required to elucidate the significance of the above findings, these data suggest that substance P and metenkephalin play a significant regulatory role in the physiological responses of the carotid body. (Supported by USPHS Research Grants NS-12636, NS-07938 and MH-37762).

41.15 SUBSTANCE P-INDUCED FACILITATION OF A SPINAL NOCICEPTIVE REFLEX: COMPARISON WITH ELEDOISIN AND PHYSALAEMIN. R. Cridland*, K. Yashpal* and J.L. Henry. Department of Physiology, McGill University, Montréal, Québec, H3G 1Y6.

We have reported that intrathecal administration of substance P to the rat induces a transient dose dependent decrease in reaction time to flick of the tail from a noxious radiant heat stimulus (Pain 14: 155-167, 1982). This transient effect, which lasts less than 5 min, is resistant to the opiate antagonist naloxone, but is followed by a rebound overshoot which is blocked by intrathecal administration of naloxone (Can. J. Physiol. Pharmacol. 61: 303-307, 1983). In view of the suggestion that different types of substance P receptors exist in peripheral tissues, because differential sensitivities exist to the substance P homologues eledoisin and physalaemin, we tested these homologues in our experimental paradigm to determine whether they might have differential effects when given centrally. Thus, eledoisin and physalaemin were each administered intrathecally to determine whether they alter reaction time in the tail flick test.

Male Sprague-Dawley rats were implanted chronically with intrathecal catheters to the lumbar spinal cord. After one week of recovery, tail flick latency was measured in trials of ten tests, one test each five min: three tests to establish control latency (usually set at 15-20 sec) and seven tests to plot the time course of effects on reaction time. Substance P, eledoisin and physalaemin were each given as 10 µg in 10 µl of artificial cerebrospinal fluid followed by a further 10 µl of the vehicle to flush the catheter. The molar dose of each peptide, then, was: substance P, 7.4 nmole; eledoisin, 8.4 nmole; physalaemin 7.9 nmole). Substance P reduced the response time to flick of the tail, to 28.9 ± 12.7 (S.D.) percent of control (n = 10). Eledoisin and physalaemin reduced this time to 25.7 ± 7.4 (n = 7) and 23.5 ± 12.2 (n = 6), respectively. The time course of the responses was similar to that reported earlier for substance P, including the rebound overshoot. Administration of vehicle had no effect on reaction time.

These results support the suggestion that substance P facilitates transmission through spinal nociceptive pathways and demonstrates that the homologues eledoisin and physalaemin have similar effects as substance P.
(Supported by the Canadian Medical Research Council).

41.16 PROINFLAMMATORY POTENTIAL OF SUBSTANCE P - GENERATION OF PHLOGOGENIC MACROPHAGE PRODUCTS. H.P.Hartung* and K.V.Toyka* (SPON: H.G.Ross). Dept. of Neurology, Univ. of Duesseldorf Medical School, W. Germany.

Besides its role as a neurotransmitter Substance P (SP) has been implicated in vasodilation and neurogenic plasma extravasation thereby promoting humoral inflammatory responses. It has recently been shown to augment endocytosis by macrophages (MØ), the major cellular component of the inflammatory process. We studied whether SP evokes heightened metabolic activities in MØ as reflected by enhanced oxidative and arachidonic acid metabolism. Adherent peritoneal MØ elicited with albumin or C.parvum were incubated in serumfree medium in the presence of SP (1 nM-1 µM). After 60 min or 12 h, respectively, release of superoxide anion (O_2^-) and H_2O_2, and prostaglandin E (PGE) and thromboxane B_2 (TXB) was determined photometrically (oxygen radicals) or by radioimmunoassay (Hartung,H.P. et al, J.Immunol., 130:1345, 1983). SP induced significant release of oxygen radical being maximally effective at 0.6 µM. Kinetics gave a plateau at 60 min (13 nmol O_2^-/7 nmol H_2O_2/10^6 MØ). SP also dose-dependently excited liberation of PGE and TXB, the latter in higher amounts (7 ng/12 h). This effect was abolished in the presence of indomethacin. Kinetics showed a steep increase within 6 h and levelling off thereafter. Exposure of MØ for 5 min sufficed to initiate 50% of maximal release. MØ could be specifically desensitized to SP. Pulse time studies and specific deactivation suggest a receptor-ligand interaction. Evidence for binding of SP to MØ will be demonstrated by immunocytochemistry. O_2^- and H_2O_2 are known to be cytotoxic to tumor cells and microbes, mediate endothelial injury and partake in inflammation. PGE and TXB are established mediators of immunoinflammatory responses contributing to vasodilation and the formation of edema, as well as triggering platelet release reaction. PGE moreover modulates MØ - T cell interaction.

We have provided evidence for novel effects of SP. SP causes MØ to set free important phlogistic compounds. We suggest that this action of SP may be relevant in the context of neuroinflammatory diease. SP liberated from damaged nerve endings would lead not only to vasodilation, as has been known before, but also via its action on MØ to further destruction of nervous tissue and augmentation of phlogistic responses due to the effects of toxic oxygen derivatives and arachidonate cyclooxygenation products.

41.17 DIRECT QUANTITATION AND CHARACTERIZATION OF THE RELEASE OF SUBSTANCE P FROM THR MYENTERIC PLEXUS. Sheri A. Baron*, Bernard M. Jaffe* and Alan R. Gintzler* (Spon: A. Wakade). Downstate Medical Center, Brooklyn, NY 11203.

It has been suggested that substance P (SP) may be an excitatory neurotransmitter in the enteric nervous system and may play a role in the local modulation of intestinal motility. There is however, only indirect evidence that SP is released from intramural neurons in the gut wall and as a consequence its role as an enteric neurotransmitter remains speculative. We report here the first direct quantitation of the release of SP from the myenteric plexus. A dynamic system has been developed in which the in vitro release of SP from 2 longitudinal muscle myenteric plexus (LM-MP) strips is obtained during continuous superfusion and measured directly by using a highly sensitive radioimmunoassay specific for SP. Electrical stimulation produced a marked increase in the rate of release of SP. The frequency-dependence profile (0.5-40 Hz) of release was multitonic rather than linear. Stimulation of 0.5 Hz increased the rate of release above basal values by approximately 133% (9 pg/min above mean basal release). Stimulation at 1 and 5 Hz also increased the rate of release but there were no significant differences among the mean increases in the rate of release of SP elicited by the above frequencies. Stimulation at 7.5 Hz produced an abrupt additional elevation in the rate of release (38.0 pg/min above mean basal release) possibly indicating recruitment of additional SP containing neurons. Increasing the frequency to 40 Hz, but not 10 or 20 Hz, produced a further increase in the rate of release (68.5 pg/min above mean basal levels). Electrically evoked release elicited by 20 Hz stimulation was reduced by 95% by omitting Ca^{++} from the superfusion buffer or by cooling the preparation to 3°C. Pretreatment with tetrodotoxin (TTx; 1 ug/ml for 20 min) substantially reduced the magnitude of the increase in the rate of release of SP but did not abolish it. Despite TTx pretreatment a significant portion (53%) of the electrically induced increase in SP release remained. Since, TTx resistant-Ca^{++} dependent action potentials are characteristic of only Type 2 (AH) cells, a portion of SP containing neurons must be of this type.

The ability of electrical stimulation to release SP from the myenteric plexus in amounts sufficient to produce a physiological response in a Ca^{++} and temperature dependent fashion in combination with data provided by indirect pharmacological experiments strongly suggests that SP functions as a neurotransmitter in the enteric nervous system. Supported by Grants DA02893, HD06488.

41.18 EVIDENCE FOR A NONCHOLINERGIC AFFERENT PATHWAY FROM COLON TO PREVERTEBRAL GANGLIA: POSSIBLE MEDIATION BY SUBSTANCE P. S. Peters* and D. L. Kreulen (SPON: J. J. Galligan). Dept. of Pharmacology, Univ. of Arizona, Coll. of Medicine, Tucson, AZ 85724.

In prevertebral sympathetic ganglia, both cholinergic and noncholinergic transmission is observed following nerve stimulation in vitro. Cholinergic (fast) excitatory postsynaptic potentials (e.p.s.p.s) are nicotinic and noncholinergic (slow) e.p.s.p.s are believed mediated by Substance P (SP). Cholinergic input also is observed, arising from a colonic afferent pathway. The degree of cholinergic activity increases when the colon is distended or induced to contract. Our objective was to determine whether a noncholinergic afferent pathway between the colon and ganglia exists which can be activated physiologically. The inferior mesenteric ganglion (IMG) and a segment of distal colon (3.5-4 cm) connected by the lumbar colonic nerves were excised from guinea pigs and pinned separately in a 2-chamber bath. Both chambers were perfused with oxygenated Krebs buffer, but drugs were administered only into the ganglion compartment. The caudal end of the colon segment was ligated and the oral end fastened to a fluid injector and pressure transducer assembly. Responses to colon distension or hypogastric nerve stimulation were recorded intracellularly from principal ganglion cells using glass microelectrodes of 49-100 M Ω resistance. Distension (5-10 cm pressure) caused a pressure-dependent increase in frequency and amplitude of cholinergic e.p.s.p.s and often a depolarization. Following cholinergic blockade with hexamethonium (10^{-4}-10^{-5}M), 9 of 27 cells (33%) depolarized an average of 3.3 ± .4 mV (S.E.M.) upon distensions of 10-15 cm H_2O pressure. Depolarizations peaked 58 ±7s (n=5) after initiation of 1-min distensions. Distensions of longer duration (3-8 min) increased the time to peak (132 ± 32s, n=5) but not the amplitude of the depolarizations. In most neurons the slow depolarizations returned to baseline within 3 min after cessation of distension. Redistension resulted in depolarizations in 3 of 4 cells. Stimulus-evoked slow e.p.s.p. amplitude did not decrease after prolonged (15-min) distension, suggesting a lack of membrane desensitization by the noncholinergic transmitter. Hyperpolarizing the membrane from -47 to -89 mV by current injection failed to enhance the amplitude of depolarization (n=1). Putative SP antagonist D-pro^4, D-$trp^{7,9,10}$-SP did not decrease the slow e.p.s.p. (1 μM, 5 min, n=2) and increased the distension-induced depolarization by 125% (1 μM, 10 min, n=1). We conclude that there exists a noncholinergic mechanosensory afferent pathway from the colon to IMG. Because the slow e.p.s.p. elicited by nerve stimulation is believed mediated by SP, it is possible that SP mediates the depolarization in the colonic mechanosensory pathway.
Support HL27781.

41.19 BOMBESIN AND SUBSTANCE P AS EXCITATORY NEUROTRANSMITTERS IN THE MUSCULARIS MUCOSA OF CANINE COLON. F. Angel*, V.L.W. Go* and J.H. Szurszewski. Dept. Physiology and Gastroenterology Unit, Mayo Medical School, Rochester, MN 55905.

In the muscularis mucosa of canine colon, transmural electrical nerve stimulation induces an excitatory response which is not mediated by cholinergic, adrenergic, purinergic or serotonergic nerves. Two peptides found in the isolated muscle, bombesin and substance P, contract the muscle when added exogenously. Substance P has a direct effect on the smooth muscle whereas bombesin acts through a nervous pathway. The aims of the study were to determine: 1) if these two peptides are the excitatory transmitters in the colonic muscularis mucosa; and 2) the relationship, if any, between bombesin- and substance P-containing neurons. Strips of muscularis mucosa were placed in a superfusion apparatus to record contractile activity. The superfusate (0.1 ml/sec) which passed over each strip was collected before and during transmural electrical stimulation (10 Hz, 200 μs, 10 V) and analyzed for bombesin- and substance P-like immunoreactivity. In control conditions, the concentration (mean ± SEM) of bombesin and substance P was, respectively, 6.0 ± 0.9 pg/ml; 5.60 ± 0.96 pg/ml. During transmural nerve stimulation the concentration of bombesin and substance P increased significantly ($p < 0.01$) to 9.41 ± 0.98 pg/ml, and 7.16 ± 0.98 pg/ml, respectively. In the presence of tetrodotoxin (10^{-6} M) and in a calcium-free solution, these increases of bombesin and substance P during nerve stimulation were blocked. Furthermore, bombesin (10^{-7} M), when added exogenously to the superfusion, released substance P into the superfusate (23 ± 1.7 pg/ml, mean ± SEM). The release as well as the excitatory effect of bombesin on the muscle was blocked by tetrodotoxin. In the presence of rabbit antiserum raised against bombesin (dilution = 1:60) there was a decrease in the excitatory effect induced by nerve stimulation. The effect of exogenously added bombesin and the release of substance P induced by bombesin in control experiments were blocked in the presence of bombesin-antibodies. Finally, in the presence of rabbit antiserum, raised against substance P (dilution 1:60), the excitatory effect as well as the increase in substance P, induced by nerve stimulation and exogenous bombesin were blocked. We conclude from these experiments that transmural stimulation releases bombesin from intramural nerves and that bombesin acts on substance P-containing nerves to release substance P, thereby causing contraction. Supported by NIH Grant AM 17238.

42.1 ENDOGENOUS LABELING OF POLYPEPTIDES BLOCKED BY CYCLOHEXIMIDE IN THE GOLDFISH MAUTHNER AXON. E. Koenig. Div. of Neurobiology, Dept. of Physiology, SUNY, Buffalo, NY 14214.

Studies in this laboratory have focussed on endogenous protein synthesizing activity in mature and growing vertebrate axons. Recently, we showed that naked goldfish retinal ganglion cell (RGC) axons regenerating in vitro incorporate amino acids into axonal protein after decentralization from the explant and that the incorporation is blocked by cycloheximide or emetine (Koenig & Adams, J. Neurochem., 39:386, 1982). Major polypeptides showing cycloheximide-sensitive labeling in RGC axons and in lumbar spinal roots of the rat include α and ß tubulins, actin and neurofilament polypeptides (Koenig, Soc. Neurosci. Abstr. 8, p.788, 1982). I now report preliminary findings regarding polypeptides exhibiting cycloheximide-sensitive labeling in the large myelinated axon (M-axon) of the goldfish Mauthner neuron. Microscopic samples of M-axon, isolated from the myelin sheath, were analyzed by a special gel microslab system after in vitro incubation of goldfish spinal cord with ^{35}S-methionine in the presence or absence of cycloheximide. Autoradiograms of gels showed that several polypeptides were labeled in the absence but not in the presence of cycloheximide. While a polypeptide of about 72K showed the heaviest labeling, tubulin and actin were also labeled but to a lesser extent. There were other unidentified polypeptides also labeled. Goldfish axons do not have major 200K and 70K polypeptides that are homologous to mammalian neurofilament polypeptides (Koenig, unpublished). They do have, however, a major 145K polypeptide that may be homologous, but there are also major 135K, 125K and 121K polypeptides that may constitute neurofilament subunits, as judged by their apparent equivalence to the 145K polypeptide. The findings that the M-axon exhibits cycloheximide-sensitive labeling of axoplasmic polypeptides is consistent with the demonstration that this axon contains RNA whose composition includes 26S, 18S, 5S and 4S species, as well as polydisperse RNA (Koenig, Brain Res., 174:95, 1979). These findings add further support to the hypothesis that local synthesis of certain cytoskeletal components of transport groups IV (SCb) and V (SCa) may be necessary to compensate for the biological decay they may undergo during transport.

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42.2 POSTTRANSLATIONAL PROTEIN MODIFICATION BY AMINO ACID ADDITION IN AXOPLASM ISOLATED FROM SQUID GIANT AXONS. N.A. Ingoglia, M.F. Zanakis, G. Chakraborty* and A. Giuditta*. Dept. of Physiology, NJ Medical School, Newark, NJ 07103 and Int. Institute of Genetics and Biophysics, Naples, Italy.

Axoplasm of the squid giant axon contains a variety of species of transfer RNA as well as their cognate aminoacyl tRNA synthetases. These axons, like all axons, are essentially devoid of ribosomes as well as significant amounts of ribosomal RNA, and have not been shown to be capable of supporting protein synthesis. The purpose of the present experiment was to determine if transfer RNA in giant axons is capable of transferring amino acids to acceptor proteins as a participant in the posttranslational modification of endogenous axonal proteins.

In the first series of experiments ^{3}H-amino acids or ^{3}H-amino-acylated tRNAs were incubated with 5 µl of axoplasm and a reaction mixture. The reaction was stopped by the addition of cold 5% TCA and radioactivity in amino acid, nucleic acid and protein fractions was determined by extraction in cold and hot TCA. Under these conditions no radioactivity was found in the protein fraction. This was also the finding following injection of ^{3}H-amino acids and ^{3}H-aminoacylated tRNA directly into the axon. Thus, under the conditions described above there is no evidence of transfer of amino acids from tRNA to proteins. In other experiments, axoplasm was pooled to a volume of 50-100 µl, homogenized gently and centrifuged at 150,000xg for 1 hour. Some of the high speed supernatant was incubated with labeled amino acids and an appropriate reaction mixture and the remainder was passed through an S200 Sephacryl column before incubation with the same reaction mixture. There was no incorporation of amino acids into protein in the high speed supernatant fraction. However, in the S200 purified fraction, a fraction devoid of molecules of less than 25,000 MW, ^{3}H arg, lys, tyr, leu and asp were all incorporated into proteins in amounts of 44, 30, 7, 5 and 3.5 xs heat inactivated controls. The sulfonic amino acid taurine was not incorporated into proteins suggesting that the binding of the carboxylic amino acids to protein is not the result of non-specific binding. The radioactivity present in the protein fraction was shown by amino acid analysis to still be present as the parent amino acid, and could be released by incubation in proteinase K. This addition of amino acids to protein is not protein synthesis since the reactions occurred in a partially purified fraction of the 150,000 g supernatant, a fraction devoid of ribosomes and free amino acids. Thus, we conclude that the reactions we are observing represent the posttranslational addition of a range of amino acids to proteins in axoplasm, and that these reactions are usually inhibited by an unknown factor(s) of less than 25,000 MW.

42.3 AXONAL TRANSPORT IN RAT SCIATIC NERVES OF A HIGH MOLECULAR WEIGHT COMPLEX CONTAINING ELEMENTS CAPABLE OF TRANSFER RNA DEPENDENT POSTTRANSLATIONAL PROTEIN MODIFICATION. M.F. Zanakis, G. Chakraborty* and N.A. Ingoglia. Dept. of Physiology, NJ Medical School Newark, NJ 07103.

Experiments were performed to determine whether a non-ribosomal tRNA dependent post-translational protein modification reaction is present in the rat sciatic nerve, and whether the elements necessary for such a reaction are transported axonally.

Ligatures were placed on the sciatic nerve (one and two cm. from the sciatic notch). Six days later, when axonally transported material would have accumulated proximal to the first ligature but not the second, segments proximal to each were removed homogenized, and centrifuged at 150,000g for 60 min. The high-speed supernatant was analyzed for the incorporation of ^{3}H-amino acids into a hot TCA insoluble product using a suitable reaction mixture. No incorporation of amino acids into proteins occurred when compared to heat-inactivated controls in the supernatant fraction. However, when the supernatant was passed through a Sephacryl S-200 column, significant incorporation into protein of ^{3}H arg, lys, leu, asp, tyr (but not tau) was found in a fraction containing molecules of greater than 125,000 daltons (F1). Furthermore, the incorporation in the proximal segment was always greater than in the distal segment, indicating axonal transport of the elements necessary for the reaction. Radioactivity in the reaction product could not be solubilized by successive washes in cold TCA or in chloroform:methanol, but was released from the pellet following incubation in protease, indicating that the ^{3}H-amino acids were covalently bound to endogenous proteins.

We next tested the reaction in regenerating nerves. Sciatic nerves were crushed bilaterally and allowed to regenerate for 7 days prior to ligation as described above. When analyses were performed as in normal nerves results showed the incorporation of all of the above amino acids into protein in F1, but in amounts varying from 2 to 20 times normals. This indicates a large increase in the activity of the elements necessary for this reaction in regenerating axons compared to normals. Other experiments were performed to determine if the reaction was tRNA dependent. SDS PAGE analysis of F1 demonstrated the presence of 4S RNA. When tRNA is removed from the reaction by DEAE cellulose chromatography, the amino acid incorporation decreased by 90%, but was restored by the addition of either endogenous or exogenous tRNA, indicating that the reaction is dependent on tRNA.

These results describe a tRNA dependent, ribosomal RNA independent posttranslational protein modification reaction within axons of the rat sciatic nerve. The activity of this reaction is increased significantly during regeneration.

42.4 POSTTRANSLATIONAL PROTEIN MODIFICATION BY tRNA DEPENDENT AMINO ACID ADDITION IN REGENERATING OPTIC NERVES OF GOLDFISH. G. Chakraborty*, M.F. Zanakis, N.A. Ingoglia. (Spon: F.P.J. Diecke) Dept. of Physiology, NJ Medical School, Newark, NJ 07103.

The experiments described below were performed to determine if tRNA found in regenerating optic axons of goldfish is participating in post-translational protein modification by addition of amino acid to endogenous axonal proteins.

The first series of experiments were performed to see if 4S RNA is transported axonally in regenerating optic axons prior to innervation of the goldfish optic tectum. Both optic nerves of goldfish were cut and regenerating nerve stumps were analysed for the presence of 4S RNA at 2,4,6 and 10 days of regeneration. In each case, ^{3}H-uridine (3-5 µci) was injected into both eyes two days prior to sacrifice. RNA was extracted by phenol extraction and 4S RNA was determined by SDS-PAGE. ^{3}H-4S RNA in regenerating optic nerves was compared with normals (intact optic nerve) and regenerating controls (both optic nerve and retinal artery were cut). No significant difference in the amount of ^{3}H-4S RNA in the nerve was detected at 2 or 4 days of regeneration but the proportion of ^{3}H-4S RNA doubled at 6 and 10 days of regeneration. This data suggests that 4S RNA (probably tRNA), is transported axonally in regenerating optic nerves of goldfish prior to reconnection.

In other experiments, both optic nerves were cut and 6-8 days later optic nerves were homogenized, centrifuged at 150,000xg and the supernatant was passed through a Sephacryl S-200 column. The supernatant and the S-200 void volume (F1, mol. wt. > 120,000 dalton) were incubated separately with ^{3}H-lys and assayed for their ability to incorporate ^{3}H-lys into protein. Supernatants exhibited no ability for such incorporation, but F1 incorporated ^{3}H-lys into protein in amounts approximately 15 times heat inactivated controls. These values (∿ 96 DPM/µg protein) are five fold greater than normal nerves (∿ 18 DPM/µg protein). Experiments using other amino acids (leu, pro, arg, asp) have shown similar results in the void volume obtained from a Sephadex G25-40 column. In all experiments the greatest incorporation is observed following incubation with the basic amino acids, less with neutral amino acids and still less with the acidic amino acid.

These experiments indicate that: (1) tRNA is axonally transported in the optic nerves of goldfish prior to reconnection of optic axons, (2) posttranslational modification of axonal protein occurs in regenerating optic nerves of goldfish (3) such modification is higher in regenerating optic nerves, compared to that of normal nerves (4) it is likely that the elements required for posttranslational protein modification are transported axonally in these regenerating optic nerves.

42.5 RETROGRADE AXONAL TRANSPORT OF FREE GLYCINE IN THE BRANCHIAL NERVE OF APLYSIA. K.E.Carlson* and C.H.Price, Dept. of Biology, Boston Univ., Boston, MA 02215.

The axonal transport(AT) of small molecules such as amino acids is a controversial subject and has received little attention compared with the AT of macromolecules. Furthermore the existence and functional significance of the retrograde AT of small molecules has been poorly documented. In this study, the retrograde AT of free glycine in the identified axons of neurons R3-14, which apparently use glycine as a chemical messenger, was characterized by biochemical and autoradiographic means. The soma of R3-14 are located in the parietovisceral ganglion(PVG) and their axons project down the branchial nerve(BN) to end in a large field in the pericardial region(PR). Using a double-chambered apparatus, the PR was incubated in media with 3H-amino acids for 4-48h while the BN-PVG tissue was isolated and perfused with plain or chemically altered media. The BN and PVG were then either rapidly frozen(for scintillation counting) or fixed for autoradiography(AR). When 3H-glycine was used, radioactivity entered the BN rapidly (the front moved at about 2 mm/h), reached the PVG by 3h, and was largely(85% or more) in the free amino acid form (TCA soluble). When anisomycin was used on the PR in parallel experiments, the amount of 3H-macromolecules in the BN and PVG was reduced by 97% compared with controls. In AR sections of the BN from a 24h incubation, more than 30% of the silver grains over the nerve were contained in R3-14 axons although these structures occupy less than 10% of the nerve area. Radioactivity accumulated in the PVG(4X greater in the right than left hemiganglion due to the presence of the R3-14 soma). The majority of this label remained in the free form but a significant amount of newly synthesized 3H-protein was always present in the nerve and ganglion. Retrograde AT of free glycine was inhibited by(in decreasing order of effectiveness): mercuric ions, vinblastine, Nocodazole, and 2,4-DNP. When other amino acids(leu, ser, glu, or ala) were used, 3-23X less label appeared in the BN (12h runs) and the R3-14 axons were not preferentially labeled in AR. Incubating the PR in 3H-GABA, in contrast, resulted in 2X more transport into the nerve than when gly was used. Most of the 3H-GABA was localized over small axons and glia while the R3-14 axons were poorly labeled. This work demonstrates the selective and active retrograde AT of a small molecule from terminal regions back to soma, a process which probably involves the compartmentalization of the soluble amino acid in a transportable organelle. The special handling of glycine by R3-14 provides further evidence for a non-metabolic role for glycine in these neurons, probably as a neural messenger. Supported by NIH #16399.

AXOPLASMIC TRANSPORT II

43.1 DIFFERENT INCORPORATION AND TRANSPORT PATTERNS OF ^{3}H-PROLINE IN THE DORSAL COLUMN NUCLEI OF NEONATAL AND ADULT CATS. N. Contos, L. Barber* and K.J. Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306.

The results of previous studies in our laboratory suggested that macroglia participate in the transfer of ^{3}H-pro-labeled molecules from the dorsal column nuclei (DCN) to their targets in the brainstem. Developmental studies by others have demonstrated that, in general, macroglia are not fully developed in neonatal cats. Thus, if the above hypothesis is correct, incorporation and transport of ^{3}H-pro-labeled molecules from DCN to their targets should differ in neonates and adults. Two studies were directed at this prediction. First, the DCN of 6 day old and adult cats were examined at the electron microscopic (EM) level to characterize differences in their macroglia and neurons. Second, the DCN of kittens ages 1,6, and 9 days as well as adult cats were injected with ^{3}H-pro, allowed to survive 24 hours, and their brains processed for light microscopic (LM) autoradiography. The labeling patterns in the neonate and adult DCN and their targets in the inferior olive and thalamus were then compared.

The EM study demonstrated striking differences both in macroglia and neurons in the neonate and adult. In the neonate, astrocytic processes were less densely filled with filaments and occupied more of the neuropil. Astrocytes were less regularly organized in their relationship to neuronal soma and myelination was rarely observed. Neurons were much smaller. In the LM autoradiographic study, the labeling patterns in developing brains of kittens aged 1-9 days did not vary much from each other but were distinct from those in the adult. At the DCN injection site, glial cells were densely labeled in both the neonate and adult, but, in the neonate, many more neurons appeared to be labeled, especially near the center of the injection sites. Corresponding differences occurred at DCN's terminal targets in the inferior olive and thalamus. Both targets were markedly more intensely labeled in the neonate. Moreover, in the neonate, terminal labeling appeared over portions of the intralaminar complex of the thalamus, (a region that has never been reported to receive DCN terminations in the adult even after extensive injections or lesions that invade regions surrounding DCN).

These results support the hypothesis that in the adult cat macroglia are involved in the transport of ^{3}H-pro-labeled molecules from DCN to their terminal targets. The results further suggest that the lack of incorporation of ^{3}H-pro by neurons in adult DCN and the corresponding attenuated terminal labeling may be related to the larger size and possible slower metabolic rate of adult neurons and/or to the more systematic associations between the neurons and their enveloping macroglial processes.

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43.2 REDISTRIBUTION OF NEURONAL DIPEPTIDYLAMINOPEPTIDASE II FOLLOWING COLCHICINE TREATMENT. M.C. Bundman, P.J. Lew* and C. Gorenstein. Department of Pharmacology, University of California, College of Medicine, Irvine, CA 92717.

Using histochemical techniques, we have previously shown that dipeptidylaminopeptidase II (DAP II) is highly localized to a small number of neuronal cell populations in rodent brains. (Gorenstein, Tran and Snyder, J. Neurosci., 10, 1096, 1981). The discrete distribution of DAP II suggests that it may play a role in the metabolism of a unique brain peptide.

In an attempt to enhance the histochemical visualization of enzyme activity in brain areas which stained marginally for DAP II we reasoned that intracerebroventricular (ICV) injections of colchicine would lead to an accumulation of enzyme in cell bodies. However, rather than an accumulation in neuronal perikarya, colchicine produced an unexpected redistribution of DAP II. Cell bodies which normally contained DAP II were depleted of staining and DAP II had, instead, accumulated in the dendrites of these cells.

Although the effect was not uniform throughout the brain, all DAP II containing neurons except those of the mesencephalic nucleus of the trigeminal nerve displayed some degree of change. The effect was dose dependent, time dependent and reversible. Progressive effects were seen with 25, 50, 100 and 400 µg of colchicine 24 hours post-injection. The effects of 100 µg colchicine were seen earliest at three hours, were maximal at 18-24 hours and had reversed by 5 days post-injection. Cresyl violet stained sections showed normal morphology in all the affected areas.

This paradoxical redistribution produced by colchicine raises some fundamental questions concerning the role of DAP II and that of other peptidases in neuronal function.

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43.3 PARADOXICAL REDISTRIBUTION OF DAP II CONTAINING ORGANELLES FOLLOWING COLCHICINE TREATMENT. C. Gorenstein, M.C. Bundman and C.E. Ribak. Departments of Pharmacology and Anatomy, University of California, College of Medicine, Irvine, California 92717

Dipeptidylaminopeptidase II (DAP II), a brain peptidase, is highly concentrated in a small number of neuronal populations. In the present study DAP II has been localized in the rat olfactory bulb at the electron microscopic level. Examination of the somata of mitral cells shows that the reaction product specific for DAP II is contained in lysosomes, lypofuscin granules, short cisternae close to the endoplasmic reticulum, and dense granules. The most intense staining appears within lysosomes and lypofuscin granules. The short cisternae appear to coil and spiral and are seen occasionally as C-shaped lamellae and ringlets. More commonly these structures are seen as spiraling lamellae that appear to have an unlabeled core. Dense granules are also commonly seen containing a dense core surrounded by an unlabeled membrane. No labeled organelles were observed in mitral cell dendrites.

DAP II activity was also studied in rats treated with colchicine. Colchicine (100µg) dissolved in distilled water was injected into the lateral ventricle of rats. Twenty-four hours later rats were perfused with 4% paraformaldehyde and 1% glutaraldehyde. Brains were dissected and 50 µm sections were obtained with a vibratome prior to staining for DAP II activity. Light microscopic studies have indicated a redistribution of DAP II label in these preparations. At the electron microscopic level, organelles containing DAP II appear concentrated in the somata at the base of dendrites rather than diffusely localized throughout the perikaryal cytoplasm as observed in untreated animals. In addition, DAP II containing lysosomes penetrate into dendrites where they appear to be more elongated and often dumbbell shaped. Some DAP II containing organelles resemble the cisternae of smooth endoplasmic reticulum that are often found in the peripheral cytoplasm of the dendrites. The distribution of other organelles such as mitochondria, rough endoplasmic reticulum and the Golgi complex appeared unaffected by the colchicine treatment.

The changes in the types of DAP II containing organelles and their redistribution in colchicine preparations suggests that colchicine has an effect on the GERL system. This effect appears opposite to that described in previous studies on protein transport within dendrites and axons where colchicine blocks the active movement of proteins and organelles so that they accumulate in the soma. The findings of the present study suggest that colchicine disrupts the intracellular matrix that sequesters lysosomes in somata.

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43.4 3,4-DIMETHYL-2,5-HEXANEDIONE IMPAIRS AXONAL TRANSPORT OF NEUROFILAMENT PROTEINS. J. W. Griffin†, D. C. Anthony*†, K. Fahnestock*†, P. N. Hoffman† and D. G. Graham*†. †Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205. and +Dept. of Pathology, Duke Univ. Sch. of Med. Durham, N.C.

3,4-dimethyl 2,5-hexanedione (DMHD) is a recently developed, more potent analogue of the neurotoxic hexacarbon, 2,5-hexanedione (HD) (Anthony, et. al., Tox. Appl. Pharm.,in press). Administration of DMHD results in intraaxonal accumulation of neurofilaments (Anthony, et. al., J. Neuropath. Exp. Neurol., in press). DMHD is known to interact with ε-amino groups of proteins to form pyrrole rings, and Anthony, et. al. have postulated that the neurotoxicity of DMHD results from oxidation of the pyrrole rings, leading to covalent crosslinking of neurofilaments. In this study we examined the effect of DMHD on axonal transport of cytoskeletal proteins in rat lumbar motor neurons and compared the changes seen to those produced by β,β'-iminodipropionitrile (IDPN), an agent known to selectively impair neurofilament protein transport (Griffin, et. al., Science, 202:633).

Rats received .6mM/kg DMHD i.p. for 5 days. ^{35}S-methionine was injected into the lumbar ventral horns of both DMHD-treated and control rats (5 weeks of age). The sciatic nerves were removed 2, 4, 12, and 21 days after labeling, and divided into 3 mm segments. The proteins in each segment were separated by SDS-PAGE and visualized by fluorography. In addition, the bands corresponding to the neurofilament proteins, tubulin, actin, and an SCb marker protein (Mr = 113 kd) were cut out, solubilized, and counted. For each of these proteins, we calculated the half velocity (HV) as the distance in mm reached by the 50th percentile of total radioactivity in that protein divided by days after injection. DMHD impaired transport of all slow component constituents, most profoundly retarding the neurofilament proteins (12 day half velocity = <.25 mm/day, compared to 2.07 in controls). Transport of tubulin, actin, and the SCb marker protein was only moderately reduced (70-90% of control velocities).

The changes produced by DMHD were essentially identical to those produced by IDPN. IDPN reduced the HV of neurofilament protein 4-10-fold, while reducing the HV of tubulin, actin and SCb marker protein transport to 50 to 80% of normal.

The similarities between DMHD and IDPN in pathologic changes and in effects on axonal transport are consistent with recent studies (Griffin, et. al., Ann. Neurol., in press) showing that HD shares with IDPN the ability to produce segregation of neurofilaments from microtubules within the axon. The similarities in effects on cytoskeletal organization and axonal transport of neurofilament proteins suggest that these agents may share biochemical actions.

43.5 ALTERATIONS IN FAST TRANSPORTED PROTEINS FOLLOWING INJURY TO HYPOGLOSSAL AXONS. J. D. Redshaw* and M. A. Bisby* (SPON: G. E. Lucier). Department of Medical Physiology, University of Calgary, Calgary, Alberta Canada T2N 4N1.

Previously we reported that a number of alterations in the composition of fast axonally transported proteins (FTP) occurred following sciatic nerve injury. The most significant of these changes involved the ratio of labelling of two polypeptide bands S_1 (MW 28,000) and S_2 (MW 23,000) which could be readily identified and compared on fluorographs of SDS-PAGE gels containing transported proteins. The S_2/S_1 ratio increased after axotomy, reached a peak by 14 days and returned toward normal values by 42 days. Motor axons prevented from regeneration and reinnervation failed to demonstrate this return toward normal values. Axotomy of retinal ganglion cell axons failed to induce any alteration in the S_2/S_1 ratio. We postulate that this change in the S_2/S_1 ratio is correlated with the ability of a neuron to regenerate its injured axon. However, Skene and Willard (J. Cell. Biol., 89:96, 1981) reported no change in GAP 23, a polypeptide probably identical to S_2, following axotomy of rabbit hypoglossal motoneurons. Therefore the purpose of the present study was to re-evaluate the S_2/S_1 ratio in normal and regenerating hypoglossal axons.

The right hypoglossal nerve of anaesthetized male rats was crushed with forceps proximal to its muscular ramifications where it crosses the digastric muscle. The rats were allowed to recover for varying periods from 1 to 28 days prior to the stereotaxic administration of 100 µCi of ^{35}[S]-methionine into the hypoglossal nuclei. Radio-labelled FTP were recovered within the hypoglossal nerves 10 mm proximal to a collection ligature four hours after isotope injection. Labelling of FTP within normal and regenerating sciatic nerves were performed as previously described (Bisby, M.A., J. Neurobiol. 11:435, 1980). Hypoglossal and sciatic nerve segments were processed for either one or two dimensional gel electrophoresis and fluorography.

Analysis of the fluorographs revealed significant alteration in the S_2/S_1 ratio, with an increase in S_2 labelling following axotomy of either hypoglossal or sciatic nerves. This increase returned toward normal values three weeks following hypoglossal nerve injury. We conclude, that in the rat, peripheral motoneurons of the hypoglossal nuclei and lumbar spinal cord respond similarly to axon injury and that the observed changes in the composition of FTP are correlated with the regenerative capacity of the axon.

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43.6 SELECTIVE INHIBITION OF RETROGRADE AXONAL TRANSPORT BY ERYTHRO-9-[3-(2-HYDROXYNONYL)]ADENINE (EHNA). D.S. Forman, K.J. Brown* and M. E. Promersberger*. Dept. of Anatomy, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Little is known about the factors that control the direction of fast axonal transport. We have found that erythro-9-[3-(2-hydroxynonyl)]adenine (EHNA) reduces the mean velocity of microscopically visible organelles that are transported in the retrograde direction, but has little or no effect on the transport of organelles in the anterograde direction. Acutely isolated giant motor axons from the walking legs of lobsters were mounted in lobster medium in a perfusion chamber. Organelle movements at 23-25°C were visualized with Nomarski optics and video microscopy, and recorded using a video tape recorder. Velocities were measured with a Colorado Video 610E Video Pointer. The following table shows the mean velocity in µm/sec ± SEM (N) of retrogradely and anterogradely moving particles before and during the perfusion of 1mM EHNA, and after washing out the EHNA:

	Retrograde	Anterograde
Control (before EHNA)	1.74±.07 (51)	0.63±0.08 (26)
1mM EHNA	0.89±.04 (56)	0.69±0.05 (34)
After removal of EHNA	1.72±.09 (43)	0.57±0.03 (29)

It can be seen that in the presence of 1mM EHNA, the mean velocity of retrograde transport was reduced by 49% (t-test;p<.01), and that this inhibition was reversible. In the same axons anterograde movements of microscopically visible particles were not obviously affected. Retrograde movements of elongated mitochondria were also inhibited reversibly, while anterograde movements of mitochondria were not affected. A higher concentration of EHNA, 4mM, produced more inhibition of retrograde particle velocity (71%), and a slight (16%) decrease in the anterograde velocity. However, 4mM EHNA produced irreversible damage to the axons after 10-15 minutes. Permeabilized axons (D.S. Forman et al., J. Neurosci., in press) were also used to demonstrate the effect of EHNA. The mean velocity of retrograde movements reactivated with 0.2mM ATP was reduced by 72% in the presence of 2 mM EHNA; the mean anterograde velocity in the same axons showed a smaller (29%) but significant (t-test;p<.05) reduction. The effects of EHNA on retrograde transport may be related to the actions of EHNA as an inhibitor of dynein and of protein carboxymethylation (P. Bouchard et al., Proc. Nat. Acad. Sci. USA, 78: 1033, 1981). EHNA is the first agent reported to affect retrograde and anterograde transport differentially. It may prove to be a useful tool for analyzing the factors that control the direction of fast axonal transport. (Supported by NIH grant GM30450.)

43.7 THE CELLULAR ORIGIN AND BIOSYNTHESIS OF RAT OPTIC NERVE PROTEINS. E.R. Lewis, J.M. Gilbert, L.I. Benowitz and P. Strocchi. Mailman Res. Ctr., McLean Hospital, Belmont, MA 02178 and Harvard Medical School, Boston, MA 02115.

High resolution 2DGE (two-dimensional gel electrophoresis) was used to characterize neuronal and glial proteins of the rat optic nerve, to examine the phases of intraaxonal transport with which the neuronal proteins are associated, and to identify the ribosomal populations on which these proteins are synthesized. Neuronal proteins synthesized in the retinal ganglion cells were identified by injecting the eye with [^{35}S]-L-methionine, followed by 2DGE analysis of fast and slow axonally transported proteins in particulate and soluble fractions. Proteins synthesized by the glial cells were labeled by incubating isolated optic nerves in the presence of [^{35}S]-L-methionine and then analyzed by 2DGE. A number of differences were seen between filamentous proteins of neurons and glia. Most strikingly, the multiple subunits of α- and β-tubulin in glia had a distinctly different 2D configuration than the tubulin subunits found among axonal proteins. There were two neurofilament proteins at 150K among the slow axonally transported proteins in the particulate fraction; this fraction also contained significant amounts of the glial filamentous proteins, GFA and vimentin which may have been labeled by transfer of amino acids from the axon to the glial compartment. The fast axonally transported proteins contained relatively large amounts of high molecular weight acidic proteins, two of which were shown to comigrate (on 2DGE) with proteins synthesized by rat CNS rough microsomes; this finding suggests that rough endoplasmic reticulum may be a major site of synthesis for fast transported proteins. In contrast, the free polysome population was shown to synthesize the principle components of slow axonal transport, including tubulin subunits, actin and neurofilament proteins. Supported by N I H grants AG02126, RO1NS16943, 5K0270713 and the Marion Benton Trust.

43.8 CENTRAL AND PERIPHERAL AXOTOMY OF DORSAL ROOT GANGLION (DRG) CELLS DIFFERENTIALLY AFFECTS SLOW AXONAL TRANSPORT. M.M. Oblinger and R.J. Lasek, Dept. of Devel. Genetics and Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

Many early reports that chromatolysis of DRG cells occurs only after peripheral branch axotomy suggested that the DRG cell selectively recognizes, and responds differently, to a signal created by axotomy of its central vs. its peripheral axon. We have examined changes in the synthesis and metabolism of cytoskeletal and associated proteins occurring in the DRG cell body after axotomy of either the peripheral or central branch. The cytoskeletal proteins were studied by labelling DRG cells and analyzing these labelled proteins after they were transported into the axons by slow axonal transport.

Slow transport profiles were compared in control animals, and animals that sustained either an axotomy of the fifth lumbar (L5) dorsal root near its entry into the spinal cord or an axotomy of the sciatic nerve 60 mm from the L5 DRG two weeks earlier. To label axonally transported polypeptides associated with the two major slow rate components, SCa and SCb, DRG cells of adult male albino rats were pulse-labelled by microinjection of ^{35}S-methionine. At post-injection times ranging from 3-28 days, animals were sacrificed and the entire L5 system removed. Consecutive 2 mm segments of nerve were cut and samples were subjected to SDS polyacrylamide slab gel electrophoresis and fluorography. Resulting changes were quantitated by excising individual protein bands from gels and determining the radioactivity by liquid scintillation counting.

A number of changes in slow axonal transport resulted from a peripheral branch axotomy; however, central branch axotomy produced no apparent alterations in the rate or composition of SCa or SCb in either dorsal root or peripheral branch axons. After peripheral axotomy several quantitative changes, including a dramatic reduction in the ratio of labelled neurofilament proteins to tubulin in peripheral branch axons, and a less robust reduction in central branch axons, occurred. Our observations indicate that while axotomy of the peripheral branch of the DRG cell influences some aspect of synthesis and/or assembly of cytoskeletal proteins, central branch axotomy does not. The differential response of the DRG cell to axotomy of its central vs. its peripheral branch may be related to differences in the information that reaches the DRG cell body by retrograde transport.

43.9 SELECTIVE ROUTING OF FAST TRANSPORTED PROTEINS IN BRANCHES OF SENSORY AXONS. R. Dino Rulli* and David L. Wilson (SPON: Ronald G. Clark.) Dept. of Physiology and Biophysics, University of Miami Sch. of Med., Miami FL 33101

Each nerve cell in the 8th and 9th dorsal root ganglia (DRG) has a single axon emerging from the cell body which bifurcates into two processes: one is directed centrally in the dorsal root (DR), and the other peripherally in the sciatic nerve (SN). We have attempted to determine the degree to which the proteins being transported in each branch can differ.

Following a ^{35}S-methionine labeling of the DRG, the fast transported proteins are collected at ligatures on DR and SN. The collected proteins are then separated by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and fluorographed. An earlier qualitative analysis of the fluorographic patterns showed no discernable differences in species of proteins shipped down the DR and the SN (Stone & Wilson, 1979).

We have performed a quantitative analysis, in which a given spot in the DR gel is punched out and counted, and its counts are compared to the counts in the spot treated in the same way from the SN gel. The null hypothesis states that each protein spot will exhibit the same ratio of cpm SN/cpm DR. The present results show that not all proteins have the same ratio. This suggests that there is some differential shipping of the proteins in fast axonal transport, and that the cell body and/or axon has some influence on the specificity of proteins being transported in the two axon branches. We gratefully acknowledge support from NSF grant BNS 81-17817 and NIH training grant NS 07044.

43.10 AXONAL TRANSPORT AND METABOLISM OF CYTOSKELETAL PROTEINS IN THE PREGANGLIONIC FIBERS OF CHICKEN CILIARY GANGLION. P. Paggi* and R.J. Lasek, Dept. of Developmental Genetics and Anatomy, Case Western Reserve Univ., Cleveland, O. 44106.

The axon and its presynaptic terminal are structurally and functionally very different. However, the cytoskeletal proteins of the axon terminal are supplied through the axon by axonal transport. Therefore, all of the proteins that are in the axon terminal must also be present in the axon. The differentiation of axoplasm into synaptoplasm must involve major changes in the composition of the axonal proteins when they enter the presynaptic terminal. In order to study the mechanisms which convert the axonal cytoskeleton of the axon into that of the presynaptic terminal, we have examined axonally transported proteins in the fibers innervating the ciliary ganglion.

Three week old chickens received injections of ^{35}S-methionine in the cerebral aqueduct and were sacrificed at different intervals of time after injection, ranging from 18 to 42 days. Labelled proteins were analyzed in segments of the oculomotor nerve, which contain both the oculomotor nerve fibers and the preganglionic fibers, and in the ciliary ganglion , which contains the terminals of the preganglionic terminals. The radiolabelled proteins were subjected to one and two-dimensional SDS-PAGE and fluorography. Axonal transport was similar to that observed in other neurons. Slow component a (SCa) was defined by the neurofilament proteins (NFP) and tubulin, and slow component b (SCb) was defined by actin and a variety of other typical SCb proteins. The cytoskeletal proteins showed different behaviours when they reached the ganglion. Actin and tubulin, which begin to appear the ganglion at day 2 remain heavily labelled in the ganglion at day 32. By contrast, NFP starts to appear in the ganglion at day 9 and slowly increases during the later times. Both actin and tubulin apparently have a longer residence time in the ganglion than the do the NF proteins. Quantitative analyses are underway to determine the differential rates of turnover of the cytoskeletal proteins when they reach the ganglion. The long term presence observed for both actin and tubulin in the ganglion is consistent with the structural features of the cytoskeleton in the presynaptic terminal. Microfilaments and microtubules are generally more abundant than neurofilaments in the synaptic terminal region. These observations are consistant with the hypothesis that differential metabolism of axonally transported cytoskeletal proteins play an important role in the conversion of the axonal cytoskeleton into that of the presynaptic terminal.

43.11 RETENTION OF AXONALLY TRANSPORTED NEUROFILAMENT PROTEINS ALONG MOUSE RETINAL GANGLION CELL AXONS. R.A. Nixon, K.A. Bishop* and W.H. Fisher*. Harvard Medical School; McLean Hospital, Belmont, MA 02178

Hypotheses have been advanced that neurofilaments are transported along axons as either a pre-assembled cytoskeletal lattice or as precursors to a stationary axonal lattice. To examine whether neurofilament proteins (NFP) may contribute to stationary axonal structures, we have quantitatively examined the fate of NFP in retinal ganglion cell (RGC) axons from 1 to 168 days (d) after synthesis. The short length (9-10 mm) of the mouse optic pathway (optic nerve and tract) permitted a detailed study of proteins retained in axons long after the main waves of transported proteins had entered synapses. Groups of 80-100 mice injected intravitreally with [^{3}H]proline were analyzed to determine changes in the absolute radioactivity (dpm) of NFP subunits (200K, 140K, 70K) and in the relative distribution of this radioactivity along RGC axons at 11 post-injection intervals from 1 to 168 d. After SDS-PAGE, changes in NFP dpm from entire optic pathways minus axon terminal regions (OP), reflecting NFP entry into and exit from axons, were quantitated in each of 8-10 mice per time point. The regional distribution of labeled NFP was also determined in 8 consecutive 1.1 mm segments of OP from 6 mice at each time point. NFP were detected in segment 8 by 17d, although the main wavefront reached this point by 25d, indicating a transport rate of 0.2-0.3 mm/d. Each major NFP subunit behaved similarly in all studies. Loss of NFP dpm between 17 and 168d was biphasic; each of the two rates was linear. Dpm declined 60% between 17 and 45d, but between 45 and 120 d only 45% of the remaining label disappeared. At 168d, 6-9% of the initially transported NFP was still present in axons. Retention of NFP was not accounted for by continual contributions from RGC perikarya. From 1 to 30d, the NFP wavefront progressed through consecutive OP segments. By 30d, segments 7 and 8 were most intensely labeled although the trailing end of the wave spanned the entire OP and contained more than half of the labeled NFP. From 6 to 15d, NFP dpm in segments 1 and 2 declined, giving the impression of a moving, trailing edge. In contrast to this early redistribution of labeled NFP, however, no change in axonal distribution occurred after 45 d even though >40% of the NFP dpm was still present along the optic pathway (>30% of it in segments 1-4). Therefore, beyond 45d post-injection, the rate at which NFP dpm declined appeared to be similar in each segment, suggesting that the radioactivity retained in the axon was not part of a moving wave. These data indicate that a substantial stationary pool of NFP exists along RGC axons which may reflect the presence of a stationary cytoskeletal structure. (Support: NS17535, RR05484, the Alfred Sloan Foundation.)

43.12 ACCUMULATION OF FAST-TRANSPORTED AChE AND ADENYLATE CYCLASE AT A NERVE LIGATURE IS UNAFFECTED BY CONDITIONS THAT INHIBIT ACCUMULATION OF ^{3}H-PROTEIN. R. Hammerschlag, F.A. Bolen* and R.C. Carlsen Div. Neurosciences, City of Hope Res. Inst., Duarte, CA 91010 and Dept. Human Physiology, Univ. Calif. Sch. Med., Davis, CA 95616

The intrasomal passage of pulse-labeled proteins to the fast transport system of the axon can be inhibited by either Co^{2+} or monensin (Stone & Hammerschlag, Cell Mol Neurobiol 1:3,1981; Hammerschlag et al, J Cell Biol 93:568,1982). This decreased export, resulting from perturbing Golgi or peri-Golgi events, was detected for all individual fast-transported species examined after two dimensional gel electrophoresis. Thus, it was a surprising finding that exposure of sensory or motor cell bodies to Co^{2+}had no effect on the accumulation of fast-transported AChE activity at a crush on the corresponding dorsal or ventral root (Larivière & Lavoie, J Neurochem 39:882,1982). We have explored further this apparent anomaly, using an in vitro preparation of bullfrog dorsal root ganglia (DRG) and spinal nerve. Effects of 0.18mM Co^{2+} or 0.1μM monensin on fast transport of AChE were examined in two types of studies. First, "in transit" AChE was allowed to clear out of the fibers prior to nerve ligation, and fast transport of ^{3}H-leu-labeled protein was monitored concomitantly. Under these conditions, Co^{2+} (n=6) led to a 23±2% (mean±SEM) decrease in accumulation of AChE activity, while ^{3}H-protein decreased 95±2%. In the presence of monensin (n=6), AChE decreased 19±4%; ^{3}H-protein decreased 76±2%. In a second approach, existing AChE activity was irreversibly inactivated by pretreatment with 1mM DFP to study effects of Co^{2+} and monensin on newly-synthesized enzyme. (This level of DFP did not inhibit transport of ^{3}H-protein.) After washing to remove unbound DFP, exposure of DRG to Co^{2+}(n=6) increased AChE 1±16%, while ^{3}H-protein decreased 83±10%. Exposure to monensin (n=6) led to a 6±5% decrease in AChE; ^{3}H-protein decreased 71±10%. Next, the accumulation of adenylate cyclase (AC) was studied as in the first protocol above to examine whether the AChE results might be observed for other fast-transported particulate enzymes. Exposure of DRG to either Co^{2+} (0±2% decrease, n=3) or monensin (8±3% decrease, n=3) had little or no effect on the build-up of AC activity at the ligature, similar to the findings in the AChE studies. Interpretation of these results depends on whether AChE or AC are among the fast-transported species that have been separated and quantitated on two-dimensional gels. If they are not, the present findings suggest that newly-synthesized fast-transport proteins reach the axon by two distinct intrasomal routes, one of which is insensitive to Co^{2+} and monensin.

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43.13 DIFFERENTIAL DELIVERY OF SULFATED PROTEINS BY FAST AXONAL TRANSPORT. GC Stone, R Hammerschlag, and JA Bobinski.* Div. of Neurosciences, City of Hope Res. Inst., Duarte, CA 91010.

Among the most abundant 150 individual proteins fast-transported in vitro in bullfrog dorsal root ganglia (DRG)/spinal nerve preparations is a subset of species that are unique in that they contain sulfate moieties while lacking any carbohydrate (Stone et al., Trans.Amer.Soc.Neurochem.,14,143). The present study has sought to confirm this observation, further characterize these proteins, and determine if their unusual property correlates with their intracellular destination. The presence of non-glycosylated, sulfate-containing fast-transported proteins (FTPs) was confirmed by finding that following double-labeling of DRG with ^{3}H-mannose and $K_2{}^{35}SO_4$, a group of acidic, low MW FTPs resolved by two-dimensional gel electrophoresis contain $^{35}SO_4$ but no detectable ^{3}H-mannose. Since mild acid hydrolysis (1N HCl, 5 min, 100°C) liberated $^{35}SO_4$ from proteins, the site of sulfate attachment is likely to be through an ester linkage to an amino acid. Previous work in non-neuronal preparations (Huttner, Nature,299,273), suggests that the sulfated amino acid residue is tyrosine. $^{35}SO_4$-containing FTPs were hydrolyzed with 0.4N $Ba(OH)_2$, to retain ester sulfate linkages, and hydrolysates were run on paper electrophoresis (pH 6.5). By this means the majority of the recovered $^{35}SO_4$-cpm co-migrated with a tyrosine-O-sulfate standard. In separate studies spinal nerves were ligated after a timed delay in order to differentiate between FTPs that had been delivered to the axolemma as opposed to those still en route to terminal regions of the neuron. Following DRG labeling, FTPs moving >50 mm/day (18°C) were permitted to pass a point on the spinal nerve prior to ligation. After applying the ligature and waiting an additional period those proteins that had been delivered to the axolemma could be found in the nerve segment immediately distal to the obstruction, while species in the segment corresponding to the "front" of FTPs were moving toward terminal regions. A comparison of gel patterns of proteins derived from these respective nerve segments revealed that the subset of non-glycosylated, sulfate-containing FTPs appear preferentially en route to terminal regions. To confirm this finding the spinal nerve was cut in segments, each of which was homogenized and exposed to mild acid hydrolysis as above. This treatment demonstrated a gradient of acid-labile $^{35}SO_4$-cpm from background levels in segments immediately distal to the ligature to maximum cpm values in the "front" of FTPs. Together these findings suggest that a unique sub-population of FTPs preferentially destined for neuronal terminals can be characterized as containing sulfated tyrosine residues. Supported by NSF 8112129 and MS-RG1296-A1.

43.14 COMPARISON OF CALCIUM AND STRONTIUM SEQUESTRATION IN NERVE AXONS IN RELATION TO AXOPLASMIC TRANSPORT, S. Ochs, R.A. Jersild, Jr.*, T. Breen* and R. Peterson*. Departments of Physiology and Anatomy, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46223.

The requirement for Ca to maintain axoplasmic transport has previously been shown using in vitro downflow of ^{3}H-labeled proteins in sciatic nerve following injection of the L7 dorsal root ganglion of cats by our usual methods. Deletion of Ca from the medium blocks transport after several hr. Additionally, high levels of Ca also block transport. Transport may continue for a while in the face of high Ca, presumably as a result of mechanisms of Ca regulation present in nerve fibers to keep free Ca at low levels. These were indicated in studies of nerves exposed to high Ca and finding organelles considered important in Ca regulation, the mitochondria and ER, as well as Ca-binding protein sites in the axoplasm, to contain increases in electron-dense particles when using a pyroantimonate method. To identify Ca in these particles X-ray microanalysis was used. Because the energy spectrum of Sb in the pyroantimonate overlaps with Ca, a special computer subtraction technique was required to show the presence of Ca. To further show the relation of Ca to these sites, Sr was employed as a tracer of Ca insofar as Sr has a chemical similarity and has similar physiological actions. In nerves exposed to high Sr (20-85 mM), an increased number of particles was seen with the pyroantimonate technique. As with Ca, they were distributed in the mitochondria, ER, axoplasm and along the axolemma. Sr has its X-ray energy peak far removed from Sb allowing its clear identification to be made with X-ray microanalysis. The particles showed both Sr and Ca present in them in most cases. In some particles Sr and in others Ca alone was seen. At high levels of Sr a block of transport was seen. This could be due to a displacement of Ca from the Ca sequestering sites or to an action of Sr on the calmodulin Ca-Mg ATPase considered to underly the transport mechanism. The presence of Ca in axons was also shown without the use of pyroantimonate. Pieces of desheathed nerves were exposed to varying concentrations of Ca from 20-75 mM and then frozen with Freon-12 cooled down to -160° C with liquid N_2 before being dried under vacuum at low dehydration temperatures. The nerves were cut into small sections and infiltrated under vacuum with Spurr either directly or after osmicating them under vacuum. Sections cut for EM and X-ray microanalysis showed high Ca in some regions within the axons, others showed high K. A low amount of Na and high levels of Cl and P were also seen. Na and Cl were present in high amounts outside the nerve fibers. Supported by NIH #NSO 8706-14 and NSF #BNS 79-14029.

43.15 INCREASED DURATION OF AXOPLASMIC TRANSPORT IN VITRO WITH ADDED GLUCOSE OR β -HYDROXYBUTYRATE, L.C.J. Gaziri* and S. Ochs. Department of Physiology and Medical Biophysics Program, Indiana University School of Medicine, 635 Barnhill Drive, Indianapolis, IN 46223.

Fast axoplasmic transport was earlier shown in this laboratory to be closely dependent on oxidative metabolism supplying ATP to the transport mechanism. It is rapidly blocked by anoxia or by agents interfering with the production of ATP. Previously we found that transport was maintained without addition of glucose or other metabolites to an in vitro medium. We have now initiated a study of transport in cat sciatic nerve, measured as customary in our laboratory by the downflow of labeled protein after injection of the L7 dorsal root ganglia with ^{3}H-leucine, to determine the length of time for which endogenous metabolites are able to maintain axoplasmic transport in vitro and the possibility of maintenance of transport for longer periods by adding metabolites to the incubating medium. Without the addition of exogenous metabolite, transport continues for close to 6 hr in vitro before a block occurs. Taking a small sample of nerve for determination of ATP and creatine phosphate (CP) by a luciferin-luciferase method, the content of high energy phosphate (~P), the combined level of ATP and CP, is seen to fall linearly in the nerves during this period of time. The block of transport occurs when the level of ~ P falls to about half of its control value, from 1.3 to 0.6 umol/g. Glycogen falls in an exponential fashion, being reduced to half its control value in about 3 hours. Glucose is more quickly depleted from the nerve with a fall to near zero in less than 2 hrs. Most of this fast fall is due to its leakage to the medium in the first 15 minutes of incubation. The addition of 5 or 15 mM glucose to the medium maintains fast transport well beyond the 6 hr time; for at least 13 hrs. The longer lasting downflow was present not only in the desheathed peroneal branch but it occurred as well in the sheathed tibial branch. Glucose therefore can readily pass through the perineurium of the sheathed nerves to enter the endoneurial space containing the fibers. With glucose present in the medium ~P levels were found to be close to control values, as were the glycogen contents. Additionally, β -hydroxybutyrate was seen also able to support fast transport for periods longer than 6 hr. Both transport and ~ P levels were maintained for at least 9 hr in vitro indicating that peripheral nerves can use β -hydroxybutyrate as an energy source in addition to glucose. This supports our earlier conjecture that in nerve glycolysis may not be the only path of supply of substrate to the mitochondrion for the production of the ATP needed by the transport mechanism.
Supported by NIH #NSO 8706-14 and NSF #BNS 79-14029.

43.16 EFFECTS OF TETRODOTOXIN UPON AXOPLASMIC TRANSPORT IN THE DEVELOPING VISUAL SYSTEM OF THE ALBINO RAT. R.V. Riccio and M.A. Matthews, Dept. of Anat., LSU Med. Ctr., New Orleans, La.

The role of retinal neuronal activity in regulating the development of postsynaptic visual relay centers in the brain is presently a topic of considerable interest. As a prelude to a morphological analysis of these regions, we have assessed the effect of tetrodotoxin (TTX)-induced impulse blockade upon axoplasmic transport in the optic nerve. Timing of toxin application was designed to coincide with proposed critical periods of development of the lateral geniculate nucleus, superior colliculus and visual cortex. Intraocular injections of 0.5-0.7 ul. TTX (in citrate buffer) at concentrations of 10-4, 10-5, and 10-6M were administered over a two minute period in rats aged 5-21 days postnatal. Control animals received an identical volume of citrate buffer, (pH 7.30). Injections for both groups were repeated every two days to insure a continuous suppression of impulse activity throughout the period examined.

In order to test the effect of intraocular TTX upon axonal transport in the optic nerve, 0.5ul. of 3H proline or 3H fucose was injected into the eye of similar groups of animals at ages 5,9,13,18, and 21 days postnatal. Following survival periods of 3,6,9, and 12 hrs. levels of radioactivity were measured within fresh samples of both the lateral geniculate nucleus and the superior colliculus employing liquid scintillation spectroscopy and autoradiography. Each dosage of TTX produced no significant difference in the rate of appearance of 3H proline labeled proteins at the optic tectum or lateral geniculate nucleus when compared to controls at each survival period tested. However, a substantial difference (approximately 35%) in 3H fucose levels is apparent on days 9-21 following three hours survival in animals treated with 10-4M TTX. These amounts decrease slightly to 26% of controls by 6 hours and maintain this approximate level throughout the survival period. A qualitative assessment of retinal ganglion cell populations suggest no difference between control and TTX-treated groups at 21 days postnatal.

Our results indicate that the effect of TTX upon a developing system of functionally-related neurons may not be restricted to impulse blockade alone but could also affect axoplasmic transport of glycoproteins some of which are known to be incorporated into synaptic membranes upon reception of appropriate visual stimuli. Morphometric studies are in progress to assess parameters of synaptogenesis, dendritic development and other indicators of neuronal differentiation within the retina and visual relay centers.

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43.17 RETROGRADE AXONAL TRANSPORT OF ANTIBODIES TO SYNAPTIC MEMBRANE PROTEINS AND GLYCOPROTEINS. R. J. Wenthold, R. A. Reale* and K. K. Skaggs* Dept. of Neurophysiology and Waisman Ctr., Univ. Wisconsin, Madison, WI 53706.

The axonal transport of horseradish peroxidase and lectins has been widely used to trace neural connections. Recent studies have suggested that the retrograde transport of lectins is dependent upon the selective binding of the lectin to glycoproteins or glycolipids on the surface of neuronal membranes. Thus glycoproteins and/or glycolipids are implied to be part of the class of endogenous molecules retrogradely transported in axons. In an effort to further characterize the nature of the endogenous molecules which are retrogradely transported, we have made antibodies against synaptic membrane proteins and studied the transport of these antibodies. One preparation of antibodies was made in rabbits against synaptic membranes from guinea pig brain. A second preparation of antibodies was made in guinea pigs against the con-A binding proteins of DOC solubilized synaptic membranes from rat.

Retrograde transport was studied in the rat hypoglossal nerve by injecting undiluted antiserum (1 to 40 ul) into the tongue. Survival times ranged from 4 hours to 4 weeks. Following fixation with 4% paraformaldehyde, antibodies were immunocytochemically localized using either the PAP technique or the indirect labeling method with HRP-conjugated antibodies. In general, results from both antisera were similar, but antibodies against synaptic glycoproteins labeled more intensely. Although cells in the hypoglossal nucleus were labeled after survival times as short as 4 hours, labeling was more intense with 6 and 12 hour survival times. Labeling is still intense with a survival time of 24 hours but visibly more diffuse than with shorter times. Progressively less intense and more diffuse labeling was associated with survival times of 48 hours and 1 week and ultimately no labeling could be demonstrated 2 or 4 weeks after the injection. Specific labeling was not observed when control antisera, including normal rabbit serum, normal guinea pig serum, rabbit anti-aspartate aminotransferase, and rabbit anti-rat albumin were used. A limited number of studies done in the guinea pig using antibodies against guinea pig synaptic membranes gave results similar to those in the rat.

These results suggest that antibodies to synaptic membrane proteins may serve as retrograde markers and be useful in neural tract tracing studies. Furthermore, by studying the transport of antibodies to individual synaptic membrane proteins, information may be obtained as to the nature of retrogradely transported proteins. (NS 18907, HD-03352, BNS 7912939)

43.18 LOCALIZATION OF AXONALLY TRANSPORTED WHEAT GERM AGGLUTININ CONJUGATED TO HORSERADISH PEROXIDASE WITHIN AXONS OF CHICK RETINAL GANGLION CELLS, J.H. LaVail and I.K. Sugino*. Department of Anatomy and Neuroscience Program, University of California, San Francisco, CA 94143.

Recently we found that affinity-purified ^{125}I-wheat germ agglutinin (WGA) was selectively taken up and transported from chick retinal ganglion cell bodies to axon terminals in the optic tectum. We have now compared the distribution of WGA conjugated to horseradish peroxidase (WGA-HRP) with that of affinity-purified ^{125}I-WGA within chick retinal ganglion cells after axonal transport. One- to two-day-old chicks were injected intravitreally with 15 µl of buffered saline containing 10 µg/µl WGA-HRP. After 23-25 hrs the chicks were reanesthetized and fixed by perfusion. The brains and injected eyes were sectioned and reacted for HRP. Unstained sections of layer d of the contralateral optic tectum were examined by EM. Various profiles were categorized as axons, axon terminals, dendrites, glia or unknown. In contrast to the labeling with ^{125}I-WGA in which the density of silver grains was highest over axons, axon terminals showed the highest labeling density with WGA-HRP (0.61 labeled organelles/µm^2 of tectal area). Axons were less frequently labeled (0.21 organelles/µm^2). The relative labeling of nonaxonal structures was also significantly different with WGA-HRP. Only 4% of the WGA-HRP labeled organelles were found in dendrites or glial and neuron cell bodies compared to 40% with ^{125}I-WGA. The intraaxonal distribution of organelles labeled with WGA-HRP was also compared to that labeled with ^{125}I-WGA. The ^{125}I-label was nonuniformly distributed within the axon. It occupied a narrow band about 62 ± 12 nm wide with a peak located about 30 nm in from the axon plasma membrane. In comparison, the conjugate also was nonuniformly distributed within axonal profiles. It occupied a band about 60 nm wide within the axoplasm; the greatest concentration was found about 70 nm in from the plasma membrane. In axon terminal profiles, the ^{125}I label was distributed as a band 62 ± 16 nm wide located just inside the plasma membrane. A peak of label was found about 30 nm in from the plasma membrane. The HRP labeled lectin occupied a band about 74 nm wide with a peak concentration located about 90 nm in from the axon plasma membrane. Based on these distributions, we have hypothesized that ^{125}I-WGA accumulates within hypolemmal cisterns of the smooth endoplasmic reticulum in the axon and terminal. The WGA-HRP labels a more extensive system of tubules and cisterns as well as lysosomes within the axon and terminal. Supported in part by grant RO1 NS 13533 from the N.I.H.

43.19 ANTEROGRADE AXOPLASMIC TRANSPORT OF WHEAT GERM AGGLUTININ IN THE EMBRYONIC AND POSTHATCH CHICK VISUAL PATHWAY W.J. Crossland, C.J. Uchwat* and Taichang Jang*. Dept. of Anatomy, Wayne State Univ. Schl. Med. Detroit, MI 48201.

We have extended our observations on the axoplasmic transport rates of the lectin wheat germ agglutinin (WGA) and have evidence of a more rapid rate of anterograde axoplasmic transport in the chick visual system than heretofore reported.

Tracer substance was injected into the anterior pole of the retina, which projects to the caudodorsal pole of the optic tectum, to take advantage of the large intraretinal and intratectal transport distance in the chick. The following substances were injected in different experiments: Wheat germ agglutinin-horseradish peroxidase conjugate (WGAHRP); wheat germ agglutinin-fluorescein isothiocyanate conjugate (WGAFITC); tritiated wheat germ agglutinin (^{3}H-WGA); and ^{3}H-L-proline. Post-hatch chicks were injected under open ether anesthesia. After appropriate survival times the animals were sacrificed and the brains fixed and cut either on a freezing microtome (WGAHRP, WGAFITC,) or after being embedded in paraffin (^{3}H-WGA or ^{3}H-proline). The length of the visual pathway was measured in each experiment. No allowance was made for incorporation time of the label or for shrinkage of the pathway during fixation.

The embryonic (stage 38, 12 days of incubation) retinotectal pathway (21.5 mm) was labeled with either WGAHRP or ^{3}H-WGA after a two hour post-injection survival period to yield a transport rate greater than 258 mm/day. WGAFITC, although a much less sensitive marker, labeled the pathway at three hours after injection (shorter survival times not attempted), yielding a rate of a least 170 mm/day.

In the post-hatch chicks WGAHRP and ^{3}H-proline were transported to the caudal tectum (32.5 mm) in 2 1/4 hours yielding a transport rate of at least 346 mm/day. These rates are greater than those previously measured in the chick using scintillation counting methods.

We conclude three things from these observations: First, WGA travels in the rapid phase of anterograde axoplasmic transport, at a rate approximately equal to that of proteins and glycoproteins which incorporate ^{3}H-proline. Second, the rapid phase of axoplasmic transport in the visual pathway of the chick is at least 50% faster than previously measured. Third, histological observation of the fate of transported substances is a more sensitive method of determining the presence of tritium label than scintillation counting.

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43.20 SLOW AXONAL TRANSPORT IN THE WOBBLER MOUSE (MURINE MOTOR NEURON DISEASE). H. Mitsumoto and P. Gambetti. Institute of Pathology (Neuropathology), Case Western Reserve Univ., Cleveland Ohio 44106

The wobbler mouse develops hereditary motor neuron disease characterized by progressive atrophy, paralysis, and contracture, predominantly affecting forelimb muscles. Detailed morphological analyses showed that the neuronal degeneration begins in the perikaryon (primary neuronopathy). In more advanced stages of the disease, proximal axonal pathology develops in the ventral roots and the brachial plexus (Mitsumoto and Bradley, Brain, 1982). We studied slow axonal transport in wobbler mice (n=9) at six weeks of age, since the disease was clearly manifested and rapidly progressing at this age. C57Bl/6J mice at same age were used as controls (n=10). Under Halothane anesthesia, C-7,8 and T-1 posterior right half laminectomy was performed. At 2 locations of each spinal level, 0.25 ul of ^{35}S-methionine adjusted to 200 uCi/ul was stereotactically microinjected into the right ventral horn. At 5 and 15 days after the injection, the right brachial nerve, with corresponding ventral roots, was dissected out and cut into 2 mm segments. Each nerve segment was homogenized, an aliquot was sampled for scintillation counting, and the remainder was processed in 10% polyacrylamide-gel-electrophoresis (SDS-PAGE) and fluorography. Major SDS-PAGE polypeptide bands were individually cut and processed for scintillation counting. In the wobbler mice, the amount of radioactivity migrating with slow transport was reduced by more than 50% at 15 days ($P < 0.01$); percent distribution of the transported radioactivity showed a significant accumulation at the ventral root level. Although all labeled polypeptides advancing with the slow component a (Sca) and slow component b (Scb) were diminished, neurofilament triplet proteins (145K and 68K) were most severely affected ($P < 0.001$). No qualitative differences were found between fluorograms from wobbler and control.

The present findings suggest that slow axonal transport in the wobbler motoneuronopathy is impaired at the ventral root level. The abnormal slow axonal transport and morphological changes in the brachial nerve of the wobbler mouse will be correlated.

(The study was supported by the ALS Society of America, Veterans Administration, and NSI4509.)

44 SYMPOSIUM. CELL RECOGNITION IN DEVELOPMENT OF THE NERVOUS SYSTEM. U. Rutishauser, Case Western Reserve University School of Medicine (Chairman); J. Silver, Case Western Reserve University School of Medicine; D.R. Bentley, University of California, Berkeley; F. Bonhoeffer+, Max-Planck-Institut für Virusforschung, Tübingen. This session will focus on recent advances in studies of nerve guidance and recognition. Dr. Silver will describe axonal growth during development and regeneration of the mammalian CNS, with emphasis on the role of neuron-glial cell interactions, fasciculation, and the influence of extracellular spaces or channels. Recent work will be discussed on the growth of axons in the developing corpus callosum and their guidance across the cerebral midline by a sling-like glial structure. Dr. Bentley's presentation will consider mechanisms for pathfinding by peripheral pioneer neurons in grasshoppers, in particular the evidence that growth cones of early fibers navigate along a chain of cells whose placement consitutes the initial guidance mechanism underlying long-distance neural projections. Dr. Bonhoeffer will describe studies demonstrating that retinoganglion cell growth cones can distinguish between cell surface membranes of retina and tectum, and that the anterior-posterior axis of the tectum displays a graded attraction for growth cones from temporal retina. His presentation will include new techniques for analyzing growth cone recognition properties, and for mapping of long range neural pathways. The chemistry and biology of a cell adhesion molecule from neural tissue, called N-CAM, will be discussed by U. Rutishauser. Topics will include the methods used to identify and purify cell adhesion molecules, a description of the structural and binding properties of N-CAM, and studies which indicate how cell adhesion involving N-CAM can contribute to the development of neural tissues and pathways.

45 SYMPOSIUM. MONOAMINERGIC INNERVATION OF CORTEX: NEW EVIDENCE OF ANATOMICAL AND PHYSIOLOGICAL SPECIFICITY. S. L. Foote, Salk Institute (Chairman); J. H. Morrison, Salk Institute; M. E. Molliver, Johns Hopkins Univ. Sch. of Med.; B. D. Waterhouse, Univ. Texas, Dallas; R. A. Nicoll, Univ. of California, San Francisco.

Recently, there has been substantial progress in characterizing the anatomy and physiology of the noradrenergic and serotonergic innervation of neocortex. It is the purpose of this symposium to summarize the most important of these developments and to synthesize them, along with previous knowledge, into working hypotheses concerning the physiological functions of these pathways. Data relevant to four basic characteristics of these locus coeruleus and raphe systems have been selected for detailed review and interpretation:
1) light-microscopic data demonstrating that terminal arborization patterns of noradrenergic and serotonergic fibers are highly systematic in the rodent and are specialized for each functional cortical region in the primate. Both density and laminar distribution vary by region in the primate, suggesting that within each region specific neuronal populations serve as targets for this innervation, 2) ultrastructural data demonstrating that many of these monoaminergic terminals form specialized synaptic contacts onto neuronal elements, 3) data suggesting that norepinephrine and serotonin, either released from terminals or applied by microiontophoresis, have specific effects on well-defined functional activity of cortical target neurons in vivo, and 4) the demonstration, in vitro, of molecular and ionic mechanisms possibly subserving these postsynaptic electrophysiological effects. These four areas have been chosen because they are ones in which substantial progress has recently been made and because the results obtained have been in many cases unexpected and have caused the most dramatic shifts in how the brainstem monoaminergic systems and their functioning are currently perceived. These four sets of data are mutually reinforcing (and somewhat overlapping) and suggest a specific interaction of these monoamine systems with neocortex to alter the latter's functioning via spatially localized and temporally discrete modification of neuronal information processing.

46.1 COLLATERAL PROJECTIONS TO STRIATE AND EXTRASTRIATE CORTICES FROM THE DORSAL LATERAL GENICULATE, PULVINAR AND EXTRASTRIATE CORTEX IN THE MACAQUE MONKEY: A DOUBLE LABEL RETROGRADE TRACER STUDY. A. Lysakowski, G.P. Standage and L.A. Benevento. Dept. of Anatomy, Univ. of Illinois Medical Center, Chicago, IL 60612.

As part of our ongoing studies on the organization of thalamocortical pathways, we made multiple injections of retrograde tracers into visual cortical areas V1 and V4, both of which are lateral geniculate (DLG) and pulvinar targets. Tracers used were HRP, Nuclear Yellow and Granular Blue in combination with Fast Blue. Injection sites for each tracer were varied between experiments.

Striate (V1) injections produced a vertical band of cells oriented in a dorsomedial-ventrolateral direction close to the lateral border of the rostral lateral pulvinar (PL8) and more diffuse clustering in the inferior pulvinar (PI). In the region between the caudal lateral pulvinar (PLγ) and PI, there seemed to be a bridge of cells moving medially from PL8-γ to PI. The DLG was heavily labelled in all layers from this V1 injection, as expected.

V4 injections produced 2-3 vertically oriented bands in PL8-γ starting medial to the V1 band laterally and extending medially to PL's border with medial pulvinar (PM) and PI. There were spaces between these bands which may be the locus for bands of cells projecting to other cortical areas. In the DLG, there were V4-projecting cells mostly in the interlaminar zones, although some were found nearby within the laminae (JCN 203:455-474, 1981) and some nearby in the white matter surrounding the DLG. In addition, V4 injections of retrograde tracers produced cells in the oral pulvinar, PM and dorsomedial nucleus. Thalamic double labelling was found only in PI (<2%). Other subcortical areas which were found to project to V1 and V4 were medial and lateral hypothalamic areas and the claustrum. The caudal claustrum projected to both V1 and V4 with a small percentage of cells (<1%) double labelled.

Layers 5 and 6 of the occipitotemporal (OTS), posterior middle temporal (PMTS), medial bank of inferior occipital, lateral bank and floor of intraparietal, and the lower bank of superior temporal (STS) sulci had one large, single labelled cell population which projected to V4 and overlapped another, smaller one which projected to V1. The area corresponding to the middle temporal region (MT) (the floor of STS, near the junction with the lateral sulcus) showed a much heavier labelling of cells projecting to V1 than to V4. STS, OTS and PMTS showed some double labelling (<1%) in layers 5 and 6, in addition to V4-projecting cells in layers 2 and 3.

It seems that separate populations of DLG cells project to V1 and V4 and that any retinal influences from the thalamus to V4 would arise from PI and regions of PL which receive superior colliculus inputs. Thus far, based on these results, collateralization appears to be a restricted characteristic of the monkey visual system. Supported by NEI EY2940.

46.2 NEURONS IN THE MONKEY VISUAL SYSTEM WHICH PROJECT TO V1 AND V2. H. Kennedy and J. Bullier, Laboratoire de Neuropsychologie Expérimentale, INSERM-U 94, Bron, France.(SPON:M. de Ryck)

In a recent study we found that a fair proportion of the neurons afferent to areas 17, 18 and 19 of the cat have branching axons which innervate more than one region. We were interested to see if a similar situation exists in the monkey. One of two retrograde fluorescent tracers (fast blue and diadimino yellow) was injected deep in the posterior bank of the lunate sulcus (V2) and the other at a shallow angle to the cortex on the operculum (V1). Injections were positioned in such a way as to include retinotopically corresponding regions.

Subcortically we found numerous double labelled cells in the lateral pulvinar, the intralaminar nuclei and claustrum and few double labelled cells in the inferior pulvinar. The dye injected in V2 was also found in some lateral geniculate neurons, principally in the interlaminar zones, with few of these cells being double labelled. It appears therefore, that, in the monkey as in the cat, V1 and V2 do share some subcortical afferents.

In the cortex labelled neurons were found in the fundus and anterior bank of the lunate sulcus, the prelunate gyrus, the posterior bank of the superior temporal sulcus, and in inferior temporal cortex. Labelled neurons were mostly in lamina 6 except in the fundus of the lunate sulcus where many filled cells were found in laminae 2 and 3. Double labelled neurons were found in 5 extrastriate sites where there was an overlap of neurons labelled by either marker. The feedback from prestriate areas to V1 and V2 arises mostly from cells in lamina 6 and shows a sizable proportion of branching axons and, in this respect, resembles the situation in the cat.

46.3 CORTICAL AFFERENTS TO VISUAL AREA PO IN THE MACAQUE. C.L. Colby, R. Gattass*, C.R. Olson* and C.G. Gross. Dept. of Psychology, Princeton University, Princeton, N.J. 08540 and Instituto de Biofisica, UFRJ, Rio de Janeiro, Brasil

Area PO is a retinotopically organized area located on the anterior bank of the parieto-occipital sulcus extending onto the medial surface. Its visual topography is unusual in that the representation of the periphery predominates (Covey, Gattass and Gross, Neurosci. Abs. 1982).

To determine the afferents to PO and the topography of those afferents, distinguishable fluorescent retrograde tracers (nuclear yellow and bisbenzimide) were injected into representations of the upper and lower peripheral visual field under physiological guidance.

Substantial label was found in V1, V2 and the intraparietal sulcus (IPS) in all cases. Label in V1 was found exclusively in the depths of the calcarine fissure representing the periphery. In V2, label was confined to the ventromedial surface and to the depth of the most medial part of the lunate sulcus which also represent the periphery. Labeled regions in IPS included two distinct areas: a caudal zone, beginning at the anterolateral surface of the annectant gyrus and extending onto the lateral bank of the IPS, and a more rostral zone in the fundus of the IPS.

Moderate label was seen in the posterior superior temporal sulcus. Some label was found in area V3, both on the anterior bank of the lunate sulcus and on the ventral surface. Sparse label was seen in and around the arcuate sulcus. Little or no label was seen in area V4 on the prelunate gyrus.

There was no intermingling of the two kinds of label in areas V1, V2 or V3. The topographic location of the label in these areas corresponded with the retinotopic location of the injection site in PO as determined by electrophysiological recording. The segregation of transported label from upper and lower visual field representations was maintained in the IPS areas which suggests that these areas may also be retinotopically organized. Within STS there were both regions of segregation and areas of overlap for the two tracers.

Several lines of evidence suggest that PO may be homologous to Area M in the owl monkey described by Allman and Kaas. Both areas are located on the medial surface, have large receptive fields and a complex retinotopic organization which over-represents the periphery relative to other visual areas. Connections of M appear to be very similar to those of PO, including the connection with V2.

The retinotopy and connections of PO suggest that it may be more involved in orientation and localization than in pattern recognition.

46.4 PROJECTIONS OF AREA V2 IN THE MACAQUE. L.G. Ungerleider, Lab. Neuropsychology, NIMH, Bethesda, MD 20205, R. Gattass*, A.P.B. Sousa*, Dept. Neurobiologia, Instituto de Biofisica, UFRJ, Rio de Janeiro RJ, Brazil and M. Mishkin, Lab. Neuropsychology, NIMH, Bethesda, MD 20205.

Cortical and subcortical projection fields of area V2 in the macaque were examined by the use of autoradiographic and degeneration tracing techniques. In one series of rhesus monkeys (N=6), the full extent of the projection fields of V2 was determined by making large sector lesions that collectively included all of V2. In a second series of monkeys (N=14), the topographic organization of the fields was determined by injecting tritiated amino acids into 16 selected V2 sites. These sites, which were identified electrophysiologically, included the representation of the center of gaze and positions ranging from about 2.5° to greater than 45° in both the upper and lower visual fields.

The results showed that V2 projects to two areas located anterior to it, Zeki's areas V3 and V4. Together, V2, V3, and V4 are arranged in adjacent cortical belts throughout most of their extents. Like V2, V3 and V4 appear to be topographically organized, with the upper field located ventrally and the lower field dorsally. The vertical meridian is represented at the V3/V4 border, while the horizontal meridian is represented at the V2/V3 border and again at the anterior border of V4, at least out to 5°. As in V2, the horizontal meridian in V4 appears to be split and doubly represented, since an injection on the horizontal meridian of V2 results in label in V4 both on the horizontal meridian representation in its upper visual field and on the horizontal meridian representation in its lower visual field. The fact that injections of V2 beyond 40° result in label in V3 but not in V4 suggests that the representation of the visual field within V4 may not extend to the far periphery.

In addition to the projections to V3 and V4, V2 was found to project topographically to area MT. Projections of V2 were also seen to area PO, but only after injections of the far periphery in V2. This finding is consistent with the large representation of the periphery in PO (Covey et al., '82). Finally, there is a projection from V2 back to striate cortex. In contrast to the forward cortical projections of V2 to areas V3, V4, MT, and PO, which terminate predominantly in layer IV and the deep part of layer III and often appear as columns, the backward projection of V2 to striate cortex terminates in layers I, II, and V.

Subcortical projections of V2 were found to the superior colliculus, the inferior and lateral pulvinar, and the reticular nucleus of the thalamus. All of these projections appear to be topographically organized.

46.5 THE CONNECTIONS OF THE VENTRAL POSTERIOR AREA (VP) IN THE MACAQUE MONKEY. A. Burkhalter* and D. C. Van Essen. Biology Division, Caltech, Pasadena, CA 91125.

Previous studies from this laboratory have indicated that a distinct visual area, VP, adjoins the anterior margin of V2 in the ventral half of the occipital lobe of the macaque. VP contains a representation of only the upper part of the visual field, whereas V3, which adjoins the dorsal half of V2, contains a representation of only the lower part of the visual field. We regard VP and V3 as separate areas because V3 receives a major input from V1 while VP does not, and because they differ in their myeloarchitecture and in their callosal inputs (Van Essen et al., Soc. Neurosci. Abstr. 5: 812, 1979; Newsome et al., Soc. Neurosci. Abstr. 6: 579, 1980). We have analyzed the inputs and outputs of VP by making injections of a mixture of horseradish peroxidase and ^{3}H-proline into VP in three hemispheres. The corpus callosum was cut, and the resultant pattern of degenerating terminals served to delineate the borders of several topographically organized visual areas.

No detectable projections were found from V1 to VP or from VP to V1, thereby confirming that there is a major dorso-ventral asymmetry in the connections of V1. Reciprocal connections were found between VP and several topographically organized extrastriate areas, including V2, MT, and V3A. In all three cases, there were patchy projections to an as yet unnamed visual area anterior to VP, which others have shown to project to inferotemporal cortex. In addition, there were projections to cortex on the lateral bank of the intraparietal sulcus, anterior to V3A and in two cases dorsal to it. In one case, there were projections to cortex on the parahippocampal gyrus, in area TF or TH. Subcortical projections were seen to the caudate nucleus, and there were reciprocal connections with the inferior and lateral subdivisions of the pulvinar.

Characteristic differences in the laminar organization of inputs and outputs were used to distinguish between ascending (forward) and descending (feedback) connections. V2 is the only cortical area that provides ascending inputs to VP. All other cortical targets receive ascending inputs from VP and can therefore be regarded as at a higher hierarchical level of processing.

The pattern of connections of VP differs in several significant respects from that reported for V3 by Zeki (J. Physiol., 277: 245, 1978). This strengthens the argument that information from upper and lower parts of the visual field is processed in distinctly different fashion in macaque extrastriate cortex.

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46.6 THE CONNECTIONS OF AREA V4 OF MACAQUE MONKEY EXTRASTRIATE CORTEX. D. J. Felleman and D. C. Van Essen, Division of Biology, Caltech, Pasadena, CA 91125.

V4 is a visual area in the macaque that was originally identified on the basis of its strong inputs from V2. Its full extent is not known, but it is generally considered to occupy most of the pre-lunate gyrus plus neighboring portions of the lunate sulcus and superior temporal sulcus (STS). Complexities in its internal organization have led to suggestions that V4 may contain distinct subdivisions.

To study the inputs, outputs, and intrinsic connections of V4, we made injections of ^{3}H-proline or a combination of ^{3}H-proline and HRP in three hemispheres, two in anterior V4 and one in posterior V4. For two hemispheres the corpus callosum was cut to allow the distribution of label to be related to the pattern of interhemispheric connections. On the basis of the laminar distribution of anterogradely labeled terminals and retrogradely labeled cells, each connection was designated as having an ascending (forward), descending (feedback), or intermediate pattern.

In all three cases, ascending inputs to V4 arose primarily from V2 and to a lesser extent from V3 and V3A. Each of these areas received feedback from V4. We did not detect any input from V1, but in one case there was a weak feedback projection to layer II of V1.

The major ascending output from V4 was to the temporal lobe, primarily in inferotemporal cortex (IT or TE). The posterior V4 injection labeled a long, narrow strip across posterior IT and extending into area TF in the occipito-temporal sulcus (OTS). The anterior V4 injections labeled one or two major foci in posterior IT and one focus in TF. There was a feedback pathway from these regions to V4. In the one V4 injection that was near the horizontal meridian representation there was also label in a topographically organized region posterior to IT.

From both of the anterior V4 injections there were ascending outputs to area MST (an MT-recipient zone in the fundus of the STS) and to area POa on the lateral bank of the intraparietal sulcus. Neither of these pathways was detected after the posterior V4 injection, however. Subcortical projections were also seen to the lateral and inferior pulvinar, claustrum, putamen, and the caudate and pregeniculate nuclei.

The intrinsic connections in V4 are highly complex, with evidence for forward, feedback, and intermediate patterns in different patches. We found no systematic relationship between the pattern within a patch and its location with respect to the injection site. It is clear from these experiments that there are anatomically distinct subdivisions within V4, but unclear whether these warrant classification as distinct visual areas. Supported by NIH Grant EY 02091.

46.7 COLOR SELECTIVITY OF V4 CELLS AND INDUCED SPECTRAL PROPERTIES. F.M. de Monasterio* (SPON: M.J. Hoffert). Section on Visual Processing, Clinical Branch, National Eye Institute, NIH, Bethesda, Maryland 20205.

I report here results obtained from single cells in the foveal representation of the prelunate gyrus of macaque cortex, area V4, using a semichronic, paralyzed and anesthetized preparation. Cell responses were evoked with narrow-band stimuli of the appropriate size, shape and orientation. These lights were matched either for an equal-quanta spectrum (13 log quanta/s.cm^2 at the screen) or a 2°-CIE equal luminosity at the low photopic range (ca. 200 td). Broad-band lights provided diffuse background illumination. Due to the significant response variability of many V4 cells, responses were summed over 10 trial blocks and, within each block, the wavelength presentation sequence could be randomized. Spectral response curves were expressed as standard deviation units from the prestimulus activity at each wavelength.

Few V4 cells showed color-opponent responses. Classification of color non-opponent cells into 'color biased' and 'color unselective' groups depended on the arbitrary selection of a spectral cutoff criterion (Schein, Desimone & de Monasterio, ARVO Abstracts 1983). The majority of V4 cells responded well to white lights. Steady-state chromatic adaptation with weak backgrounds showed that V4 cells receive input from 2 or 3 cone types. While these inputs were not antagonistic in color non-opponent cells, both the peak sensitivity and badwidth of responses mediated by signals from green- and red-sensitive cones indicated that antecedent spectral interactions affected cell inputs. When using weak chromatic backgrounds in non-equilibrium conditions (i.e. simultaneously flashing test and background lights), a transient but marked desensitization of some cone inputs could be evoked. In color non-opponent cells, this desensitization produced a transient color-opponent behavior similar to that found in retinal ganglion cells. The antecedent opponent mechanism underlying the transient desensitization also induced a transient suppression of the responses to the simultaneous presentation of end-spectral lights.

The results are noteworthy in at least two respects. First, they demonstrate the existence of transient adaptational anomalies in cells of a central level of the primate visual system. Like the similar retinal anomalies, they are consistent with recent psychophysical findings in humans. Second, the results are relevant to problems of neuronal taxonomy in area V4. Because of antecedent spectral interactions, stimuli delivered in close spatial or temporal presentation may induce transient color opponency in color non-opponent cells. On this basis it is possible not only to misclassify V4 cells, but even also to draw elaborate erroneous conclusions regarding 'higher level' properties of the neurons.

46.8 RECEPTIVE FIELD PROPERTIES OF NEURONS IN VISUAL AREA V4 OF THE MACAQUE. R. Desimone and S.J. Schein#. Laboratory of Neuropsychology, NIMH and #Section on Visual Processing, National Eye Institute, Bethesda, MD. 20205.

V4 is a visuotopically organized prestriate area that receives major input from V2 and is a major source of visual input to inferior temporal cortex. The color properties of V4 cells have been described by us and others previously. In the present study we report the spatial properties of V4 cells.

Single neurons were studied within the representation of the central 5° of V4 in 4 anesthestized (N_2O), paralyzed macaques, with a semi-chronic preparation. We mapped the excitatory receptive field (RF) by hand and then measured the response to sine-wave gratings and bars. The cells could be divided into two groups based on their responses to gratings. Both groups had similar RF widths, equal to 0.9° + 0.4 x Eccentricity. The optimal spatial frequency for cells in the first group, however, had a period greater than the RF width, while that of cells in the second group had a period equal to or less than the RF width. Thus, the first group appeared to show summation within the RF, while the second group appeared to show spatial interactions within the RF. Some (but not all) of the properties of the two groups appear to be analogous to properties of striate simple and complex cells, respectively. Cells of the first group were tuned to low spatial frequencies (mean 0.4 cpd), were often single-phase sensitive to flashed gratings of optimal frequency, gave modulated responses to drifting gratings (35% mean modulation), and responded best to wide bars (mean 2.0 x RF width). By contrast, cells of the second group were tuned to higher spatial frequencies (mean 3.1 cpd), were rarely single-phase sensitive, gave unmodulated responses to drifting gratings (10% mean modulation), and responded best narrow bars (mean 0.4 x RF width).

Cells of both groups normally had a large suppressive zone located around the RF extending up to 10-20 degrees from the RF center. Although dark bars, light bars, annuli and gratings failed to elicit any response outside the RF, such stimuli often reduced the response to an RF stimulus by 80% or more.

Although the two groups of cells in V4 may represent a continuum rather than a dichotomy, the similarity to the simple/complex division in striate cortex suggests that the latter duality might be carried far into the visual pathway. In addition, the powerful interaction between the RF and the large silent suppressive surround suggests that V4 cells respond best to a difference in visual content between the RF and its surround.

46.9 EFFECTS OF VISUAL CORTICAL LESIONS ON VISUAL TRACKING IN CATS. R.J. Tusa, J.L. Demer* and S.J. Herdman, Wilmer Eye Institute, Johns Hopkins Hosp. and Univ. of Md., Baltimore, Md., 21205.

Left hemisphere cortical lesions were made by subpial aspiration in 6 adult cats under general anesthesia. Ablations included all visual cortex in 2 cats (VC); PMLS (Clare-Bishop) in the lateral suprasylvian sulcus in 2 cats (PMSL); areas 17 & 18 in one cat (17/18); and 17 & 18 + corpus callosum section in one cat (17/18 + CC). Slow phase eye velocity in response to a full field optokinetic nystagmus (OKN) drum, which was slowly accelerated (.27 deg/s^2) or stepped from 4-120 deg/s, was measured using the magnetic search coil during both binocular and monocular viewing. Eye velocity was plotted against retinal slip velocity (target velocity minus eye velocity). Visual-vestibular interactions were determined by measuring vestibulo-ocular reflex (VOR) gain (eye vel/head vel) in the dark during sinusoidal head rotation (.05 and .25 Hz, 30 deg/s), compared to VOR gain in the light with an OKN drum moving in or out of phase with the head.

Measurements were made 5 days, 3 weeks and 7 weeks post-operatively and compared to pre-operative performance. The extent of the lesions were confirmed histologically.

In cats with VC lesions, peak eye velocity to the left (towards the lesion) for binocular OKN was 50% of pre-operative measurements and this peak eye velocity occurred at a lower retinal slip velocity. OKN in the other direction was unchanged and this directional asymmetry persisted through the study. The cat with the 17/18 lesion had no deficit in OKN, whereas the cat with the 17/18 + CC lesion had a deficit similar to the cats with the VC lesions. Cats with PMLS lesions also had an OKN deficit similar to cats with VC lesions, but they completely recovered by 3 weeks. During monocular OKN viewing, there was a significant deficit in peak eye velocity to the left for nasal-temporal and temporal-nasal stimuli; and to the right for nasal-temporal stimuli only. This occurred in the cats with the VC lesion, 17/18 + CC lesion and transiently in cats with the PMLS lesion. Alterations in visual-vestibular interactions following cortical lesions were found to be predictable from the individual VOR and OKN responses.

Our preliminary results suggest that OKN deficits in cats are similar to those found in primates following large, unilateral cortical lesions. We hypothesize that the cortical contribution for OKN in via areas 17/18. This region appears to enhance slow phase eye velocity ipsilaterally through extrastriate cortex. Following a unilateral 17/18 lesion, the contralateral 17/18 areas can be used to enhance slow phase eye velocity toward the side of the lesion through the corpus callosum. Although PMLS also appears to be involved with OKN, other areas are able to compensate following its removal.

46.10 **FUNCTIONAL NEUROANATOMY OF VISUALLY GUIDED REACHING IN THE MONKEY.** R. J. W. Mansfield and D. N. Pandya. Department of Neurology, Boston University School of Medicine, Boston, MA 02118 and Rogers Memorial Veterans Administration Hospital, Bedford, MA 01730.

A central question in the study of primate vision is the relation between defined anatomical structures and specific behavior. To address this question we have developed a double isotope 2-deoxy-D-glucose (2DG) method of labelled local cerebral glucose utilization and applied it to the analysis of visually guided reaching in the Rhesus monkey. In the paradigm task a monkey fixated then grasped with its right hand small targets that appeared at random intervals displaced 10^o to the right of its midline. With only the target zone illuminated a bolus intravenous injection of [^{14}C]-2DG (100 µCi/kg) was given and rapid trials conducted for 45 minutes. Subsequently following a second intravenous bolus injection of [^{3}H]-2DG (3 mCi/kg) the task was continued for 45 minutes but in the absence of illumination. The animal was deeply anesthetized, perfused with 3.3% buffered formalin, the brain blocked and rapidly frozen in Freon 22. Cryostat sections (20 µm) cut serially at 200 µm intervals were developed separately for tritium and carbon-14 autoradiography to yield a ten-fold isolation of both tritium and carbon 14 images. Using calibrated standards and a computer image processing system the optical densities of the autoradiographs could be referenced back to levels of glucose metabolism. To differentiate in a given region between intrinsic task-specific metabolic activity and extrinsic input from other regions of enhanced metabolism, adjacent sections were stained for cytochrome oxidase.

The autoradiographs revealed regions of differential glucose metabolism both cortically and subcortically that were not coextensive with dense cytochrome oxidase labelling. In both the visually guided reaching task and in its dark control limbic and motor regions such as cingulate gyrus and basal ganglia had increased glucose metabolism but in the visually guided task increased labelling was found in the majority of regions known to receive a more direct visual input. In V1 the region of increased glucose metabolism was confined to a portion of the opercular surface representing the central 5^o with the densest label in layer IVC. In the more anterior portions of the visual system the region of increased metabolism was enlarged so that in the inferotemporal cortex most of area TE was engaged consistent with the larger foveal representation in that area. In general the results are consistent with a graded activation of cortical regions along the visuo-motor pathway in a focal visually guided task.(Supported by F.R.N.S. and NIH Grant EY 04854; and by Veterans Hospital, Bedford, MA, and NIH Grant NS 16841.)

46.11 DEFICITS IN PURSUIT EYE MOVEMENTS AFTER CHEMICAL LESIONS OF MOTION-RELATED VISUAL AREAS IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE MONKEY. W.T. Newsome, R.H. Wurtz, M.R. Dursteler*, and A. Mikami. Lab. Sensorimotor Res., National Eye Institute, NIH, Bethesda, MD 20205.

Neurons in identified visual areas (including MT and MST) of the superior temporal sulcus (STS) are selective for the direction and speed of stimulus motion. We produced chemical lesions of these motion-related areas to determine whether cells in these areas provide information about stimulus motion for the generation of smooth pursuit eye movements.

We trained monkeys to fixate a spot of light and measured eye movements using the magnetic search coil technique. After a monkey had begun to fixate, the spot of light went out and another spot of light at a variable location on the horizontal meridian began to move towards or away from the fixation point at 16^o/sec (a step-ramp paradigm). The monkeys were required to shift fixation and pursue the moving spot. A monkey's response could generally be divided into three phases: first, a brief interval (60-100 msec) of nonfoveal pursuit before making a saccade; second, a saccadic eye movement to bring the fovea onto the moving target; third, post-saccadic foveal pursuit of the moving target for the duration of the trial. Our analysis is concerned with the pre-saccadic pursuit and with the first 100 msec of post-saccadic pursuit--those intervals of pursuit which must depend upon visual motion information derived from a known location on the peripheral retina. A microliter syringe was insulated so that recording of multiple unit activity could be obtained from the tip. Motion related visual areas were identified and injections were made near the representation of the horizontal meridian. For reversible lesions we injected one ul of muscimol (1ug/ul) into the area. For irreversible lesions we injected 4 ul of ibotenic acid (15ug/ul).

Following injection of muscimol, presaccadic initiation of pursuit to targets moving in affected regions of the visual field had a longer latency (by 50-120 msec) or did not occur at all. Latency of saccades to the target remained about the same, as did initiation of pursuit to targets in the ipsilateral visual field. On the day following the injection, pursuit initiation in the test hemifield returned to the preinjection levels. Following injection of ibotenic acid in one monkey the velocity of pursuit was severely reduced for about 200 msec after the saccade. This monkey did not initiate presaccadic pursuit before or after the injection. The monkey recovered from the deficit within a week. These results suggest that motion information from visual areas of the STS is important for the generation of pursuit eye movements in response to targets moving on the peripheral retina.

46.12 FINE-RESOLUTION, QUANTITATIVE ACTIVITY-LABELING OF MACAQUE BRAIN WITH 2-DEOXYGLUCOSE. S.J. Schein & F.M. de Monasterio*. Section on Visual Processing, Clinical Branch, National Eye Institute, Bethesda, MD 20205.

Activity-labeling with 2-deoxyglucose (Sokoloff et al 1977) results in intracellular accumulation of water-soluble 2-deoxyglucose-6-phosphate (2-DG-6-P). Standard tissue processing (rapid freezing, cryomicrotomy, mounting and rapid heat-dehydration) produces autoradiograms (ARGs) with resolution of ~50µm (Goochee, Rasband & Sokoloff 1980). An early attempt to obtain fine-resolution maps of galactose, another water-soluble molecule, used freeze-drying of a small piece of intestine and plastic embedding (Stirling & Kinter 1967). A similar procedure, but using freeze-substitution or solvent immersion, was applied to a snail nerve ganglion (Sejnowski et al 1980). In addition to the unsuitability of these methods for a large brain, they provide only qualitative ARGs (Stirling & Kinter; Reingold et al 1981), whose interpretation may be complicated by the finding that some of the retained label is chemically bound to glycogen (Witkowski & Yang 1982).

A previous report (Schein & de Monasterio 1981) presented empirical studies of the process of freeze-substitution. Here, we present the underlying physical principles of the process, principles necessary for the design of conditions (solvent, temperature and time) which minimize loss of 2-DG-6-P and allow complete replacement of water. We also present the chemistry of withdrawal of tissue label, which dictates some of the conditions for freeze-substitution, the choice of embedding material and the method for mounting of sections on slides. Autoradiography uses sheet film.

Tissue processing which follows these principles produces sharp ARGs with minimal loss or movement of 2-DG-6-P. The resolution of ^{14}C-ARGs is roughly equal to section thickness. Full-size sections of macaque brain can be obtained at a thickness of 7µm. ARG with ^{3}H gives still finer resolution but at a sacrifice: Only the surface of such 'thick' sections is imaged on film. Results with this new method for the ocular dominance system of macaque striate cortex will be presented.

Conversion of film density into glucose utilization relies on use of the operational equation (Sokoloff et al) and calibration of film density as a function of tissue radioactivity. We present a new calibration method which can be used with ^{14}C or ^{3}H, on sections of any thickness, prepared by this or any other method.

46.13 THE PATTERN OF VISUAL CALLOSAL CONNECTIONS IN THE CAT AS REVEALED IN TANGENTIAL SECTIONS OF THE UNFOLDED CORTEX. J. Olavarria* and R.C. Van Sluyters (SPON: E. Marg). Neurobiology Group and School of Optometry, University of California, Berkeley, CA 94720.

We have studied the distribution of visual callosal cells in sections cut parallel to the brain surface after unfolding and flattening the entire cortex of the cat. Callosal cells were retrogradely labeled by implanting 15% horseradish peroxidase (HRP) gels in the sectioned corpus callosum of 5 cats. After a survival period of 48 hrs, the cats were perfused briefly with 2% glutaraldehyde (300 cc in 7 min) in order to achieve fixation of cortical grey matter without fixing the underlying white matter. The cortex was separated from the rest of the brain and the hippocampus was resected. Unfolding was facilitated by gently removing the white matter, incising the pia overlying the sulci and cutting the cortex at selected places along its margin. The resulting sheet of tissue was flattened between two glass slides, placed overnight in fixative, frozen, sectioned tangentially (60-80 um) and reacted for HRP. This procedure introduced minimal distortion since cortical cytoarchitectonics appeared normal in "coronal" sections of the flattened tissue. The complete pattern of visual callosal connections was reconstructed from consecutive tangential sections. This pattern was related to the locations and topographic maps of known visual areas utilizing gyral and sulcal landmarks that were preserved in the flattened tissue by dying the surface of the brain prior to unfolding. In addition, the exact location of striate cortex was determined in selected tangential sections stained for myelin.

Labeled cells formed an elaborate pattern whose organization was similar in all cats studied. The location of this pattern correlates well with the representation of the vertical meridian in the various visual areas mapped by Tusa, Palmer and Rosenquist, and others. Thus, a densely labeled band extends along the entire lateral border of area 17. A second band, located more laterally, virtually surrounds a broad region of cortex containing areas 18, 19, 21b and 20a. Two densely labeled areas bridge the gap between these two bands and apppear to correspond to patches of callosal projections previously reported within area 18. A large labeled region covers most of areas 21a, DLS and VLS. This region also covers the posterior portions of the PMLS and PLLS areas, while anteriorly, near the fundus of the lateral suprasylvian sulcus, it narrows and lies in the region of the border between these two areas and the AMLS and ALLS areas. The present approach has provided detailed information regarding the overall organization of visual callosal connections in the normal cat, thereby encouraging its application to studies of the development and modifiability of this pathway.

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DRUGS OF ABUSE: NONOPIATES I

47.1 THE EFFECTS OF DIAZEPAM WITHDRAWAL ON FLURAZEPAM, BICUCULLINE AND PICROTOXIN SEIZURE THRESHOLD IN DIAZEPAM DEPENDENT RATS. C.E. Reigel, Jr.* and W.M. Bourn, Dept. of Pharmacology, Toxicology and Nuclear Pharmacy, School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209

Benzodiazepine (BZ) binding to the BZ receptor increases GABA receptor affinity for endogenous GABA, thus enhancing GABAergic function. GABAergic function may also be manipulated at two other distinct sites: the GABA receptor itself and the GABAergic chloride ionophore. Bicuculline (BCC) and picrotoxin (PTX) produce convulsions through actions at the GABA receptor and the GABAergic chloride ionophore, respectively. Recently flurazepam (FZP) has been demonstrated to produce convulsions in rats, possibly through actions at a BZ receptor.

In the present study FZP, BCC and PTX seizure thresholds were determined in diazepam (DZ) dependent, withdrawn rats and vehicle withdrawn control rats in an attempt to implicate the possible site(s) responsible for DZ physical dependence and the hyperexcitability seen during withdrawal. Rats with chronically implanted cortical electrodes were assigned to two groups for each seizure threshold study. Each group received a series of 30 intraperitoneal injections, one every 12 hours. DZ dependent rats received 100 mg/kg DZ suspended in 1% Tween 80. Vehicle control rats received only the 1% Tween 80 vehicle. FZP, BCC and PTX seizure thresholds were determined by continuous tail-vein infusion 36 hours after the last injection of DZ or vehicle, a period previously determined to be the peak of DZ withdrawal as assessed by weight loss and increased irritability. Only FZP and PTX seizure thresholds were decreased during DZ withdrawal (see below).

	Seizure Threshold: mg/kg	
	DZ Withdrawn	Vehicle Withdrawn
FZP	41.4 ± 2.1 (11)	63.3 ± 2.8 (11)†
BCC	0.090 ± 0.005 (10)	0.082 ± 0.003 (11)
PTX	13.10 ± 0.35 (11)	14.00 ± 0.18 (11)††

† Significant at $p < 0.001$, two-tailed Student's t

†† Significant at $p < 0.05$, two-tailed Student's t

Results were compared to FZP, BCC and PTX seizure thresholds in barbital dependent, withdrawn rats.

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47.2 SYNAPTIC EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL AND ITS 11-HYDROXY METABOLITE ON CAT SPINAL MOTONEURONS. S.A. Turkanis and R. Karler*. Dept. of Pharmacology, Univ. of Utah, School of Medicine, Salt Lake City, Utah 84132.

Delta-9-tetrahydrocannabinol (delta-9-THC), the major psychoactive constituent of marihuana, elicits a variety of central depressant and excitatory effects, which range from sedation and ataxia to frank convulsions. The purpose of the present work was to identify possible synaptic mechanisms of action for these central effects of delta-9-THC. To this end, conventional electrophysiological techniques were used to uncover synaptic effects of delta-9-THC and its principal active metabolite, 11-hydroxy-delta-9-THC, (0.01-0.1 mg/kg i.v.) on the spinal cord of adult cats with their spinal cords severed and their brains destroyed ischemically. The results of the investigation demonstrated that both drugs enhanced the excitatory postsynaptic potential (EPSP) amplitude to suprathreshold values. Subsequent experiments showed that delta-9-THC and its metabolite under the same conditions increased the motoneuron membrane resistance, an effect which may account for the rise in EPSP amplitude. Moreover, the EPSP enhancement does not appear to be associated with an increase in the afferent input because neither drug affected the compound action potential recorded from the dorsal roots near the point where they enter the spinal cord. In contrast to the increase in the EPSP, delta-9-THC reduced the amplitude of the inhibitory postsynaptic potentials (IPSPs), but the metabolite exerted no effect. In addition to excitation, both cannabinoids elicited depression, as evidenced by an increase in the firing threshold for the motoneuron action potential. The data are compatible with well-established properties of delta-9-THC in conscious animals because the drugs evoke a complex mixture of central effects.

Delta-9-THC and the 11-hydroxy derivative exert, in general, similar effects on the spinal cord; likewise, the comparative results reported previously from other test systems also indicated a similarity in their pharmacological properties. The data demonstrate that the metabolite may contribute to the activity of the parent drug. The findings with EPSPs and IPSPs suggest possible sites and mechanisms of drug action, such as alterations in postsynaptic receptor sensitivity, transmitter equilibrium potentials and transmitter release; whereas the increases in membrane resistance and motoneuron firing threshold are probably related to drug-caused postsynaptic conductance changes. Regardless of the precise mechanisms, if the above effects are applicable to higher sites in the CNS, they may contribute to delta-9-THC's central excitatory and depressant properties. (Supported by NIDA research grant DA-00346).

47.3 DOSE-RELATED EFFECTS OF PHENCYCLIDINE ON SPONTANEOUS AGGRESSIVE BEHAVIOR IN THE RAT. B.D. Greenberg*, J.W. Russell*, and D.S. Segal. Psychiatry Dept., Sch. of Med., Univ. of Calif., San Diego, La Jolla, CA 92093.

Phencyclidine (PCP) abuse has been frequently associated with violent behavior in humans. There have been a number of studies investigating the effects of PCP on experimentally-induced aggressive behavior in animals, with findings which vary depending upon the methodology used. It is unclear from the available results whether PCP will alter species-specific aggressive interactions under conditions in which animals exhibit a relatively low level of spontaneous aggression. In several previous studies the effects of PCP were examined in pairs or groups of drug-treated animals. This approach does not allow characterization of the response of the drug animal to the pattern of behavior of its untreated partner, and vice-versa. We therefore investigated the effects of PCP in pairs of rats in a paradigm in which spontaneous aggression was infrequent and when only one animal of a pair was drug-treated.

Male Wistar rats were habituated individually for 7 days to sound-attenuated chambers identical to the test chamber. On the eighth day, pairs of rats, selected randomly, were placed into the test chamber for the first time 5 min after one animal received saline and the other animal was injected with saline or 0.25, 0.5, or 1.0 mg/kg PCP (s.c.). Testing took place in the dark phase of the illumination cycle. Animals were videotaped for 60 min after injection, and the following behaviors were subsequently rated: boxing (mutual upright posture), attack, submissive behavior, and allogrooming.

Phencyclidine induced dose-related alterations in the pattern of spontaneous aggressive behavior. At the lowest dose tested (0.25 mg/kg), PCP produced attacks by the drug-treated animal and a corresponding increase in submissive behavior by the untreated partner, as well as an increase in boxing behavior by both animals. In contrast, the highest dose of PCP tested (1.0 mg/kg) elicited attacks and allogrooming by the untreated animal. The lowest dose effect is interpreted as the result of PCP-induced distortion of perception of social cues, while the highest dose result appears to be due to a general disruption in social communication by ataxia in the PCP-treated animal.

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47.4 DOSE AND TIME REQUIREMENTS FOR BENZODIAZEPINE PHYSICAL DEPENDENCE PRODUCTION. H.C. Rosenberg, W.M. Gilliam* and T.H. Chiu. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

Studies were performed to determine the dose and treatment duration required to produce physical dependence on benzodiazepines in cats. Dependence was evaluated by rating 20 possible abstinence signs after giving the antagonist, Ro15-1788. Following chronic daily intragastric administration of flurazepam (FZP), Ro15-1788 produced tremor, increased muscle tone, panting, salivation, pupillary dilation, piloerection, and defecation (Eur. J. Pharmacol. 81: 153, 1982). Ro15-1788 alone or immediately after an acute dose of benzodiazepine did not produce any abstinence signs. The incidence and severity of abstinence signs was dependent on the antagonist dose, but was maximal at doses of 2 mg/kg and above.

To determine the dose requirement of dependence, animals were treated 5 weeks with 1, 5 or 20 mg/kg FZP. All animals had an abstinence syndrome upon antagonist administration. Abstinence signs were only slightly more severe after chronic doses of 5 and 20 mg/kg than after treatment with 1 mg/kg.

To determine the treatment duration required for dependence, cats were given 5 mg/kg FZP for 1, 3, 7, 35 or 70 days. The abstinence syndrome was very similar after 35 and 70 days of treatment, and only slightly less intense after 3 and 7 days of treatment. One of the three cats given a single 5 mg/kg FZP dose had a very mild abstinence syndrome precipitated by Ro15-1788. Five cats given a single 40 mg/kg FZP dose all had a mild abstinence syndrome precipitated 24 hrs later.

Physical dependence was measured 1,2,7 and 14 days after 5 weeks of 5 mg/kg FZP daily treatment. Abstinence precipitated 2 days after the end of chronic treatment was the same as that at 1 day. That at 7 days was less severe, and there was no observable abstinence 2 weeks after chronic treatment. This time-course correlated with the slow disappearance of plasma desalkylflurazepam and the slow reversal of tolerance in cats treated by this method (Eur. J. Pharmacol. 81:357, 1982).

In animals treated with diazepam, 5 or 20 mg/kg daily for 5 weeks, Ro15-1788 precipitated an abstinence syndrome that was essentially the same as that seen in cats treated with FZP.

Dependence resulting in spontaneous abstinence signs usually requires prolonged treatment with large doses of benzodiazepines. In contrast, dependence as measured by an antagonist precipitated abstinence syndrome is possible 24 hrs after a single dose, and is almost as intense after chronic treatment with a rather low dose (1 mg/kg FZP) as after much higher doses (20 mg/kg).

Supported by DHHS grants DA02194 and NS16595. FZP, diazepam and Ro15-1788 were generously supplied by Hoffmann-LaRoche, Inc.

47.5 NEUROPHARMACOLOGICAL PROFILE AND TOLERANCE ASSESSMENT FOR ACUTE AND CHRONIC EFFECTS OF 2,5-DIMETHOXY-4-METHYLAMPHETAMINE (DOM) IN THE RAT. B. Hine+ and M. Torrelio*. Millhauser Labs, Dept. of Psychiatry, New York Univ. Med. Ctr., New York, NY 10016.

Phenethylamine hallucinogens have been considered agents that produce, in animal studies, a unique neuropharmacological profile of amphetamine-like stimulant effects at low doses and responses characteristic of indolealkylamine hallucinogens at high doses. The present experiments used a variety of response measures to assess this generalization after intraperitoneal injections of racemic DOM hydrochloride (0.25-4.0 mg/kg) over a 15-day period in male Sprague-Dawley rats. Receptor blocking studies also were performed to further characterize the involvement of dopamine and serotonin in these responses.

Acute DOM produced a bidirectional effect on vertical activity (rearing), with maximal increases above saline controls at 0.25-0.5 mg/kg and significant decreases at 1.0 mg/kg and higher doses. Maximal production of head-twitch responses occurred at a DOM dose of 1.0 mg/kg. An increase in the number of head twitches and rearings occurred for several dose levels after 5 daily injections, followed by a decreasing number of responses over the next 10 days. Unidirectional increases in rectal temperatures occurred at 0.25 mg/kg and higher doses, with a similar pattern of increases and then decreases in the hyperthermic response over 15 days. At 2.0 mg/kg, only a progressive increase in hyperthermia occurred with daily injections. DOM produced a dose-related induction of palpebral ptosis to which tolerance did not develop over 15 days. Although no changes in water consumption occurred after acute DOM, a progressive increase occurred, with increasing doses, over the 15-day period. The involvement of dopamine in DOM-induced head twitches and rearing was demonstrated by the blockade or attenuation of these responses after pretreatment with haloperidol (0.25 and 0.5 mg/kg). However, although methysergide pretreatment at 10 mg/kg had no effect on DOM-induced rearing, head twitches and ptosis were significantly attenuated. Haloperidol increased DOM-induced ptosis.

These data indicate that DOM can induce both amphetamine- and indolealkylamine-like effects over a range of doses in rats, and repeated administration may produce changes in the expression of these responses that do not reflect a tachyphylaxis or unidirectional tolerance. In addition to extending existing data on the involvement of dopamine in DOM-induced responses, the results suggest the desirability of including a variety of measures when developing profiles for pharmacological actions of agents such as DOM.

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47.6 PHENCYCLIDINE-INDUCED LOCOMOTOR ACTIVITY IN THE RAT IS BLOCKED BY A 6-HYDROXYDOPAMINE LESION OF THE NUCLEUS ACCUMBENS OR VENTRAL TEGMENTAL AREA: COMPARISONS TO OTHER PSYCHOMOTOR STIMULANTS. Edward D. French and Guido Vantini*, Maryland Psychiatric Research Center, P.O. Box 3235, Baltimore, MD 21228.

A considerable volume of evidence now exists documenting the effects of phencyclidine (PCP) on central neurotransmitter systems, in particular dopamine (DA). Behaviorally and biochemically, PCP shares some of the actions produced by amphetamine (AMPH). Several groups have reported that forebrain DA systems mediate the AMPH hyperactivity since 6-OHDA lesions of either the mesolimbic DA containing nucl. accumbens (NAc) or A10 neurons of the VTA projecting to forebrain can block this AMPH effect. Also there recently has been found an interaction between PCP and SKF10047 (a σ opiate receptor agonist) in receptor binding analyses and in operant discriminative stimulus paradigms. Interestingly, SKF also produces in the rat behavioral changes similar to those produced by PCP. The goal of the present study was to examine the role of the mesolimbic NAc system in mediating the locomotor stimulant actions of PCP and SKF.

Using photocell equipped cages and gross behavioral observations the motor activating and behavioral effects of AMPH (1.5mg/kg), SKF (25mg/kg), scopolamine (1mg/kg) and caffeine (10mg/kg) were measured and compared to PCP's (5mg/kg) actions. 5mg/kg was selected as the optimal dose of PCP based upon a dose-response determination of maximum locomotor activity with minimal interference from an accompanying ataxia. While all compounds produced a moderate to large degree of hyperactivity with varying time courses for the effects, gross behavioral observations indicated a greater similarity between PCP and SKF than between any of the other drugs. Following bilateral 6-OHDA lesions of the nucleus accumbens (4ug/ul-2ul vol.) the robust locomotor stimulating effect of PCP was blocked. Such lesions also successfully prevented AMPH- and SKF-induced hyperactivity but not the behavioral activation caused by scopolamine or caffeine. In another set of experiments rats received bilateral 6-OHDA (4ug/ul-1ul vol.) or vehicle injections into the VTA and were subsequently administered AMPH, PCP, and SKF. As with NAc lesions such treatment significantly attenuated the locomotor stimulating effects of all three compounds. In both lesion experiments NAc DA content was measured and found to be reduced by 87-91%.

These results strongly suggest that the locomotor hyperactivity produced by PCP, SKF, and AMPH is subserved by a common presynaptic mechanism present within the mesolimbic DA system.

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47.7 COMPARATIVE EFFECTS OF SUB-CHRONIC BUSPIRONE OR NEUROLEPTICS ON RAT BRAIN DOPAMINE FUNCTION. Brian A. McMillen, Dept. Pharmacol., School of Medicine, East Carolina Univ., Greenville, NC 27834.

Buspirone is an anti-anxiety drug without any other benzodiazepine activity. Previous reports showed buspirone to be an antagonist of rat dopamine (DA) presynaptic autoreceptors (1). In addition, buspirone reverses catalepsy due to either DA receptor blockade or DA depletion (2). Thus, it was hypothesized that long term buspirone treatment could alter brain DA receptors or metabolism. Buspirone was compared to known inhibitors of DA receptors, haloperidol or trifluoperazine. For sub-chronic studies, buspirone (1.0 mg/kg/day) or haloperidol (0.3 mg/kg/day) were administered s.c. via 2 week osmotic minipumps (Alza). Without drug washout, rats were challenged with either 1.0 mg/kg buspirone or 0.1 mg/kg haloperidol. The table shows that haloperidol, not buspirone, caused a marked sub-sensitivity to both challenge drugs.

	striatal DOPAC ug/g	s.e.m.	
2 weeks	no drug	1.0 bus	0.1 halo
incision only	1.61 ±0.11	4.67 ±0.16	4.09 ±0.16
1.0 buspirone	1.47 ±0.07	3.88 ±0.34*	3.62 ±0.24
0.3 haloperidol	2.40 ±0.18*	3.13 ±0.15+	3.04 ±0.12+

*p<0.05, +p<0.01; different from respective controls (Dunnett's t test), 6 to 8 rats per group.

Results from 3 months of daily administration p.o. of either 3.0 mg/kg buspirone or 1.0 mg/kg trifluoperazine were simiar to these data from the sub-chronic experiment. Buspirone, 3.0 mg/kg/day, altered neither tyrosine hydroxylase nor pre-synaptic DA receptor sensitivity, tested in vitro after a 4 day drug washout. The Bmax and Kd for 3H-spiperone binding to striatal membranes was significantly less after buspirone than after 3 months of trifluoperazine (Bmax= 314 ±15 vs 404 ±18 fmoles/mg and Kd= 0.18 ±0.01 vs 0.28 ±0.01 nM, respectively). These data are in harmony with those of Hyslop et al. (this volume). Co-administration of buspirone for two weeks with trifluoperazine prior to the 4 day drug washout significantly reversed the effect of trifluoperazine on striatal 3H-spiperone binding. These data suggest that: (1) buspirone should be free of extra-pyramidal risk as neither sub-sensitivity to haloperidol challenge nor super-sensitivity of D2 receptors occured after chronic treatment; (2) buspirone did not alter DA autoreceptor function; and (3) trifluoperazine-induced alterations of D2 receptors may be reversed by co-administration of buspirone. This latter effect may be secondary to other effects of buspirone on the extrapyramidal system (1,2).

(Supported by a contract from Bristol-Myers Co.)

1. McMillen, et al., J. Neurosci. 3:733, 1983.
2. McMillen, and McDonald, Neuropharm. 22:273, 1983.

47.8 EFFECTS OF DIFFERENT CLASSES OF PSYCHOPHARMACEUTICALS ON CEREBRAL CAPILLARY PERMEABILITY AND BLOOD FLOW. J. Schwartzman*, S. Preskorn, T. Kent*, S. Goldberg* and R. Glotzbach*. Departments of Psychiatry and Pharmacology. University of Kansas Medical Center, Kansas City, Kansas 66103.

Antidepressant drugs such as amitriptyline increase the effective permeability (PS) of cerebral capillaries to water without altering cerebral blood flow (CBF). This effect occurs in both primates and rodents at clinically relevant concentrations and is enhanced by chronic treatment similar to their clinical use. This increase appears to be dependent on an intact central adrenergic system supporting the concept that one function of this system is regulation of the cerebral microcirculation. These observations do not tell us whether the effect is a general phenomenon observed following any psychopharmaceutical. In this set of studies, we examined the effects of a novel antidepressant, mianserin, in a dose-dependent fashion (1, 10, 100 and 1000 ug/kg i.v., and 1 ug/kg i.c.v.) as well as two other classes of drugs: antipsychotic and anti-anxiety agents. For the former, we did a dose-response (0.4, 1.0 and 10 mg/kg i.v.) study with haloperidol (H) and also examined effects of fluphenazine (F), thiothixene (TX) and thioridazine (TR) at a fixed dose (0.4 mg/kg i.v.). For the latter, we have to date examined only one dose of diazepam (D), 1 mg/kg i.v. We tested the effects of these treatments on PS and CBF as a function of CO_2 administration in 225-250 gm, male Sprague-Dawley rats using a dual label radioisotope technique previously described (Brain Res. 249:23-30, 1982). Results:

Table: Effect of different classes of drugs on CBF and PS at hypocapnia and hypercapnia.

	C*	M***	H**	F	TX	TR	D
CBF at CO_2 = 20	163	179	57	45	52	19	49
CBF at CO_2 = 80	398	410	345	303	368	459	229
PS at CBF = 1	144	354	174	130	138	141	157
PS at CBF = 4	334	398	354	349	354	354	364

* C = control animals; ** pooled results for all haloperidol doses; *** 1 ug/kg i.c.v., results which were the most profound.

The results show that, unlike amitriptyline, neither antipsychotic nor anti-anxiety agents increase PS but do reduce CBF especially under hypocapnic conditions. Conversely, the novel antidepressant increased PS but did not alter CBF. These results indicate that PS and CBF can be separately affected and that these changes distinguish different classes of psychopharmaceuticals. To date, the effect on PS appears to be restricted to drugs with mood effects. Supported in part by MH-00272, NS-1752 and the PMAF.

47.9 ROLE OF CATECHOLAMINES IN PLACE PREFERENCE CONDITIONING WITH PSYCHOSTIMULANTS, M.T. Martin-Iverson, R. Ortmann* and H.C. Fibiger, Div. Neurol. Sci., Dept. Psychiat., University of British Columbia, Vancouver, B.C., Canada,V6T 1W5.

It has been shown that whereas d-amphetamine-induced place preferences can be attenuated by dopamine (DA) receptor blockade, or by lesions of DA-containing terminals in the region of the nucleus accumbens (Spyraki, C. et al., Brain Res., 253:185,1982), place preferences induced with cocaine are resistant to such treatments (Spyraki, C. et al., Brain Res., 253:195, 1982). The present study extends these findings to two other psychostimulants, methylphenidate (MPD) and nomifensine (NFS).

Place preference conditioning was conducted following the procedure of Spyraki et al. (1982), using different doses of MPD (2.5, 5.0 and 10.0 mg/kg, i.p.) and NFS (5, 10 and 20 mg/kg, i.p.). All doses of MPD and NFS produced significant place preferences, but rats receiving vehicle injections exhibited no preferences. Other rats were conditioned with MPD (2.5 or 5.0 mg/kg, i.p.) 30 min after receiving haloperidol (HAL: 0.25-0.5 mg/kg, i.p.), or received HAL (0.2 mg/kg) prior to conditioning with NFS (5 mg/kg, i.p.). In all cases, HAL failed to attenuate place preferences.

Central CA depletions were induced in other rats by intraventricular injections of 6-hydroxydopamine (6-OHDA, 250 ug, base, dissolved with 0.3 mg/ml ascorbate in 10 ul of 0.9% saline) after treatment with pargyline (50 mg/kg, i.p.). One week later, place preference conditioning tests were conducted. Although DA was reduced by 30% (n. accumbens) to 19% (striatum) of control values, and whole brain noradrenaline was reduced to 13% of control values, no attenuation of place preferences was observed.

Both treatment with HAL (0.2 mg/kg, i.p.) and CA depletions induced with 6-OHDA were effective at attenuating the locomotor stimulant effects of MPD (5.0 mg/kg, i.p.). HAL (0.2 mg/kg, i.p.) also blocked the motor stimulant effects of NFS (5 mg/kg, i.p.).

The present study demonstrates that the reinforcing properties and motor stimulant effects of MPD and NFS are mediated by different neural substrates. Taken together with previous experiments, it appears that the neural substrate(s) mediating the rewarding effects of MPD, NFS and cocaine are distinct from the system(s) mediating the reinforcing effects of d-amphetamine.

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47.10 COCAINE WITHDRAWAL: EFFICACY OF TYROSINE. M. S. Gold[1], A. L. C. Pottash[1], W. J. Annitto*[1], K. Vereby*[2], D. R. Sweeney[1], Fair Oaks Hospital, Summit, N.J. 07901[1] and N.Y. State Drug Testing Laboratories, Brooklyn, N.Y. 11217[2].

In a preliminary report we have described a cocaine withdrawal syndrome consisting of drug craving, irritability, anergia, depression, fearfulness, hypersomnia, shaking, nausea, which may be accompanied by an autonomic discharge.[1] In all cases where this withdrawal syndrome has been demonstrated the cocaine user had virtually unlimited access to cocaine on the basis of personal or family wealth, profession, or distributing the drug. The post-cocaine crash or cocaine withdrawal is frequently given by addicts as the reason they can not stop self administration of cocaine. In animal models of cocaine self administration cocaine is preferred to food and with unlimited access animals may self administer cocaine to the end points of seizures and death.

In an attempt to treat the cocaine withdrawal syndrome as we had previously accomplished with the narcotic withdrawal syndrome[2], we hypothesized that severe cocaine withdrawal is related to depletion of catecholamines. Support for this hypothesis has come from one demonstration of low 3-methoxy 4-hydroxyphenylglycol and tyrosine in cocaine addicts. As a preliminary test of the catecholamine depletion hypothesis of cocaine withdrawal we administered the amino acid precursor (0.1 gm/kg) tyrosine to six cocaine addicts in an open, non-blind trial. Patients were rated on a twice daily basis for withdrawal signs and symptoms. Tyrosine had consistent anti-withdrawal effects. In addition to allowing successful detoxification, tyrosine may be a useful post-detoxification pharmacotherapy. These clinical data support a functional catecholamine depletion hypothesis for cocaine withdrawal on the basis of tyrosine's ability to increase DA, NE, and E. Though other treatments may be found to be equally efficacious (eg. methylphenidate, amphetamine, MAO inhibitors) tyrosine as an amino acid catecholamine and thyroid hormone precursor has no apparent abuse potential and offer the hope of restoring hemostatic balance to the cocaine deranged catecholamine systems. Double-blind placebo controlled trial of tyrosine in cocaine withdrawal is in progress and will be discussed.

1. Gold, M.S., Symposium on Cocaine Proceedings, N.Y., N.Y., May 3-4, 1982.
2. Gold, M.S., et al N Eng J Med, 302:1421-1422, 1980.

47.11 ESTROGEN MODULATES THE EFFECTS OF AMPHETAMINE ON LOCOMOTOR ACTIVITY IN MALE RATS. Richard P. Michael and Charles H.K. West. Department of Psychiatry, Emory University School of Medicine and Georgia Mental Health Institute, Atlanta, GA 30306.

Behavioral and neurochemical studies in rats have shown that exogenously administered estrogen can alter the effects of dopamine agonistic drugs, thereby suggesting that estrogen may modulate dopamine neurotransmission under certain circumstances. Recent work has shown that the effects of estrogen depend upon the time between its administration and the behavioral test. The long time-course for these effects is consistent with a genomic mechanism of action, but short-term behavioral effects have not been examined. This study investigates the change in the locomotor activity increase induced by d-amphetamine (d-A) following treatment with estradiol benzoate (EB). Male rats were administered EB (50 µg/kg, s.c.) and were subsequently tested at various times from 45 min to 20 days for changes in locomotor activity (infrared beam interruptions, 10 min sessions) induced by acute (15 min pretreatment) administration of d-A (0.25 mg/kg, s.c.). Tests with d-A were separated by at least 2 days since control tests had demonstrated no change in response to d-A with this treatment regimen. The EB + d-A scores were compared with scores from control tests with sesame oil vehicle + d-A. Throughout each 20 day test period following EB, the baseline activity scores after saline injections did not differ significantly from control scores. Therefore, the data for d-A are expressed as a percentage of saline scores collected within a few days of each d-A test. The EB effect on the sensitivity of the animals to d-A could be divided into 3 phases: (1) an acute (45 min) increase in sensitivity, (2) a later (1 day) decrease in sensitivity and (3) a slow time course (2 - 16 days) increase in sensitivity to d-A. Control tests with d-A yielded increases in activity scores (mean ± SEM) ranging from 308 ± 21% to 328 ± 28% of saline baseline values. When activity was monitored 45 min after EB administration, the response to d-A was significantly increased to 435 ± 49% over saline baseline. In contrast, 1 day after EB treatment, the d-A response was significantly reduced to only 279 ± 20%. Tests 2 to 16 days after EB treatment all revealed significant increases over baseline in the response to d-A (2 days: 422 ± 48%, 4 days: 395 ± 39%, 8 days: 431 ± 63%, 12 days: 459 ± 48%, 16 days: 392 ± 30%). By 20 days, the response to d-A was slightly but not significantly above control values. These results show that EB can alter the hyperactivity response to d-A, probably via more than one mechanism of action. Furthermore, a short latency behavioral effect of EB has been demonstrated, implying a non-genomic mechanism.

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CHARACTERIZATION OF CHOLINERGIC RECEPTORS

48.1 MONOCLONAL ANTIBODIES AGAINST THE α-BUNGAROTOXIN BINDING PROTEIN OF CHICK OPTIC LOBE. H. Betz and F. Pfeiffer* Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, D-8033 Martinsried, FRG.

α-Bungarotoxin (α-BTX) binds with high affinity and nicotinic-cholinergic specificity to a membrane protein of the chick visual system. Several properties of this α-BTX receptor are consistent with the characterization of this protein as a neuronal nicotinic acetylcholine receptor. Also, the toxin binding site region of this protein has been localized on a polypeptide of M_r = 57,000 (Betz et al., J. Biol. Chem., 257:11390, 1982).

In order to investigate the function and cholinergic ligand site(s) of the α-BTX receptor, monoclonal antibodies (mAbs) have been produced against the detergent-solubilized protein after >1000 fold purification on α-BTX-derivatized agarose and wheat germ agglutinin-sepharose. From one fusion of the spleen of a hyperimmunized mouse with the myeloma cell line X63-Ag8.653, fourteen independent stable hybridoma clones have been established which secrete mAbs against the solubilized chick α-BTX binding component. None of the mAbs crossreacts to a significant extent (<1-2%) with solubilized AChR from chick muscle or Torpedo electric organ. Some, however, recognize the α-BTX receptor of the rat phaeochromocytoma cell line PC12.

About half of the mABs inhibit α-BTX binding to membrane fractions or detergent extracts of chick optic lobe. The binding of a few, but not all of the latter class of mAbs is blocked by nicotinic-cholinergic antagonists. The mAbs are thus concluded to recognize at least three different epitopes on the chick α-BTX binding protein. Several of the non-blocking mAbs produce a down-regulation of α-BTX binding sites in chick retina cultures by increasing the degradation of the toxin receptor. Also, the mAbs are shown to be useful reagents for the immunoaffinity isolation/precipitation of the α-BTX receptor from chick and rat (PC12) cells.

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48.2 MONOCLONAL ANTIBODY (mcab) 247G: EXAMPLE OF A FUNCTIONAL PROBE FOR THE ACETYLCHOLINE RECEPTOR (AcChR) MOLECULE. M. Mihovilovic* and D. Richman* (SPON. J. Crayton). Dept. of Neurology, The University of Chicago, Chicago, Illinois 60637.

Binding of mcab 247G to purified, solubilized AcChR from Torpedo californica results in stable AcChR-mcab 247G complexes in which 1 mcab molecule binds per every 2 α-bungarotoxin (αBgtx) binding sites. The immune complex binds only one αBgtx molecule: binding is characterized by a second order rate constant, k_1, of 1.92 ± 0.30 x 10^5 M^{-1} s^{-1}, which compares to the kinetically resolved fast αBgtx binding component of native solubilized AcChR (k_1 = 1.62 ± 0.11 x 10^5 M^{-5} s^{-1}). The kinetically resolved slow αBgtx binding component of the solubilized AcChR (k_2 = 0.35 x 10^5 M^{-1} s^{-1}) is not expressed in the immune complex.

Inhibition of αBgtx binding to AcChR by benzoquinonium reveals that the ligand binds to two sites of the AcChR characterized by I_{50} values of 5.0 ± 1.0 x 10^{-7} M and 3.5 ± 1.5 x 10^{-4} M, while AcChR-mcab 247G complexes express only the low affinity benzoquinonium binding site. Thus our data indicate a correspondence betweeen the low affinity benzoquinonium binding site and the kinetically resolved fast αBgtx binding component of the AcChR molecule. These results demonstrate the ability of anti-AcChR mcabs to functionally probe the AcChR molecule.

48.3 THE STRUCTURE AND TRANSMEMBRANE NATURE OF THE ACETYLCHOLINE RECEPTOR IN AMPHIBIAN SKELETAL MUSCLE AS REVEALED BY CROSS-REACTING MONOCLONAL ANTIBODIES. Bruce E. Hedges*, Peter B. Sargent, Larisa Tsavaler*, Lydia Clemmons*, and Jon M. Lindstrom (SPON: Carla J. Shatz). Dept. of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305, and Receptor Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

A collection of 126 monoclonal antibodies (mAbs) made against acetylcholine receptors (AChRs) from the electric organs of Torpedo californica or Electrophorus electricus was tested for cross-reactivity with AChRs in cryostat sections of skeletal muscle from Rana pipiens and Xenopus laevis by indirect immunofluorescence. Fifty mAbs (40%) cross-reacted with AChRs from Rana; 25 mAbs (20%) cross-reacted with AChRs from Xenopus. mAbs specific for each of the four subunits of electric organ AChR (α, β, γ, δ) cross-reacted with AChRs from each amphibian species. mAbs cross-reacting with Xenopus AChRs were, with one exception, a subset of the mAbs cross-reacting with Rana AChRs. The major difference detected between the two species was in binding by mAbs specific for the main immunogenic region (MIR) of the α-subunit. Whereas 23 of 33 anti-MIR mAbs cross-reacted with Rana AChRs, only one of these mAbs cross-reacted with Xenopus AChRs.

Some (32) of the cross-reacting mAbs were tested for binding to AChRs in intact muscle. Of these 21 mAbs bound to AChRs only when membranes were made permeable with saponin. Electron microscopy using immunoperoxidase or colloidal gold techniques revealed that these mAbs recognize cytoplasmic determinants and that mAbs which do not require saponin in order to bind AChRs in intact muscle recognize extracellular determinants.

These results suggest that AChRs in skeletal muscle of Rana and Xenopus are composed of subunits corresponding to the α, β, γ, and δ subunits of AChRs from fish electric organs. The subunit specificity of mAbs whose binding was examined by electron microscopy suggests that parts of each subunit (α, β, γ, δ) are exposed on the cytoplasmic surface and that, as in AChRs from fish electric organs and mammalian muscle, the MIR on α subunits of Rana AChRs is exposed on the extracellular surface.

Supported by the National Science Foundation, the National Institutes of Health and the Muscular Dystrophy Association.

48.4 PROTEOLYTICALLY DERIVED FRAGMENTS OF THE ALPHA SUBUNIT OF THE ACETYLCHOLINE RECEPTOR FROM TORPEDO CALIFORNICA THAT BIND ^{125}I-ALPHA-BUNGAROTOXIN. B. Oblas,* N. D. Boyd* and R. H. Singer.* (SPON: J. Walsh) Depts, of Anatomy and Physiology, Univ. of Massachusetts Med. Ctr., Worcester, Massachusetts 01605

We have previously reported a nitrocellulose protein transfer technique which we have used to confirm that the alpha-bungarotoxin binding site resides on the alpha subunit (40K) of the Torpedo californica acetylcholine receptor. (Oblas, B., et. al., Anal. Biochem., 130:1, 1983). We are now extending the use of this technique to define further the location of this binding site on the 40K subunit by studying the ability of proteolytically derived fragments of this subunit to bind ^{125}I-alpha-bungarotoxin.

The alpha subunit was solubilized and digested with various proteolytic enzymes including Staphylococcus aureus V_8 protease, papain, chymotrypsin and trypsin. The resulting peptide fragments were separated on a 20% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred in a renaturing environment to a nitrocellulose sheet which was then incubated with ^{125}I-alpha-bungarotoxin. Autoradiography (2-6 days) revealed different patterns of binding for each enzyme treatment. After proteolytic digestion with V_8 protease, ^{125}I-alpha-bungarotoxin bound to a single peptide fragment of apparent molecular weight 17K, while proteolytic digestion with papain resulted in two fragments of 33 and 27K which bound the labeled toxin. None of the fragments of the alpha subunit obtained by chymotrypsin digestion was found to bind ^{125}I-alpha-bungarotoxin. Limited digestion by trypsin converted the 40K subunit into two fragments of apparent molecular weights of 37 and 33K which have affinity for the labeled toxin. More extensive digestion by this enzyme resulted in a decrease in fragments and a concomitant appearance of a 17K fragment that bound the labeled toxin. No labeling was observed for these fragments when a 100 fold excess of unlabeled toxin was added to the incubation mixture.

The entire amino acid sequence of the Torpedo californica alpha subunit has been reported (Noda, M., et. al., Nature, 299: 793, 1982) and shown to contain two pairs of basic residues, Arg-Lys and Lys-Arg which upon trypsin digestion would yield two fragments with molecular weights similar to the two larger molecular weight fragments reported here. The isolation of the smallest fragment will allow the determination of that region of the alpha subunit in which the alpha-bungarotoxin site resides.

48.5 PROTEIN-BLOTTING ANALYSIS OF α-BUNGAROTOXIN BINDING TO THE ACETYLCHOLINE RECEPTOR: SPECIES COMPARISON, PROTEOLYTIC FRAGMENTS, AND MBTA SPECIFICITY. Thomas L. Lentz, Jonathan M. Gershoni*, Paul Wilson*, Donna Klimowicz*, Linda M. Hall, and Edward Hawrot. Depts Cell Biology and Pharmacology, Yale Univ. Sch. Med., New Haven, CT and Dept. Genetics, Albert Einstein Coll. Med., Bronx, NY.

We demonstrated previously the direct binding of α-bungarotoxin (BuTX) to the α-subunit of the acetylcholine receptor (AChR) from Torpedo electric organ after immobilization onto protein blots (J. Cell Biol., 95:422a, 1982). Further studies have been carried out to determine whether BuTX binding to protein blots could be used to detect AChR from other sources. Membrane fractions were prepared from Torpedo and Electrophorus electric organ and Drosophila brain. Enriched extracts were prepared by cobratoxin column chromatography of goldfish brain, embryonic chick muscle, a mouse muscle cell line (BC3H1), and denervated rat skeletal muscle. Samples were solubilized in LDS sample buffer and electrophoresed on 10% polyacrylamide gels. Protein blots were prepared by electrophoretically transferring the resolved AChR subunits from the gels onto Zetabind, positively charged nylon membrane filters. The filters were then quenched with 1% hemoglobin, incubated with ^{125}I-BuTX, washed and autoradiographed. In all cases, one polypeptide band (40-44kd depending on species) bound labeled toxin. These results suggest that species over a wide spectrum of taxonomic classes contain a receptor component analogous to the α-subunit of Torpedo. To further characterize the BuTX binding site on Torpedo AChR, proteolytic fragments of the α-subunit were prepared and tested for BuTX binding. After excising the α-subunit from gels treated with sodium acetate, specific proteolytic fragments were prepared by digesting with different proteases. The fragments were electrophoresed, transferred to filters, and incubated with ^{125}I-BuTX. Distinct and reproduceable peptide maps were obtained for each protease. BuTX bound to 28 and 19kd fragments of α-subunit produced by V-8 protease; to 29, 18, and 14kd fragments after papain digestion; and to 28, 20, and 17kd fragments after bromelain digestion. To test whether the BuTX binding detected on protein blots correlates with the physiologically-relevant acetylcholine binding site, the effect of 4-(N-maleimido)benzyltrimethylammonium iodide (MBTA) on toxin binding was tested. Various concentrations of MBTA (0.5-10μM)(gift of A. Karlin) were incubated with Torpedo membrane samples containing 0.3μM AChR. Aliquots from each sample were either solubilized in Triton X-100 and assayed for their ability to bind ^{125}I-BuTX or were solubilized, electrophoresed, transferred to filters, and incubated with ^{125}I-BuTX. Prior affinity alkylation with MBTA was found to inhibit to the same degree binding of BuTX to the Triton-solubilized intact AChR and to the isolated α-subunit immobilized on the filters.

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48.6 IDENTIFICATION OF AN M_r 43,000 PROTEIN KINASE IN ACETYLCHOLINE RECEPTOR-ENRICHED MEMBRANES. A.S. Gordon, D. Milfay*, and I. Diamond. Dept. of Neurology, Univ. of Cal. Sch. of Med., San Francisco, CA 94143.

We have shown that acetylcholine receptor (AChR)-enriched membranes from Torpedo californica have an endogenous protein kinase activity which phosphorylates the AChR in situ. We have now used 8 azido ATP, an ATP photoaffinity analog, to identify the kinase in these membranes. We first determined that 8 azido ATP reacts with the ATP binding site of the receptor kinase by studying its effect on receptor phosphorylation. AChR-enriched membranes were preincubated for 20 min with increasing concentrations of 8 azido ATP before assaying for protein kinase activity. Corresponding decreases in receptor phosphorylation were observed, suggesting that 8 azido ATP binds to the receptor kinase. Incubation of AChR-enriched membranes with [α-^{32}P] 8 azido ATP and subsequent irradiation with UV light resulted in the covalent labeling of one major band of M_r 43,000. Alkaline stripped membranes which show a selective reduction in the M_r 43,000 polypeptide also show a corresponding reduction in incorporation of photoaffinity label. This was recovered in the neutralized alkaline extract which showed one band at M_r 43,000 when labeled with the photoaffinity ligand. We also found that endogenous protein kinase activity decreased in the alkaline extracted membranes in proportion to the loss of M_r 43,000 peptide. Moreover, the alkaline extract was able to phosphorylate casein in an exogenous assay system. These results suggest that an M_r 43,000 polypeptide in AChR-enriched membranes is the protein kinase that phosphorylates the AChR.

48.7 TETRACAINE BINDING TO TORPEDO POST-SYNAPTIC MEMBRANES. Nina P. Strnad & Jonathan B. Cohen. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

The binding of [^{3}H]-tetracaine ([^{3}H]-TET) to nicotinic postsynaptic membranes from Torpedo electric tissue has been studied at 4°C by ultracentrifugation and ultrafiltration. Tetracaine had been identified as a ligand that displaces bound [^{3}H]-histrionicotoxin ([^{3}H]-HTX) only weakly (IC_{50}=100 μM) when cholinergic sites are occupied by carbamylcholine (carb), but potently (IC_{50}=0.4 μM) in the absence of cholinergic ligands. The nonspecific interaction of [^{3}H]-TET with the membranes is characterized by a partition coefficient, P=80 mol/g protein per mol/ml. In the absence of cholinergic ligands [^{3}H]-TET binds with high affinity (Keq=350nM) to a number of sites equal to 0.48±0.11 the number of [^{3}H]-AcCh binding sites. This binding is inhibited by HTX and meproadifen. The ratio of high affinity binding sites for three non-competitive antagonists, [^{3}H]-TET, [^{3}H]-proadifen, and [^{3}H]-HTX, is equal to 1 : 1.1±.3 : 1.3±.2. Cholinergic ligands alter the affinity of [^{3}H]-TET binding, but not the number of high affinity sites. With receptor sites fully occupied by α-bungarotoxin (α-BgTx) or tubocurarine (50 μM), Keq equals 0.4 and 0.9 μM respectively, while in the presence of carb (100μM), Keq~30 μM. Since [^{3}H]-TET binds with the same affinity in the presence or absence of α-BgTx, we conclude that 1) the binding of α-BgTx produces no local change of receptor conformation as seen by the anesthetic site, and 2) the weak binding in the presence of carb is a consequence of an allosteric effect.

The kinetics of [^{3}H]-TET binding have been analyzed by ultrafiltration to define tetracaine's effect on receptor conformational equilibria. The kinetics of [^{3}H]-TET dissociation at equilibrium was measured as the rate of exchange for unlabelled tetracaine. In the absence of cholinergic ligands or in the presence of α-BgTx, dissociation kinetics were characterized by a single rate constant ($t_{1/2}$=35 min) for greater than 80% of the exchange reaction. Inclusion of tubocurarine (50 μM) or carb (100 μM) changes the [^{3}H]-TET dissociation rate to $t_{1/2}$=15 min and <0.5 min, respectively. The association rate constant for [^{3}H]-TET binding measured in the absence of cholinergic ligands is 4.5 x $10^4 M^{-1} min^{-1}$, or an order of magnitude slower than the rate of α-BgTx binding. We conclude that tetracaine induces a conformational change in the nicotinic receptor which is distinct from the high-affinity or desensitized state induced by cholinergic agonists. (Supported by USPHS Grant NS-19522 and Predoctoral Training Grant GM-07805.)

48.8 NICOTINIC AGONIST BINDING INCREASES IN RAT HIPPOCAMPUS FOLLOWING FIMBRIAL TRANSECTION. A.L. Morrow, R. Loy and I. Creese. Dept. of Neurosciences, Univ. of Calif., San Diego, Sch. of Med., La Jolla, CA 92093.

The regulation of cholinergic receptors in the hippocampal formation of the rat has been studied by removal of the septal and diagonal band afferents and by muscarnic receptor blockade. While chronic treatment with atropine or scopolamine increases ^{3}H-QNB binding (a muscarinic ligand), removal of the cholinergic afferents does not. Nicotinic cholinergic binding sites labeled with ^{125}I-α-bungarotoxin are likewise unaltered. As it is unclear if this ligand labels all nicotinic receptors in the brain, we examined the binding of ^{3}H-acetylcholine (ACh), under conditions which exclude muscarinic receptor labeling. Nicotinic ^{3}H-ACh binding (Kd=15.2+1.9) was performed according to Schwartz et al. (Mol. Pharm. 22:56, 1982) using eserine to prevent hydrolysis. Eight days following knife transection of the fimbria/fornix there is a 49% increase in the specific binding of 10 nanomolar ^{3}H-ACh ($p<.001$, 2-tailed Students t-test). Preliminary saturation studies indicate that the increased binding is due to a 45% increase in the affinity of ^{3}H-ACh for its binding site with no change in Bmax ($p<.05$, 1-tailed Students t-test). Using Munson and Rodbard's LIGAND computer program we have confirmed a change in affinity by simultaneous analysis of the saturation data for operated and unoperated samples. Constraint of the affinity of ^{3}H-ACh to be identical for control and operate tissue significantly worsens the fit in each of four such comparisons.

This is the first demonstration that ^{3}H-ACh binding is altered by cholinergic denervation and supports the hypothesis that the septal cholinergic projection terminates at nicotinic sites in hippocampus. Supported by PHS NS14372 (R.L.), NS17860 (I.C., R.L.), March of Dimes (I.C., R.L.) and NIMH Predoctoral Fellowship MH08898 (A.L.M).

48.9 NICOTINIC-INDUCED RELEASE OF ENDOGENOUS DOPAMINE FROM RAT STRIATAL SLICES FOLLOWING CHRONIC TREATMENT WITH NICOTINIC AGONISTS. T. C. Westfall and H. Perry*. Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

Several investigators have reported that activation of nicotinic-cholinergic receptors within the striatum results in the release of labelled dopamine (DA) following incubation of tissue with radioactive amine (Neuropharmacol. 13:693, 1974; Anesthes. 48:118, 1978; Brain Res. 106:117, 1976). Although the use of labelled compounds to assess transmitter release has been a useful tool, there are nevertheless problems that make this technique less reliable than measuring the release of the endogenous transmitter itself. The purpose of the present study was to examine the release of endogenous DA from slices following acute activation of nicotinic-cholinergic receptors and in slices obtained from rats chronically treated with the nicotinic agonist dimethyl phenylpiperazinium (DMPP, 17 mg/kg/day for 7 or 14 days) or saline by means of Alzet osmotic pumps placed subcutaneously. Animals were decapitated and striata rapidly removed in the cold room. Striata were dissected with glass manipulators, sliced sagittally with a MacIlwain tissue chopper to a thickness of 0.5 mm. Following a preincubation period in Krebs-Ringer solution at 37° for 15 min, the slices were placed in wire baskets and incubated for various times in 750 μl of buffer in the absence or presence of varying concentrations of DMPP or nicotine for 5 min. Dopamine released from the tissue into the incubation medium was measured by injection of 20 μl aliquots of the incubation media directly into the HPLC. Dopamine was quantified with an electrochemical detector maintained at 0.7402 (Model LC-4A glassy carbon electrode). The routine solvent was 12% methanol, 175 mM acetic acid, 1 mM EDTA and 2 mM heptane sulfonic acid and the pH adjusted to 3.8 with 10 M NaOH.

DMPP in concentrations of 100, 500, 750 and 1000 μM produced an increase of 1.06±.24, 4.73±5.3, 6.67±.53 and 6.84±.59 of DA/g/min from control slices. The DMPP-induced release from slices obtained from animals chronically treated with the agonist for 7 days was 1.95±.43, 5.83±.48, 7.77±.58 and 7.16±.35, and were not different from control. The DMPP-induced release from slices obtained from animals chronically treated with DMPP for 14 days was significantly lower than from control slices or slices from animals treated with DMPP for 7 days. There was an increase of 1.36±.14, 2.45±.31, 4.66±.28 and 5.06±.67 for the 4 concentrations, respectively.

These results demonstrate that the chronic treatment of animals with a nicotinic agonist results in slices which are subsensitive to the DMPP-induced release of endogenous DA. Studies are underway to define the mechanism of this response.

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48.10 SPONTANEOUS OPENINGS OF THE ACETYLCHOLINE RECEPTOR CHANNEL, Meyer B. Jackson. Dept. of Biology & Mental Retardation Research Center, Univ. of California, Los Angeles, CA 90024.

Patch clamp recordings from embryonic skeletal mouse muscle in culture revealed infrequent channel currents of short duration in the absence of cholinergic agent. The amplitude of these channel currents was identical with the amplitude of channel currents elicited by the cholinergic agonist suberyldicholine. This equality was maintained over a range of voltage, using either sodium or cesium as the major cation of the patch electrode filling solution and the cell bathing solution. Treatment of cells with α-bungarotoxin blocks these channel currents. The receptor binding site was chemically modified by treatment first with dithiothreitol and then with N-ethylmaleimide. This treatment blocked agonist elicited openings but not spontaneous openings. Thus, spontaneous openings are not prevented when the binding site of the receptor is interfered with. These experiments indicate that an acetylcholine receptor channel complex fluctuates briefly and infrequently into the open state in the absence of cholinergic activation.

48.11 BARBITURATES ALLOSTERICALLY REGULATE HIGH AFFINITY ACETYLCHOLINE BINDING TO NICOTINIC RECEPTORS, B.A. Dodson* and K.W. Miller* (SPON: G.J. Crosby). Depts. of Anaesthesia and Pharmacology, Harvard Medical School, Massachusetts General Hospital, Boston, MA 02114 USA.

Barbiturates have been shown to modify acetylcholine (ACh) binding to the acetylcholine receptor (AChR)-rich membrane from Torpedo. Amobarbital decreased [^{3}H]ACh binding by 70% with an apparent K_d = 18 ± 3.5 μM. Secobarbital, which itself has no effect on [^{3}H]ACh binding, antagonized the effect of amobarbital in a competitive manner with an apparent K_d = 84 μM (Dodson and Miller, Soc. Neurosci. Abstr., 8:340, 1982). A pentobarbital binding site has been characterized, which appears to interact allosterically with the ACh binding site on the AChR-rich Torpedo membrane (Miller et al., BBRC 105:659-666, 1982). Here we will show that it is through this barbiturate binding site that the barbiturates are able to allosterically regulate the binding.

AChR-rich membranes were prepared from the electroplaques of freshly killed T.nobiliana by differential and gradient centrifugation. The change in displaceable [^{14}C]amobarbital (6 μM) binding to AChR (4 μM) by amobarbital (0-1 mM), with and without 0.1 mM carbamylcholine and/or 10 μM αbungarotoxin (αBTX), or by secobarbital (0-2 mM) was determined by centrifugation assay at pH 7.0. All binding data were fitted to mass action curves by a non-linear least squares method.

Both amobarbital and secobarbital displaced [^{14}C]amobarbital in a competitive manner with K_ds of 26 ± 4.2 μM and 94 ± 9.1 μM, respectively. These K_ds are comparable to the apparent K_ds (above) obtained by [^{3}H]ACh binding studies. αBTX alone had no effect on [^{14}C]amobarbital binding, but carbamylcholine decreased [^{14}C]amobarbital binding by 50%. This carbamylcholine sensitivity was, however, completely blocked by αBTX. These data are consistent with the barbiturate binding site being on the AChR protein, but not on the ACh binding site itself. Furthermore, the concentration dependence of both the decrease in [^{14}C]amobarbital binding by carbamylcholine and the decrease in [^{3}H]ACh binding by amobarbital follows mass action and as such is consistent with allosteric regulation. Thus we have presented data supporting the hypothesis that there exists a barbiturate binding site on the AChR protein, but not on the ACh binding site itself, which can allosterically regulate ACh binding.

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48.12 CHARACTERIZATION AND PARTIAL PURIFICATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS SOLUBILIZED BY ZWITTERIONIC DETERGENT. M. Gavish and M. Sokolovsky+. Department of Pharmacology, Rappaport Family Research Institute, Faculty of Medicine, Technion, POB 9697, Haifa, and +Department of Biochemistry, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel.

The muscarinic acetylcholine receptors were solubilized from rat brain cortex by Zwitterionic detergent 3-|(chloramidopropyl) dimethylammonio|-1-propanesulfonate (CHAPS). About 35% of the binding activity was solubilized and 40% retained in the pellet. Binding of the muscarinic antagonist |^{3}H|-N-methyl-4-piperidyl benzilate (4NMPB) was saturable. Scatchard analysis revealed a single population of binding sites with K_D value of 0.7nM and B_{max} value of 340 fmoles/mg protein. The affinity of the CHAPS soluble receptors to the agonists oxotremorine and carbamylcholine and for the antagonists QNB and atropine was found to be similar to that of the membrane bound receptors. The molecular weight of the CHAPS soluble muscarinic receptors as determined by chromatography on Sepharose-6B was found to be 320,000 daltons. The soluble receptors were purified 10 fold by chromatography on DEAE-cellulose or hydroxylapatite.

BLOOD-BRAIN BARRIER I

49.1 COLLOIDAL CARBON AS A COMBINED OPHTHALMOSCOPIC AND MICROSCOPIC PROBE OF THE RETINAL-BLOOD BARRIER INTEGRITY. Manuel del Cerro, Donald A. Grover* and Wesley M. Williams*. Center for Brain Research, University of Rochester School of Medicine, Rochester, N.Y. 14642.

The retinal vasculature is characterized by having a barrier restrictive of the free movements of molecules between blood and retinal tissue (BRB). Anatomically and physiologically, this barrier is the ocular counterpart of the blood-brain barrier (BBB). While studying the effects of an experimental immunopathy, we searched for a probe that would permit us to test the barrier in the same eye by ophthalmoscopy, light microscopy, and electron microscopy. Colloidal carbon, administered intravenously, permitted us to achieve these goals. The tracer can be seen in the choroidal and retinal circulation, and abnormal deposits of it can be visualized in vivo. The ophthalmoscopic findings can be corroborated and extended by stereomicroscopy and by light microscopy. Subsequently, the plastic embedded specimens can be used for a transmission electron microscopical study. This tracer, thus, permits a sequential clinical, histological, and even ultrastructural evaluation of the status of the BRB.

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49.2 BRAIN CAPILLARY PERMEABILITY TO INORGANIC IONS. Q.R. Smith, C.-Y. Tai* and S.I. Rapoport (SPON: R.M. Steinman). Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, NIH, Baltimore City Hospitals, Baltimore MD 21224

The cells of the blood-brain barrier are connected by rings of tight junctions, which restrict intercellular diffusion and limit exchange of inorganic ions between plasma and brain. Ions move from plasma to brain directly across cerebral capillaries into brain extracellular fluid (ECF), or indirectly across the choroid plexus or arachnoid membrane into cerebrospinal fluid (CSF), with subsequent diffusion into brain ECF. To determine the ion transport properties of the cerebrovascular endothelium, we measured initial uptake, following an i.v. bolus injection of ^{22}Na, ^{42}K, ^{45}Ca, ^{28}Mg or ^{36}Cl, into 12 brain regions and CSF of the rat. Initial uptake analysis, by minimizing the time for diffusion between CSF and brain ECF, made it possible to distinguish ion flux at the cerebral capillary from the contribution of CSF to brain radioactivity.

Radioisotopes (20-200 μCi/kg) were injected i.v. into pentobarbital-anesthetized rats, and timed arterial blood samples were collected for 5-60 min, when the rats were killed. Samples from brain, cisternal CSF and plasma were collected and analyzed for radioactivity. Transfer constants (k) for unidirectional uptake were calculated from brain parenchymal and CSF radioactivities at the time of death divided by the plasma concentration integral of unbound tracer.

Ion fluxes into CSF were 10-40 times greater than into brain tissue, so that a concentration gradient from CSF to brain was rapidly established for each tracer. Regional brain k's were greater for regions closest to ventricular CSF. For ^{22}Na, ^{45}Ca and ^{36}Cl, brain k's ranged 3-6 fold, with minimum values in the frontal-parietal cortex. For ^{42}K and ^{28}Mg, k's ranged less than 3-fold. Acetazolamide-induced inhibition of ^{36}Cl entry into CSF did not alter the cerebral cortex k for ^{36}Cl, but in periventricular regions k fell by up to 50%. When uptake time was limited to 30 min or less, frontal cortex k reflected movement primarily across the brain capillaries, with a minimal contribution from CSF. In the frontal cortex cerebrovascular permeability (cm/sec) was 60 x 10^{-8} for K, 17 x 10^{-8} for Mg, 8 x 10^{-8} for Na, 5 x 10^{-8} for Cl and 2 x 10^{-8} for Ca. The calculated electrical resistance of the brain capillary was 12,500 Ω·cm^2. The permselectivity ($P_K > P_{Na}, P_{Cl}$) and large electrical resistance of brain capillaries suggest that ion movement across the capillaries is predominantly transcellular, across the vascular endothelium. Furthermore, 50% or more of the ion influx into rat brain may be from tracer that first enters CSF.

49.3 VESICLES WITHIN ENDOTHELIAL CELLS IN FROG SCIATIC NERVE DO NOT TRANSPORT HRP IN EITHER LUMENAL OR ANTILUMENTAL DIRECTIONS. M.E. Michel*, N.L. Shinowara and S.I. Rapoport.(SPON:J.Thompson) Lab.Neurosciences, and Section Experimental Morphology, National Institute on Aging, Gerontology Research Center, Baltimore, MD 21224.

The endothelium of blood vessels within sciatic nerve of Rana pipiens is replete with vesicles at both their lumenal and ablumenal surfaces. With electron microscopy these vesicles appear as either round bottomed flasks attached by a narrow neck to the cell surface, or as free vesicles within the cytoplasm near the cell surfaces. Anastomosing vesicles are not uncommon, but transcellular channels formed by chains of fused vesicles have not been found. The contribution of these two vesicular populations to an endothelial blood-nerve barrier has not been described. To test the transport capabilities of the lumenal vesicles we injected horseradish peroxidase (HRP, 25 mg/ml; 0.1 ml) into the dorsal lymph sac of adult female Rana pipiens and allowed the tracer to circulate for 5-60 min. To examine potential transport by vesicles at the ablumenal (endoneurial) endothelial surface we slowly injected HRP (5 mg/ml; 1-3 µl) into the endoneurium via a small slit in the surrounding perineurial sheath. These frogs were sacrificed 5-30 min after endoneurial injection. Fixed sciatic nerves were chopped into 80-100 µm sections, reacted with 0.05% diaminobenzidine with 0.01% H_2O_2 for 1 hr at room temperature on a covered shaker, and processed routinely for electron microscopy.

Intravascular HRP was confined to the lumen of endoneurial blood vessels at all post-injection times. Vesicles at the lumenal surface were lined by the dense reaction product at both 5 and 60 min, and no progressive filling of cytoplasmic or ablumenal vesicles at the later times could be discerned. No tracer appeared at the endoneurial surface of the endothelial cells and no filled vesicles were seen attached to this cell surface. HRP injected into the endoneurium penetrated the basal lamina of endoneurial endothelial cells and filled vesicles at the ablumenal cell surface. Tracer filled the interendothelial cleft within junctional regions but appeared halted by regions of membrane apposition near the lumen. No differences in the location of tracer-filled vesicles were seen in tissues taken at 10 and 30 min after injection of HRP. Tracer was not associated with the lumenal cell surface; tracer-filled vesicles were seen only in the vicinity of the ablumenal surface. We conclude from these studies that the endoneurial endothelial vesicles do not transport HRP in either a lumenal or antilumenal direction.

49.4 [14C]SUCROSE PERMEABILITY OF ENDONEURIAL CAPILLARIES IN THE FROG SCIATIC NERVE. S.I. Rapoport and A. Weerasuriya*. (Spon. R.E. Taylor) Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224 USA

The endoneurial space in a vertebrate peripheral nerve fascicle is separated from blood and extracellular tissue fluid by the endoneurial vasculature and the perineurium respectively. Functionally, these two barriers constitute the blood-nerve barrier. The perineurium of the frog sciatic nerve is relatively impermeant to small hydrophilic solutes ([14C]sucrose permeability - 5.6×10^{-7} cm/sec) and has a moderate electrical resistance (478 Ωcm^2), indicating that it presents a significant hindrance to the transperineurial passage of substances. A characterization of the dynamics of fluid volume and solute concentrations within the endoneurial space also requires a description of the permeability of the other part of the blood-nerve barrier - the endoneurial capillaries. We estimated the permeability-surface area product (PA) of endoneurial capillaries of frog sciatic nerve to [14C]sucrose at room temperature with an in vivo perfusion arrangement. The perfusion medium (frog Ringer with 2% bovine serum albumin) containing [14C]sucrose and a tritiated blood space indicator, was infused into both legs of an urethane-anaesthetized frog via the descending aorta, at a pressure head of 16-23 mm Hg. An outlet was provided by cannulating the ventral abdominal vein. Perfusion times ranged from 4 to 8 min. At the end of the perfusion period the nerves were removed from the frog and desheathed. After their dry weights had been determined, the perineuriums and desheathed nerves were dehydrated, solubilized and their radioactivities measured with conventional procedures. PA of the endoneurial capillaries to [14C]sucrose was estimated to be 5.0 ± 0.79 (S.E.M., n=20)$\times 10^{-5}$ sec^{-1}. In a one cm length of nerve with a diameter of 0.7 mm, the calculated PA for [14C]sucrose of the endoneurial capillaries is 1.9×10^{-7} cm^3/sec and that of the investing perineurium is 1.2×10^{-7} cm^3/sec. This supports the hypothesis that, under normal conditions, the endoneurial capillary wall is the major site of passage for substances entering and leaving the endoneurial space, and that the perineurium probably plays a relatively passive role in defining and tightly limiting the endoneurial space within which the endoneurial capillaries exercise their regulatory functions.

49.5 PERMEABILITY ROUTES OF HORSERADISH PEROXIDASE ACROSS THE FROG PERIPHERAL NERVE SHEATH. N.L. Shinowara, M.E. Michel* and S.I. Rapoport. Sec. Experiment. Morphol. & Lab. of Neurosciences, NIA-Gerontol. Res. Ctr., Balto. City Hospitals, Baltimore, MD. 21224

Normally, intravascular horseradish peroxidase (HRP, Sigma Type VI, 20 mg/kg body wt., 1 hr circulation time) is kept out of the endoneurium of the frog nerve by the perineurium and endoneurial blood vessels. We examined the possible routes of HRP movement across and within the nerve sheath (epineurium and perineurium) when it was applied directly to the epineurial surface of the nerve (in vitro, in physiological saline, or in vivo with gelfoam) or to the endoneurium by injection through a perineurial perforation. Nerves were fixed by immersion, in 2-3% glutaraldehyde in buffer or Ringer's, reacted with diaminobenzidine, postfixed and processed for electron microscopy. The tightest region of the perineurium to HRP was the inner 1-2 layers of perineurial cells; the arrangement of multiple layers of cells joined by tight junctions and interspersed with connective tissue markedly retarded the intercellular movement of tracer which was applied to the nerve exterior. When HRP had extension lateral access to sheath layers at the site of injection into the endoneurium, tracer moved readily between layers, indicating that the tight junctions, which joined adjacent layers, were incomplete and ineffective in retarding the lateral and circumferential diffusion of HRP within the perineurium. HRP, which leaves a trail by binding to cell membranes and connective tissue components, reached the endoneurium when applied to the nerve for 2 hr or more, probably damaging perineurial cells. Entry of injected HRP into the inner perineurial layers from the endoneurium also was associated with damaged cells, which often contained HRP. Numerous vesicles within perineurial cells did not appear to contribute to tracer movement. In rare cases occasional tracer-filled vesicular pits were found in regions free of HRP in the adjoining extracellular space. This suggests possible vesicular uptake of HRP, followed by vesicular movement from sites of uptake; however, there was no evidence of HRP discharge from the vesicle. In conclusion, the outer perineurial layers have incomplete barrier layers. Cellular integrity appeared compromised by HRP over time and cellular damage was associated with HRP movement across the inner layers. The presence of some isolated, HRP-filled vesicles was not clearly related to transcellular transport and discharge. Vesicles were not responsible for the majority of HRP movement across the perineurial cell layers.

49.6 FURTHER EVIDENCE FOR OPENING THE BLOOD-BRAIN BARRIER (BBB) BY DMSO AND HYPEROSMOTIC STRESS. R. Broadwell, B. Balin*, M. Salcman, and R. Kaplan*, University of Maryland School of Medicine, Baltimore, Maryland 21201

We have reported that within 2 hr. of intravenous administration of HRP in the company of DMSO in mice, the BBB is opened transiently to the circulating HRP (Science 217:164, 1982). Such opening could be attributed to vesicular transport through the cerebral endothelial cells, the formation of patent transendothelial channels, and/or a widening of tight junctions between contiguous endothelial cells. In an effort to ascertain how the BBB is modified by DMSO, mice were injected intravenously with 10-20% DMSO/HRP and fixed 5 min or less after injection. Mice so injected exhibited numerous focal exudates of HRP surrounding blood vessels throughout the brain. Ultrastructural inspection of these "leaky" vessels revealed that the vessels were undamaged and contained numerous HRP-positive vesicles, tubules and dense bodies. The latter were demonstrated by acid phosphatase cytochemistry to be lysosomes. Tubules that could be mistaken for a transendothelial channel were shown on a goniometer tilt stage to have no membrane continuity with the luminal and abluminal membranes of the endothelial cells; however, a few tight junctions did appear open and filled with HRP that extended into the basement membrane and perivascular space. Labeled vesicles lying in proximity to the cytoplasmic side of the abluminal membrane may be indicative of pits in the abluminal surface exposed to the exudated HRP and, therefore, would not be vesicles engaged in active transport. To test this possibility, normal mice and mice hyperosmotically stressed by imbibing 2% NaCl for 5-7 days were injected with HRP/saline into the lateral cerebral ventricle and fixed 1-24 hrs. later. Peroxidase filled the extracellular clefts throughout the brain within 3 hrs. post-injection. Cerebral vasculature bathed extracellularly in HRP through 24 hrs. contained labeled vesicles, remarkably few labeled dense bodies, and no labeled tubules. Serial thin sections revealed that many of the vesicles were indeed invaginations of the abluminal surface membrane. The luminal surface of endothelial cells from only salt-stressed mice was coated with HRP reaction product. Inspection of some of these cells disclosed that the tight junctions were open and flooded with HRP. These observations suggest that: (1) DMSO acts on the BBB by opening the tight junctions; (2) the movement of HRP and associated cell surface membrane into the cerebral endothelium is vectorial, predominantly from the luminal surface as opposed to the abluminal surface; (3) the negligible uptake of HRP by the cerebral endothelium from its abluminal side supports the existence of a brain-blood barrier; and (4) hyperosmotic stress induced by salt-loading opens cerebral endothelial tight junctions, perhaps as a consequence of shrinkage of the endothelial cells.

49.7 VASCULAR INFUSION WITH SITS RESULTS IN LABEL OF CIRCUMVENTRICULAR, LIMBIC, AND OTHER BRAIN STRUCTURES. L.C. Schmued* and J.H. Fallon, UCI Dept. of Anatomy, University of California, Irvine, CA 92717

Although most cerebral blood vessels are considered impervious to exogenous tracers such as HRP, Evans Blue, Pontamine Sky Blue, Reduced Silver, and India Ink, it is apparent that certain restricted regions do possess permeable capillaries. These regions are often referred to as circumventricular organs and have been described by a number of authors including Reese and Karnovsky, ('67) and Broadwell and Brightman ('76) These studies employed HRP to indicate capillary permeability and subsequent neuronal uptake.

In our study we used the fluorescent retrograde tracer SITS to study regional permeability differences in the blood brain barrier. This tracer has been previously employed to demonstrate axonal retrograde transport by covalent binding of the tracer to nerve terminals (Schmued and Swanson, '82). In this experiment, a 0.1% solution of SITS in 0.9% saline is injected into the rat heart. After 10 minutes, the rat is perfused with saline containing 0.1% SITS followed by 10% neutral buffered formalin. Frozen sections are cut and counterstained with Ethidium Bromide.

Our results indicate selective labeling of several distinct classes of CNS structures. As expected, all vascular endothelial cells and the choroid plexus are heavily labeled. Also, consistent with the findings of Broadwell and Brightman, is the labeling of circumventricular organs and other structures including the arcuate, supraoptic, paraventricular, diagonal band, cranial motor, and cranial parasympathetic nuclei. We also found labeling, in distinct cytoarchitectonic subdivisions of a number of other nuclei, particularly those comprising limbic structures. Some of these limbic structures include the islands of Calleja, cingulate cortex, anterior thalamus, midline thalamus, perirhinal cortex, dorsolateral septum, amygdala, lateral habenula, subiculum, and hippocampus. Most hippocampal labeling was found in the inferior, deep granule cells of the dentate gyrus with some cells labeled in the hilus and isolated pyramidal cells throughout Ammon's horn.* Other labeled structures which are not classically considered limbic include the locus coeruleus, pretectum, dorsal raphe, some cerebellar Purkinje cells and midbrain central grey. Regions which exhibit extensive vascular staining but virtually no neuron perikaryal label include sensory nuclei, neocortex, and extrapyramidal motor structures.

It is not clear why we see SITS uptake in structures not included in the classical circumventricular organ system. It may be the result of a more sensitive method, or it may be the result of the physical, chemical, and pharmacological properties of the tracer employed. We are currently investigating the possibility that limbic structures as well as hypothalamic structures are able to sample trophic factors from the peripheral environment. (Supported by NIH Grants NS 16017 and 15321).

49.8 INCREASED PERMEABILITY OF C-6 ASTROCYTOMA VASCULATURE MONITORED WITH AN ULTRASTRUCTURAL TRACER. C.L. Edmonds*, R.R. Shivers and R. Del Maestro*, Departments of Zoology and Clinical Neurological Sciences, University of Western Ontario, London, Ontario, Canada.

Capillaries of brain gliomas have been shown to lack a blood-brain barrier (BBB). Also, peri-tumoral neuropil is frequently edematous which suggests that it too is hyperpermeable in the presence of tumors. In spite of these observations, little is known about the anatomical basis of tumor and peritumoral capillary hyperpermeability or the functional relations between them. To determine the ultrastructural basis for lack of a BBB in tumors and peritumoral neuropil, a protein tracer was injected into the brain vasculature of rats which had been innoculated with C-6 astrocytoma cells. At various times during tumor development, horseradish peroxidase was injected into the external jugular vein. At 10, 30 and 60 min. post-injection the brains were perfused and tumor, peritumoral and contralateral areas were examined with 60kv and 1000kv (high voltage) electron microscopy. Single and complex vesiculo-tubular channels filled with HRP reaction were observed in the endothelial cytoplasm of peri-tumoral and contralateral areas of tumor-bearing rats. Similar structures were not seen in brains of control rats. Often, the vesiculo-tubular channels spanned the endothelium to form a continum from the luminal to abluminal surface. Tight junctions of both tumor-bearing and control tissue appeared intact and free of reaction product. Large, thin-walled sinusoidal blood vessels were seen in the tumors. HRP reaction product was present in pinocytotic vesicles, extracellular compartment, and in pinocytotic vesicles and multivesicular bodies of the tumor cells. Results of this study support the notion that the HRP-filled vesiculo-tubular channels of peritumoral and contralateral tissue form an unrestricted route for transport of material across the endothelium. Supported by the N.S.E.R.C. of Canada.

49.9 AUGMENTED UPTAKE OF A NEUTRAL AMINO ACID BY THE PITUITARY NEURAL LOBE IN DEHYDRATED RATS. P.M. Gross, R.G. Blasberg*, C.S. Patlak* and J.D. Fenstermacher*. (SPON:J.H. Hurst). National Cancer Institute and National Institute of Mental Health, Bethesda, MD 20205

The pituitary neural lobe is a dense terminal field of projections from hypothalamic cell bodies that synthesize and elaborate the vasoactive hormone, vasopressin, during conditions such as dehydration. The mechanism of vasopressin secretion involves a vesicular exocytotic process at the interface of nerve terminals and capillary bed in the neural lobe. It is not known to what extent vasopressin secretion influences the rate of solute transport across neural lobe capillaries. In this study, we examined extraction by the neural lobe of a small neutral amino acid, α-aminoisobutyric acid (AIB, MW=103) during normal and dehydrated (5 days of water deprivation) conditions in conscious albino rats. In order to determine the extraction fraction (E) for AIB by the expression, $E = K_i/F.V_c$ (where K_i= the tissue transfer rate constant for AIB corrected for vascular space, F = rate of blood flow and V_c = plasma fraction of arterial blood), the following four measurements were necessary in separate groups of normal and dehydrated animals: 1) plasma volume, V_p, was evaluated with ^{125}I-albumin given by iv injection in 2 minute experiments; 2) K_i was estimated from 12 second studies using ^{14}C-AIB, which was given by iv pulse injection, and included rapid arterial blood sampling; 3) F was determined with ^{14}C-iodoantipyrine, administered by iv ramp infusion in 20 or 60 second experiments; and 4) arterial hematocrit was determined conventionally. Horizontal intact sections (20 μm thick) of frozen whole pituitary glands were autoradiographed to film. Measurements in the neural lobe of V_p, K_i and F were quantified from computerized image analysis. In the neural lobes of normal animals, we found that V_p, K_i, F and E were 34 μl/g, 0.67 ml/g/min, 5.0 ml/g/min and 23%, respectively. Values for normal brain tissue (e.g., caudate nucleus) in conscious rats are 5 μl/g, 0.0004 ml/g/min, 1.3 ml/g/min and 0.1%, respectively. Our results in the neural lobe of control rats reflect the special characteristics of this structure's circulatory bed, viz, high capillary density, fenestrated endothelia, and extremely rich perfusion under normal conditions. In dehydrated rats, each of the measurements was increased. K_i was elevated 4 to 5-fold, indicating a markedly augmented extraction of AIB by the neural lobe. These findings demonstrate that transport processes across neural lobe capillaries and/or pituicyte cellular membranes are greatly stimulated during conditions of high vasopressin secretion.

49.10 EFFECT OF 2450 MHz MICROWAVE ENERGY ON THE BLOOD-BRAIN BARRIER TO HYDROPHILIC MOLECULES. W. M. Williams*. Center for Brain Research and Dept. of Radiation Biology and Biophysics, Univ. of Rochester School of Med. and Dent., Rochester, NY 14642.

Microwave energy at 2450 MHz was found ineffective in increasing the permeability of the blood-brain barrier to the hydrophilic tracers HRP and [^{14}C] sucrose. Furthermore, a diminished permeability to HRP and sodium fluorescein was apparent after 180 minutes of exposure to microwaves at an incident power density of 20 mW/cm^2. Colonic temperature, as well as temperature within the cerebral cortex, hypothalamus, cerebellum and medulla, was elevated by less than 1°C over those of sham-exposed rats. A significant ($p < .05$) decrease in the permeability to HRP and [^{14}C] sucrose occurred after exposure to an incident power density of 65 mW/cm^2 for 30 minutes. These exposures induced a moderate hyperthermia with brain and colonic temperatures increasing to ~40 to 41°C. Extending exposure time to 90 minutes also resulted in diminished permeability to HRP concomitant with severe hyperthermia and brain and colonic temperatures that exceeded 42°C. Permeability to HRP was similarly affected by exposure to ambient heat (42±2°C) which produced brain and colonic temperatures that were comparable to those observed after 90 minute microwave exposures.

Reduction in permeability to HRP correlated with a suppressed incorporation of the tracer by pinocytosis in cerebral microvessels. Suppression of blood-brain barrier permeability to hydrophilic tracers was most pronounced at brain temperatures exceeding ~40°C and is demonstrated to be temperature dependent.

49.11 TRAUMATICALLY INDUCED CHANGES IN EXTRACELLULAR SPACE IN THE RAT SPINAL CORD. N.R. Clendenon, W.A. Gordon*, M.E. Nesham*, and J.N. Allen, Jr.*, Dept. of Neurology, Ohio State Univ., Col. of Med., Columbus, OH 43210.

Previous studies utilizing the rat model of experimental spinal cord trauma assessed the sequential development of edema formation, electrolytic alterations and infiltration of blood during the acute period subsequent to injury (Clendenon, N.R. et al., Soc. Neurosci. Abst. 7:87, 1981). Trauma was induced by the weight drop method (5g,18cm) onto the dura exposed cord. Control spinal cords were removed by the non-traumatic hydraulic pressure technique (DeSousa, B.N. and Horrocks, L.A., Dev. Neurosci. 2: 115, 1979). Laminectomy controls showed non-significant increases in tissue water, sodium, and chloride levels and a significant increase in blood infiltration. Increased tissue water was significant at 10 min after injury and continued to rise. Blood infiltration into traumatized cord at 15 min was 5-fold greater than in laminectomized controls. Sodium and chloride levels were also increased at 15 min and potassium levels were decreased at 30 min.

Using the same model of spinal cord injury changes in extracellular fluid volume were examined by utilizing the inulin space method. Inulin, having a low molecular weight (approx. 5,000), is an appropriate extracellular marker and its distribution in brain is well documented. The method of Ross and Mokotoff (J. Biol. Chem. 190:659, 1951) was used to assay inulin in blood and spinal cord samples from nephrectomized rats given in i.v. bolus of marker (400 mg/kg body wt) 3 to 5 hrs prior to sacrifice. Inulin space for control spinal cords was 13.3%±0.70 (S.E.M.). Laminectomy resulted in a non-significant decrease in extracellular space to 86% of control. By 15 min following injury, the extracellular space was significantly ($p<0.001$) reduced to 56% of the laminectomy control. A similar decrease was found at 30 min.

These results, together with the rapid loss of Na^+,K^+-activated ATPase activity at the site of maximal injury (Clendenon, N.R. et al., J. Neurosurg. 49:563, 1978), suggest an intracellular uptake of fluid and metabolites from the extracellular compartment and a loss of membrane integrity affecting ionic pumps. (Supported by NIH grant NS-10165).

49.12 BLOOD-BRAIN BARRIER DYSFUNCTION DURING SEIZURES AND NOREPINEPHRINE INFUSION IS ENHANCED BY LOCUS CERULEUS LESION. S. I. Harik and T. McGunigal, Jr.* Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, Ohio 44106.

The functions of the putative noradrenergic innervation of cerebral microvessels from the locus ceruleus (LC) remain ambiguous. Although such innervation does not seem to control cerebral blood flow, there is increasing evidence that it may modulate transport and permeability functions of the blood-brain barrier (BBB). Here, we studied the effect of LC lesion on the leakage of albumin across the BBB under steady state conditions and during seizures and hypertension, both of which are known to be associated with increased leakage of macromolecules across the BBB. Two weeks after unilateral chemical LC lesion, awake but restrained rats were given ^{125}I-radioiodinated serum albumin (RISA), 100 µCi/Kg, intravenously and then infused with saline or 10 µg/kg/min of one of the following agents for 30 min: norepinephrine (NE), isoproterenol (ISO) or angiotensin (AT). In another group we studied the effect of 15 min of bicuculline-induced seizures in anesthetized, paralyzed and artificially ventilated rats. At the end of the experiment, the brain was immediately perfused in situ, to remove intravascular RISA. Bilateral samples from the frontal and parietal cortex and hippocampus were measured for their RISA and NE content. Data was analyzed by comparing the results of NE-depleted structures, ipsilateral to LC lesion, with those from the contralateral side by the paired t test.

The magnitude and the duration of hypertension induced by NE, AT and seizures were similar. Saline caused no change in blood pressure while ISO produced hypotension. Unilateral LC lesion caused severe depletion of NE in the ipsilateral cortex without affecting the contralateral cortex. After NE-induced hypertension and seizures, RISA content of NE-depleted regions was significantly higher than those of the contralateral side. Also, the leakage of RISA into the parietal cortex was higher than into the frontal cortex. Infusion of AT, ISO, or saline did not cause significant differences in RISA content between the 2 sides. These results suggest that noradrenergic innervation of cerebral microvessels from the LC help preserve the integrity of the BBB during pathophysiological conditions associated with increased circulating catecholamines.

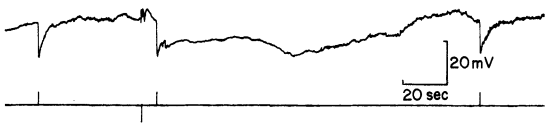
SENSORY TRANSDUCTION I

50.1 KINETIC MEASUREMENT OF PHOTOCURRENTS, NUCLEOTIDE CHANGES AND PROTEIN PHOSPHORYLATION DURING LIGHT ACTIVATION OF FROG PHOTORECEPTORS. M. Biernbaum*, R.H. Cote*, E. Brewer, C. Schobert*, H. Hamm, and D. Bownds. Lab. of Molecular Biology and Dept. of Zoology, Univ. Wis., Madison, WI 53706.

Purified suspensions of frog rod outer segments still attached to the mitochondria-rich inner segment portion of the receptor cell (o.s.-i.s.) can be prepared by gentle suction treatment of the isolated retina, followed by Percoll gradient centrifugation. These structures maintain normal dark currents and light responses for several hours. The quantities of o.s.-i.s. material obtained (0.1 mg/retina) permit simultaneous chemical analysis. Nucleoside triphosphate and cyclic GMP levels similar to those of the living retina are observed. A 20-60% decrease in cyclic GMP can be observed within 100-300 msec after onset of illumination using either rapid freezing or acid quenching techniques, providing a conclusive demonstration that cyclic GMP levels can change on the time scale of excitation and adaptation processes. Uptake and light-induced extrusion of ^{45}Ca is also observed. GTP and ATP undergo a slower light-induced decrease, and their recovery after bright flash illumination lags the return of the dark current but correlates with the return of light sensitivity. Rhodopsin (38K) and one small protein (12.5K) are reversibly phosphorylated upon illumination, and four proteins (36K, 13K, 12.3K, and 12K) are dephosphorylated. The phosphorylation of rhodopsin continues during the return of conductance after bright illumination, after the dephosphorylation reactions have terminated. Current studies are comparing the protein phosphorylation changes with concurrent conductance and sensitivity changes.

50.2 INJECTION OF TRYPSIN ACTIVATED PHOSPHODIESTERASE (PDE) INTO ROD OUTER SEGMENTS (ROS) CAUSES LIGHT-DEPENDENT HYPERPOLARIZATION. J.B. Hurley*, and Y. Shimoda* & W.H. Miller. Div. of Biology, California Inst. of Tech., Pasadena, CA 91125 and Dept. of Ophthal. & Visual Science, Yale Med. School, New Haven, CT 06510.

Previous biochemical and physiological studies have shown that light activates ROS PDE to hydrolyze cyclic GMP and hyperpolarize the ROS. Cyclic GMP PDE was extracted from ROS membranes, activated by trypsin, purified to homogeneity (with a specific activity of 1000 µmol cyclic GMP/min/mg) and injected into ROS of the isolated retina of the toad, Bufo marinus. Brief injections of PDE caused a marked light-dependent hyperpolarization lasting for minutes. The middle light response on the record below is to a light flash given after a 200 msec injection of PDE indicated by the downward spike on the signal trace below the record. The initial hyperpolarizing phase of this receptor potential has the same amplitude and latency as the controls, but except for the voltage dependent depolarization just following the "nose", the recovery phase of the light response is inhibited for about 2 min while the membrane potential remains hyperpolarized by 10 to 20 mV. Injection using the same pipette that had been heated to denature the trypsin-activated PDE showed no light-dependent effect. The injection of activated PDE would be expected to increase the rate of cyclic GMP hydrolysis causing the ROS to slowly hyperpolarize. Light would be expected to accelerate the hyperpolarization by increasing the rate of cyclic GMP hydrolysis. The paucity of inhibitor relative to PDE following its injection may be an important factor in the marked prolongation of the light response. (Supported by USPHS Grants AI-19296 (M. I. Simon, Cal. Tech.) and EY-03196 (W.H.M.) and fellowships from Fight for Sight, Inc., New York City (Y.S.) and the Alex. B. Cox Memorial Fund, Yale Medical School (Y.S.).)



50.3 INJECTION OF CYCLIC GMP-DEPENDENT PROTEIN KINASE INTO ROD OUTER SEGMENTS (ROS) CAUSES DEPOLARIZATION THAT IS INHIBITED BY ILLUMINATION. Y. Shimoda*, W. H. Miller, R. M. Lewis, A. C. Nairn* and P. Greengard. Depts. Ophthalmology and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

Previous biochemical and physiological studies have suggested that cyclic GMP depolarizes ROS and that cyclic GMP hydrolysis, regulated by a light-dependent phosphodiesterase, causes hyperpolarization. We have investigated the role of cyclic GMP-dependent protein phosphorylation in this system. Cyclic GMP-dependent protein kinase purified to homogeneity from bovine lung (specific activity; 5 μmol/min/mg using histone h2b as a substrate) was injected from glass micropipettes into ROS of isolated Bufo marinus retina. Injection of the protein kinase causes (1) a depolarization and (2) a reduced amplitude of the light-induced hyperpolarizing response. Both the depolarization and the reduced amplitude of the light response were found to recover on a time scale of minutes. Injection of protein kinase that had been inactivated by heating to 60°C for 20 minutes caused a slight hyperpolarization during injection and no detectable change in the amplitude of response. The application of a light flash during the depolarization caused by injection of cyclic GMP-dependent protein kinase immediately prevented further depolarization.

The above data indicate that the activity of the injected kinase depends on the high endogenous ROS level of cyclic GMP, which is significantly reduced by activation of the light-dependent phosphodiesterase. The data are consistent with the interpretation that endogenous ROS cyclic GMP normally depolarizes the ROS through the activation of an endogenous cyclic GMP-dependent protein kinase. (Supported by USPHS Grants EY-03196 (W.H.M.) and NS-08440 (P.G.) and fellowships from Fight for Sight, Inc., New York City (Y.S.) and Alexander B. Cox Memorial Fund, Yale Medical School (Y.S.).)

50.4 INTERNAL DIALYSIS OF SOLITARY SALAMANDER ROD PHOTORECEPTORS. P. R. Robinson, P. R. MacLeish and J. Lisman, Dept. of Biology, Brandeis University, Waltham, MA., and Dept. of Neurobiology, Harvard Medical School, Boston, MA.

The study of phototransduction in vertebrate rod photoreceptors would be greatly facilitated if the composition of the cytoplasm could be reliably manipulated. Recent work shows that the internal dialysis method(1) can be used to alter the cytoplasmic composition of Limulus photoreceptors allowing several types of molecules, including proteins, to be introduced into the cell's cytoplasm (2). We have applied a similar method to solitary vertebrate rods prepared by enzymatic dissociation of the dark-adapted tiger salamander (Ambystoma tigrinum) retina. Using electrodes with a 1um tip diameter and resistances between 5 and 15 megaohm, seals of 1-50 gigaohm were obtained on either the inner or outer segment of solitary rods suspended in an amphibian salt solution with 1mM Ca. After the patch membrane broke spontaneously or was disrupted with large voltage pulses, the cell was studied in the "whole cell" recording mode. Total membrane current was measured under voltage-clamp conditions using a current-to-voltage transducer with series resistance compensation. In order to find conditions which support transduction in dialyzed rods, several electrode solutions were tested. Since the internal composition of rods is not well defined, the design of the electrode solution was somewhat arbitrary. Satisfactory results were obtained with a dialysis solution containing 92mM K-aspartate, 5mM $MgCl_2$, 7mM NaCl, 1mM $NaHCO_3$, 10mM taurine, 1mM Na-ATP and 1mM Na-GTP (pH 7.4). When the cell was clamped at the resting potential (-20 to -40 mV), the peak response to a saturating light was 30-40 pA. Such responses were stable for an hour or more. Current-voltage relations in the dark showed both inward and outward rectification and a slope resistance of approximately 1 gigaohm at rest. These properties are in reasonable agreement with those obtained using conventional intracellular recording techniques. It appears that this method will be useful for introducing molecules into functioning vertebrate rod photoreceptors while examining their response properties under high resolution voltage-clamp conditions.

1) Lee et al, J. Gen. Physiol. (1978),71, 489-507
2) Stern and Lisman, PNAS (1982),79,7580-7584
(Supported by EY-01496 and EY-03665)

50.5 BUFO ROD RESPONSES REVEAL A CONTRIBUTION OF THE SODIUM PUMP. K.N. Leibovic. Department of Biophysics, State University of New York at Buffalo, Buffalo, NY 14214.

High intensity light flashes elicit a response from vertebrate photoreceptors, characterized by a hyperpolarizing spike which is followed by a plateau and relaxation to the resting potential.

We have found that perfusion of the isolated retina with ouabain reduces and abolishes the spike and --more slowly-- the remainder of the response. At the same time the resting potential remains almost constant. These data were obtained from intracellular recordings in the isolated retina of Bufo marinus.

Our interpretation of these data is as follows: Ouabain specifically inhibits the Na+-K+ ATPase. As the pump is inactivated by ouabain so the spike disappears. Therefore we propose that the spike arises from an electrogenic Na+-K+ pump. During the normal physiological response to a bright flash, as the outer segment conductance is shut off, so the pump relaxes and this explains the response relaxation from the spike to the plateau. The contribution of the pump to the membrane potential is therefore equal to the difference between the saturated (largest) spike and the plateau. During the normal light response the pump follows the outer segment conductance. On the other hand, during ouabain perfusion the experimental observations lead us to the conclusion that the pump also affects outer segment conductance. This would explain why the plateau amplitude decreases as the spike is reduced and why the resting potential remains steady.

In the light of our data we will also show why the spike cannot be due to any simple voltage sensitive conductance, which may be activated by a given membrane hyperpolarization.

Supported in part by NIH grant R01-EY03672-01A1.

50.6 REVERSAL OF THE REACTION WHICH INACTIVATES METARHODOPSIN CAN BE A SIGNIFICANT SOURCE OF SPONTANEOUS QUANTUM BUMPS IN LIMULUS MEDIAN PHOTORECEPTORS. John Lisman, Ellen Armstrong*, and Laur Blumberg*. Dept. Biol., Brandeis Univ., Waltham, MA 02254.

Many photoreceptors produce observable responses to single photons. The similar events which occur spontaneously in the dark could potentially have three sources: 1. Spontaneous isomerization of rhodopsin; 2. On the assumption that a photon converts rhodopsin to an active pigment state (M^*) which is then converted to an inactive state (M), spontaneous events could be generated if M spontaneously reverts to M^*; 3. A reaction which is normally turned on by M^* might turn on spontaneously. To examine the role of these reactions, we have measured the rate of spontaneous quantum bumps in the UV receptors of Limulus median eye. These cells have a rhodopsin with peak absorbance near 360 nm and a metarhodopsin with a peak near 470 nm. Measurements using the early receptor potential indicate that there is little or no dark regeneration of metarhodopsin to rhodopsin over the time course of 8 hours. It was therefore possible, by chromatic adaptation, to produce stable variation in the ratio of rhodopsin to metarhodopsin (R/M). After such adaptations, cells were dark-adapted until the spontaneous quantum bump rate became stable. If reaction 1 were the sole source of spontaneous events, their rate should be highest when R/M is high. If reaction 3 were the source, the rate should be independent of R/M. We found, however, that the rate is highest when R/M is low, consistent with reaction 2 as a source. The results therefore suggest that the reaction which inactivates metarhodopsin is reversible and that such reversions can be a significant source of spontaneous quantum bumps when the metarhodopsin concentration is high.

50.7 CALCIUM MEDIATES THE LIGHT-DEPENDENT DECREASE OF THE MAINTAINED VOLTAGE-DEPENDENT K CURRENT IN LIMULUS VENTRAL PHOTORECEPTORS. K. Chinn* and J. Lisman. (SPON: M. Woodruff). Dept. of Biol., Brandeis Univ., Waltham, MA 02154.

In addition to its effect on the light-activated Na conductance, light reduces the maintained voltage-dependent K current (I_k) in Limulus ventral photoreceptors (Sci. 212:1273, 1981). We have investigated the mechanism underlying this light-dependent decrease in I_k. To test whether the light-induced rise of Ca_i might be responsible, we buffered Ca_i by pressure-injecting the Ca buffer EGTA (pCa 7.1) while monitoring the injection volume using dye absorbance. At intracellular concentrations of about 60 mM EGTA the effect of light on the maintained outward current was inhibited. In the EGTA experiments the ASW bathing media contained 10 mM Ni to block the voltage-dependent inward current (I_{in}). This was necessary because in EGTA-injected cells we found a maintained inward current that was reduced by light. The EGTA experiments suggest that Ca has a role in the light-dependent decrease in I_k. To further test this idea we performed a three microelectrode experiment, using two microelectrodes to clamp the cell and the third to iontophoretically inject Ca. We found that injection of Ca in the dark reversibly reduced the voltage-dependent outward current. This was not due to an activation of I_{in} because Ca injection reduced I_k even under conditions where I_{in} was blocked with Ni. We also investigated whether changes in H were important in the light dependent decrease in I_k, by pressure injecting pH buffer (MOPS, pH 7.2) into photoreceptors. MOPS (100-200 mM) did not affect the light-induced reduction of I_k, indicating that changes in pH are not involved and that protons do not induce Ca release. These experiments show that the light-induced decrease in I_k occurs via a Ca-dependent mechanism.

50.8 CLUMPING OF AUDITORY STEREOCILIA DURING TEMPORARY THRESHOLD SHIFT. M. J. Mulroy. Dept. of Anatomy, Sch. of Med., Medical College of Georgia, Augusta, GA 30912.

The objective of this study was to determine if anatomical changes in the stereocilia of auditory hair cells occur during the period of temporary threshold elevation (TTS) following exposure to loud noise. Alligator lizards with normal right and left ears were used. The functional state of the ears was assessed by measuring their averaged evoked response (N_1 of the cochlear potential) to acoustic clicks. The left ear was exposed to moderately intense broadband noise for a short duration and the recovery time of N_1 to its preexposure level was measured to ensure that the TTS was of sufficient duration to permit fixation of the ear during TTS. The ear was exposed to the noise again, its response was measured, and the ear was fixed immediately by cochlear perfusion. The inner ear was removed, mounted in a depression slide and observed under a light microscope. It was then processed for observation with a scanning electron microscope. In unexposed ears the individual stereocilia in a tuft are separated from each other. In noise-exposed ears the stereocilia adhere to adjacent stereocilia within the tuft. The phenomenon is unambiguous and easily seen with the light and scanning electron microscope in the basal region of the basilar papilla where the stereocilia are freestanding (uncovered by a tectorial membrane) and very long. When examined with the transmission electron microscope the cell membranes of the stereocilia are intact, that is, the stereocilia are not fused. I interpret these results to indicate that during exposure to noise, which is intense enough to induce TTS, the hair cell actively protects itself by clumping its stereocilia together. This could modify the mechanical response of the stereocilia and thus could temporarily elevate the threshold of the hair cell.

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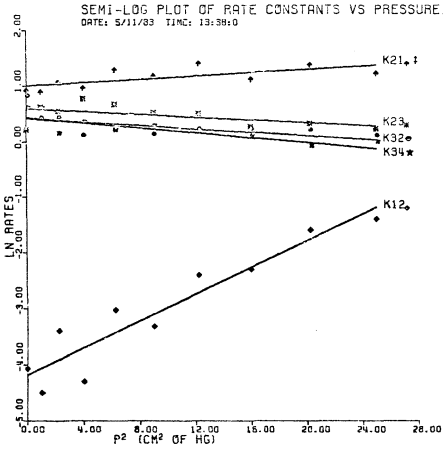
50.9 STRETCH ACTIVATED SINGLE ION CHANNEL CURRENTS RECORDED FROM EMBRYONIC CHICK SKELETAL MUSCLE. F. Guharay* and F. Sachs* (SPON: R. McIsaac). Dept. of Biophysical Sci., SUNYAB, Buffalo, NY 14214.

After forming a giga-seal, suction (-5 cm of Hg) applied to the interior of the patch pipette causes an increase in channel activity. The stretch activated channel is cation selective but discriminates poorly between sodium and potassium ions (rev. potential≈-30 mV). The open channel conductance is 70 pS with 150 mM KCl on both sides of the patch. A suction of 8.4 cm^2 of Hg produces an e-fold change in the probability of the channel being open. Treatment of the cells with cytochalasins A,B and E makes the cells more sensitive to stretch, 0.28 cm^2 of Hg producing a similar change in the probability of the channel being open. Kinetic analysis shows that the channel has one open and three closed states. By matching the open and closed time distributions to a four state sequential model C(1)-C(2)-C(3)-O(4), the effect of applied stretch is reflected only in one rate constant (K_{12}). The increase in the rate constant K_{12} with increasing stretch underlies the observed increase in channel activity with increasing stretch. Supported by NINCDS 13194 to F.S.



50.10 BEHAVIOR OF A SINGLE NEURON IN A RECURRENT EXCITATORY LOOP. O. Diez-Martínez*, and J.P. Segundo of Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, México, D. F. 04510. MEXICO; Department of Anatomy and Brain Research Institute, University of California at Los Angeles, CA 90024, USA.

This communication examined the practical possibility and consequences of recurrent excitation, or positive feedback on the rate of impulse discharge of an isolated nerve cell. Inhibitory recurrent circuits have been explored extensively. Excitatory ones, however, although occurring in the Central Nervous System, have been largely ignored. Since there are no excitatory fibers converging upon the neuron of the slowly adapting organ (SAO), a recurrent excitation loop was constructed by enabling each impulse from the SAO of crayfish to trigger through an electronic circuit a brief stretch or "tug" of the receptor muscle. Each tug, when applied independently influenced the discharge as would an EPSP generated by the activation of a "recurrent collateral circuit". Introduction of recurrent excitation led to characteristic discharge timings; hence, even an isolated neuron can have intrinsic mechanisms that prevent positive feedback from freezing it in a extreme non-operational state. Such timings depended critically on the "phase" i.e., on the time elapsed between a SAO impulse and the tug.

When the control discharge was stationary because the SAO muscle length remained invariant, phases of a few ms, simply changed the pattern to one of doublets, and affected little the average rate. As the phase increased, bursts appeared and bursts and interburst intervals became more prolonged. With the largest phases examined (40 ms), the discharge consisted of a slow alternation of about 30 s high rate bursts separated by equally long intervals. When the discharge was modulated by .2 cps sinusoidal length varia -- tions, with recurrent excitation the peak-to-peak rate (i.e., the receptor's overall sensitivity) and the proportion of the cycle without afferent discharges increased, and the rate vs. length display was distorted even though remaining with a "loop-plus-extension" shape.

These changes were phase dependent: for example, loops could have a sharp high peak at one phase and be flat-topped at another. When a length jitter was superimposed upon either an invariant or a sinusoidal varying length, exaggerating interspike interval variability, recurrent excitation exerted fewer, weaker and somewhat different effects: e.g., it reduced the overall intensity of the invariant cases and shortened the proportion of the cycle with discharges in the modulated ones.

50.11 MEMBRANE POTENTIAL REGULATION OF cAMP: CONTROL MECHANISM FOR SWIMMING BEHAVIOR IN THE CILIATE PARAMECIUM. M. C. Gustin*, N. M. Bonini*, and D. L. Nelson* (Spon: G. Felsten). Dept. of Biochemistry and Neurosciences Training Program, Univ. of Wisconsin, Madison, WI 53706.

The membrane potential of the excitable cell Paramecium tetraurelia controls the frequency and direction of the ciliary beat, thus determining its swimming behavior. Hyperpolarization results in increased forward swimming speed, whereas depolarization, after transiently reversing the direction of the power stroke, slows forward swimming. The reversal in swimming direction is known to be mediated by Ca^{++}, which enters through voltage-sensitive channels of the ciliary membrane. We have found that swimming speed can be controlled by changing intracellular cAMP, and further that the membrane potential itself regulates cAMP levels. Membrane potential was manipulated by varying the external K^+/Ca^{++} ratio and cyclic nucleotides were measured by radioimmunoassay. In a representative experiment, the resting level of cAMP, 95 pmoles/10^6 cells, increased to 126 pmoles upon hyperpolarization and decreased to 78 pmoles upon depolarization. These changes in cAMP were correlated with increased and decreased forward swimming speed, respectively. IBMX, a phosphodiesterase inhibitor, at 1 mM potentiated the changes in cAMP levels. Cyclic AMP in the extracellular medium was found to be low under all conditions of hyperpolarization and depolarization; therefore changes in intracellular levels cannot be explained by altered membrane transport of cAMP. Extracellular cAMP alone had no effect on swimming speed; however, the membrane-permeant derivative monobutyryl cAMP induced a 2-3-fold increase in swimming speed, and was reversible upon dilution of the drug. IBMX could also stimulate cells to swim forward at twice the control level. Although paramecia contain substantial amounts of cGMP (100-200 pmoles/10^6 cells), the levels did not vary significantly upon membrane potential manipulation, even with IBMX present. Permeant derivatives of cGMP were without effect on swimming speed.

Our results suggest that the membrane potential regulates intracellular cAMP in Paramecium. Alteration of cAMP may in part mediate the effect of membrane potential on swimming speed since cAMP itself is capable of increasing swimming speed. Our data also indicate that the regulation of cAMP levels by membrane potential must occur at the level of the adenylate cyclase or phosphodiesterase.

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50.12 PSYCHOPHYSICAL ASSESSMENT OF SENSATION MAGNITUDE ELICITED BY IMPULSE TRAFFIC IN SINGLE RAPIDLY ADAPTING LOW THRESHOLD MECHANORECEPTOR SENSORY AXONS IN AWAKE HUMANS. W. J. Culp*(1), J. Ochoa (1), E. Torebjork*(2), H. Woehlck (1) and J. Baird*(3). (1) Dartmouth Medical School, Hanover, NH; (2) University Hospital, Uppsala, Sweden; (3) Dartmouth College, Hanover, NH.

Work using intraneural microstimulation (INMS) has demonstrated that it is possible to excite single identifiable myelinated sensory axons in awake humans. Afferent impulses in single sensory axons from glabrous skin give rise to specific sensations that are 'pure' in quality and projected by the brain to the cutaneous receptor field innervated by that axon (1,2). Natural cutaneous stimuli adequate for low threshold mechanoreceptors leads unavoidably to multi-unit activation. Here we report results of psychophysical studies assessing information transfer using INMS to drive single axons of identified RA and Pacini units.

Stimulation of single axons of RA type evokes a painless sensation of flutter-vibration. Magnitude estimation experiments in which the subject ranks the perceived frequency of 'vibration' associated with impulse trains of various frequencies demonstrate that RA units behave linearly, giving a Stevens' power function exponent near 1.0. These results confirm related findings from other INMS studies (3). Just noticeable difference (JND) experiments show that the minimum detectable change in impulse frequency is a constant proportion of the modulus frequency. Thus sensation magnitude in single sensory RA 'channels' appears to follow Weber's law. Available information indicates that estimates of sensation magnitude as a function of afferent impulse frequency in single RA sensory axons are as precise as similar frequency estimations using multi-unit afferent signals elicited by vibration stimuli applied to the skin. Thus impulse activity in a single RA sensory afferent provides sufficient information for accurate central decoding of magnitude.

In contrast, similar experiments using INMS to drive single axons of Pacini units demonstrate that impulse frequency in single axons is not a sufficient signal for subjective estimation of vibration frequency. This is so in spite of the fact that the sensation elicited by INMS of a single PC unit is perceived by the subject as being qualitatively identical to that evoked by a single RA. The exponent for the Stevens' power function derived from magnitude estimation in single PC units show values ranging from 0.4 to near zero. JND measurements show that human subjects are nearly unable to correlate sensation magnitude with the frequency of impulse activity in single PC axons.

(1) Torebjork, H. E. and Ochoa, J. L. (1980) Acta Physiol. Scand. 110, 445-447. (2) Ochoa, J. L. and Torebjork, H. E. (1983) J. Physiol. (in press). (3) Torebjork, H. E., et al. (unpublished).

INVERTEBRATE LEARNING AND BEHAVIOR I

51.1 MODIFICATION OF B-PHOTORECEPTOR ADAPTATION PREDICTS DIFFERENTIAL LIGHT RESPONDING FOLLOWING CONDITIONING IN HERMISSENDA. T. Crow, Dept. of Physiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Conditioning produces a number of cellular neurophysiological changes in the B-photoreceptors of Hermissenda (Crow and Alkon, 1980; Crow, 1982). However it is unclear how cellular changes in the B-photoreceptors are related to the actual change in phototactic behavior produced by conditioning since photoresponses are enhanced by conditioning and normal positive phototactic behavior is suppressed following conditioning. In order to further investigate this apparent paradox I have investigated the role of the B-photoreceptors in mediating differential responding to different light intensities following conditioning.

The amount of time during an observation period that Hermissenda remain in a test light is a graded function of light intensity. This measure of phototactic behavior is positively correlated with the intensity of the test light used to measure phototactic behavior ($n=25$, $r=.73$, $p<.01$). Following pre-test measurements at three different light intensities animals received 150 pairings of light and rotation ($n=20$) or 150 random presentations of light and rotation ($n=15$). Twenty-four hours after conditioning both paired and random groups were again tested at the same three light intensities. The paired group ($n=20$) remained in the 'dim' test light significantly longer than their baseline tests ($p<.01$) or the random controls ($p<.01$). However, the paired group remained in the 'bright' test light significantly less that the baseline measures ($p<.01$) or the random controls ($p<.05$). Random controls did not exhibit differential responding to the different light intensities after random presentation of light and rotation.

Following conditioning and post-test measures of phototactic behavior recordings from light adapted B-photoreceptors revealed changes in the photoresponses to light that parallel the behavior results. The conditioned group ($n=12$) exhibited a significant increase in spike activity in response to the 'dim' test light ($p<.05$) and a decrease in activity in response to the 'bright' test light ($p<.05$) as compared to the photoresponses at the same light intensities of the random controls ($n=10$).

The finding that conditioning produces differential responding to different light intensities that is associated with similar changes in the B photoresponse suggests that the conditioned change in phototactic behavior may be explained by cellular changes intrinsic to the B-photoreceptors. Conditioned changes in photoreceptor adaptation (Crow, 1983) may underly differential phototactic suppression observed after conditioning.

51.2 CHANGES IN HERMISSENDA TYPE B PHOTORECEPTORS INVOLVING A VOLTAGE-DEPENDENT CA^{++} CURRENT AND A CA^{++}-DEPENDENT K^+ CURRENT DURING RETENTION OF ASSOCIATIVE LEARNING. J. Farley*, Dept. Psychology, Princeton U., Princeton, NJ 08544 and D.L. Alkon, Sect. on Neural Systems, Lab. of Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543 (SPON: E.F. MacNichol, Jr.).

A persistent, associative-training produced reduction of a fast, voltage-dependent K^+ current (I_A) has previously been reported in Hermissenda Type B photoreceptors as a biophysical correlate of the associative suppression of phototaxis (Alkon et al. Science, 215:693, 1982). Type B cells, in turn, have been shown to be causally related to this simple form of learning (Farley et al., Science, 1983, in press). To assess the possible contribution(s) of other long-lasting conductance changes accompanying associative learning, we minimized, by pharmacologic and electrophysiologic means, the contribution of I_A to training-produced differences in Type B membrane properties.

Peak and steady state light-evoked generator potentials of ligated B cells were significantly greater for associatively-trained ($n=13$) vs. random-control animals ($n=7$) [37.9 ± 2.5 vs. 25.4 ± 1.9 mV (peak), $p<.01$; 28.10 ± 2.2 vs. 19.9 ± 2.1 mV (steady state), $p<.05$], one and two days following training, when 15 mM 4-aminopyridine (4-AP) ion - which under voltage-clamp selectively blocks I_A - was added to the bathing solution. Significantly greater input resistances were also observed for B cells in 4-AP from animals exposed to paired vs. random-control condition [61.4 ± 5.7 vs. 25.22 ± 6.2 MΩ; $p<.05$]. Training produced differences in the long-lasting depolarization (LLD) response of the Type B cell following light offset were abolished, however. Type B cells from paired animals were also significantly more likely to exhibit a transient, regenerative depolarization which depends on Ca^{++} influx (9/13 vs. 1/7 cells, $p<.05$) during its response to light, as well as in the dark in response to applied depolarizing current ($p<.05$). In addition, large depolarizing currents gave rise to a significantly larger after-hyperpolarization [-9.82 vs. -6.9 mV; $p<.05$] which other results indicate arises from activation of a Ca^{++} dependent K^+ current (I_C). Similar results in the absence of 4-AP have been observed when I_A's contribution to the generator potential was minimized by administering a 10 sec, 40 mV depolarizing current step prior to light. The results indicate that changes of other currents, probably involving $I_{Ca^{++}}$ and $I_{Ca^{++}\text{-}K^+}$ (cf. Alkon et al., Soc. Neurosci. Abstr., 1983), in addition to I_A, account for learning-induced changes of the transient and steady-state Type B light response. These results also indicate that learning-induced changes of I_A are largely responsible for the previously observed learning-induced changes of the LLD.

51.3 EFFECTS OF INTERSTIMULUS INTERVAL AND CONTINGENCY ON CLASSICAL CONDITIONING IN APLYSIA. R. D. Hawkins, T. J. Carew and E. R. Kandel. Center for Neurobiology & Behavior, Columbia Univ., and N.Y. State Psychiatric Instit., N. Y., N. Y. 10032.

The siphon withdrawal reflex of Aplysia undergoes differential classical conditioning with cutaneous stimulation of the siphon or mantle shelf as the conditioned stimuli (CS^+ and CS^-) and shock to the tail as the unconditioned stimulus (US) (Carew et al., 1983). This reflex has proven to be a useful system for analyzing the neural mechanisms of conditioning (Hawkins et al., 1983). To test the generality of this system, we have begun to compare the properties of conditioning in Aplysia with those of conditioning in vertebrates. We first examined the effect of the interstimulus interval (ISI) by varying the time between presentation of the CS^+ and the US in different groups of animals ($N \geq 12$ per group). Significant differential conditioning was obtained 15 minutes after five training trials when the onset of the CS^+ preceded onset of the US by 0.5 seconds ($p < .01$), and marginal conditioning was obtained when the ISI was 1.0 second ($p < .05$, one tail). By contrast, no significant learning occurred when the CS^+ preceded the US by 2, 5, or 10 seconds, when the onsets of the stimuli were simultaneous, or when US onset preceded the CS^+ by 0.5, 1.0, or 1.5 seconds (backward conditioning). Thus, conditioning in Aplysia resembles many instances of vertebrate conditioning (e.g., Gormezano, 1972) in having a steep ISI function, with optimal learning occurring when the CS precedes the US by approximately 0.5 seconds.

Conditioning in vertebrates has been shown to depend not only on the temporal relation of the CS and US (contiguity) but also on the degree of correlation or contingency between the stimuli (e.g., Rescorla, 1968). We tested the importance of contingency in Aplysia by giving one group (N=20) five trials of normal differential conditioning with a 5 minute intertrial interval (ITI), and a second group (N=20) the same training but with five additional USs inserted between the paired trials. Presentation of these additional USs reduced the degree to which the US was contingent on the CS, but did not change the number of pairings (the amount of contiguity). When tested 24 hours after training, animals receiving normal training showed significant learning ($p < .05$), whereas animals receiving additional USs did not. The lack of learning in the reduced contingency group was not simply due to the increased temporal density of USs, since another group (N=24) which received five trials of normal training with the same US density (2.5 minute ITI) also showed significant learning ($p < .01$). Thus, the occurrence of USs which are not signaled by the CS reduces conditioning.

These results indicate that conditioning of the withdrawal reflex shows effects of interstimulus interval and contingency similar to those seen in vertebrates. It therefore may be possible to analyze the neural mechanisms of these features of conditioning in Aplysia.

51.4 A TEST OF HEBB'S POSTULATE AT IDENTIFIED SYNAPSES WHICH MEDIATE CLASSICAL CONDITIONING IN APLYSIA. T. J. Carew, T. W. Abrams, R. D. Hawkins, and E. R. Kandel. Center for Neurobiology & Behavior, Depts. Physiol., Neurol., & Psychiat., P&S, Columbia Univ., and N. Y. State Psychiatric Instit., N. Y., N. Y., 10032.

Hebb postulated that classical conditioning is produced by alterations in the strength of synapses when use of those synapses contributes to the generation of action potentials in a postsynaptic neuron. Despite its major impact on theories of learning, Hebb's postulate has not yet been tested directly in a neural circuit known to mediate associative learning. Recently Aplysia has been shown to exhibit differential classical conditioning of a simple withdrawal reflex (Carew et al., 1983), and a specific associative change has been localized to an identified set of synapses between sensory and motor cells for the reflex (Hawkins et al., 1983). Thus, this system has provided an opportunity to test the Hebb postulate directly.

We first asked whether postsynaptic activity is sufficient to produce associative changes. We used a differential training procedure in which action potentials in one sensory neuron (SN) are paired with an unconditioned stimulus (US) and action potentials from another SN are specifically unpaired (Hawkins et al., 1983). The experiment had two phases: in Phase I we paired activity in one SN with action potentials in the postsynaptic motor neuron (produced by injected current), and produced unpaired activity in another SN (5 trials, 5 min. iti). This was followed by a test to assess EPSP amplitude from each SN. In Phase II, activity in both SNs was produced either paired or unpaired with tail nerve stimulation, followed by a second test. After pairing with postsynaptic spikes (Phase I; N=10) EPSPs from both SNs showed synaptic depression ($\bar{x}$ EPSP amplitude = 50% of pretraining control). By contrast, after pairing with tail-nerve stimulation (Phase II; N=8), there was clear differential facilitation ($\bar{x}$ paired EPSP = 151% control, unpaired = 47% control, $p < .01$). Thus, at the same synapses where postsynaptic activity was not effective as a US, tail-nerve stimulation was effective.

We next asked whether postsynaptic activity is necessary for associative changes to occur. In 10 experiments the postsynaptic cell was held hyperpolarized during training so that it did not fire action potentials to the US (tail-nerve stimulation). Despite the lack of postsynaptic activity, differential facilitation still occurred ($\bar{x}$ paired EPSP = 175% control, unpaired = 86% control, $p < .005$).

Our results show that postsynaptic activity is neither sufficient nor necessary to produce associative changes at synapses in Aplysia. However, this does not imply that a Hebb-type mechanism might not contribute to other instances of learning. It will be of interest to assess the relative importance of presynaptic activity and postsynaptic factors in different forms of learning in the several vertebrate and invertebrate systems where a cellular analysis is now possible.

51.5 ASPECTS OF THE CELLULAR MECHANISM OF TEMPORAL SPECIFICITY IN CONDITIONING IN APLYSIA: PRELIMINARY EVIDENCE FOR Ca^{2+} INFLUX AS A SIGNAL OF ACTIVITY. T. W. Abrams, T. J. Carew, R. D. Hawkins and E. R. Kandel. Center for Neurobiology and Behavior, Columbia Univ., College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

In conditioning of the siphon withdrawal reflex of Aplysia, the unconditioned stimulus (US), a tail shock, produces presynaptic facilitation of the synaptic connections from the siphon sensory neurons (SNs) in the conditioned stimulus (CS) pathway. The facilitation is amplified if a SN has fired action potentials immediately prior to receiving facilitatory input from the US, as occurs during conditioning when the CS precedes the US. This activity-dependent amplification of presynaptic facilitation provides a mechanism for temporal specificity in conditioning of the reflex (Hawkins et al., 1983).

To investigate this activity dependence, we bypassed the circuit carrying the facilitatory input from the tail by substituting for the tail shock brief ($\sim$1 sec) puffs of serotonin (5HT) onto somata of siphon SNs. We first tested whether preceding spike activity enhances the response of SNs to 5HT. We used an A-B-A protocol in which single cells alternately received either A) 5HT puffs without paired activity or B) paired 5HT puffs delivered immediately following a 0.5 sec train of intracellularly stimulated spikes at 10 Hz. Spike durations were tested before and after training, and spike broadening was used as an index of the 5HT response. In 10 experiments, SNs showed more spike broadening after paired 5HT puffs than after 5HT puffs in the absence of paired activity ($\bar{x}$ spike duration = 117% vs. 107% of pretraining control, $p < .001$).

We next investigated the possibility that the brief elevation of intracellular Ca^{2+} produced by activity in SNs transiently increases their response to 5HT and thereby serves as a signal for temporal specificity. To test this hypothesis we eliminated Ca^{2+} influx by reducing external Ca^{2+}, without directly altering the Na^+ or K^+ fluxes or the voltage changes that occur during the spike. In 5 experiments, we paired 5HT puffs with 0.5 sec spike trains in SNs in both normal SW (10^{-2} M Ca^{2+}) and in Ca^{2+}-free SW (10^{-7} M Ca^{2+}), using a protocol similar to that described above. All pre- and post-tests were conducted in normal SW. After pairing in normal SW containing Ca^{2+}, cells exhibited significantly more spike broadening than they did after pairing in Ca^{2+}-free SW ($\bar{x}$ spike duration = 118% vs. 94% of pretraining control, $p < .002$). These preliminary results suggest that Ca^{2+} influx in SNs of the CS pathway may serve as a temporally discrete intracellular signal indicating that these cells have recently been active. We speculate that Ca^{2+} influx may provide temporal specificity by transiently modulating the cyclase system through which 5HT exerts its action.

51.6 LONG-TERM POTENTIATION, ACTIVITY-DEPENDENT NEUROMODULATION, AND ASSOCIATIVE INFORMATION STORAGE IN APLYSIA. E.T. Walters and J.H. Byrne. Dept. of Physiol. and Cell Biol., Univ. Texas Med. Sch., Houston, TX. 77025.

Long-term potentiation (LTP) has attracted considerable attention as a possible neural substrate for learning and memory, but neither its mechanisms nor functions have been analyzed conclusively. Stimulation procedures commonly used to produce LTP may cause concurrent homosynaptic activation and heterosynaptic modulation of the test synapses. Therefore activity-dependent neuromodulation (amplification of heterosynaptic modulation by paired spike activity in modulated cells) may be a mechanism underlying LTP. This possibility is also interesting because activity-dependent neuromodulation can underlie associative conditioning (Walters and Byrne, 1983; Hawkins et al, 1983).

To test this hypothesis we examined the interaction of homo- and heterosynaptic facilitation using a training procedure in Aplysia similar to that used to produce LTP in vertebrate preparations. Brief, high frequency stimulation of individual peripheral nerves produced LTP of connections from the stimulated nerve but only transient facilitation of connections from adjacent nerves. To separate the homo- and heterosynaptic effects we examined monosynaptic connections from tail sensory neurons to motor neurons and selected a peripheral nerve that did not activate the sensory neurons. Two or more sensory neurons were stimulated artificially to produce single test EPSPs every 10 min. After 2 baseline EPSPs the peripheral nerve was stimulated with ten 20 Hz trains delivered at 5 sec intervals for 45 sec (100 pulses total). LTP was only produced when this heterosynaptic stimulation was paired with artificial intracellular activation of 100 spikes in the sensory neuron. These coactivated neurons (N=8) showed significantly more facilitation after 2 hours than sensory neurons exposed to nerve shock alone (N=12, $p<.01$), intracellular activation alone (N=6, $p<.025$), or test stimuli alone (N=11, $p<.005$). While neither nerve shock nor intracellular activation alone produced synaptic facilitation 2 hours after training (LTP), both nonassociative forms of training produced weak facilitation that lasted at least 30 min.

The cellular properties of LTP in this preparation resemble the associative changes produced by pairing sensory neuron activation with tail shock. Both processes may involve the Ca^{++} dependence of serotonin evoked cAMP synthesis (Walters and Byrne, 1983; Hawkins et al, 1983; Ocorr et al, this volume). The simple training procedure described here should facilitate further subcellular analysis of long-term neuronal modifiability. In addition, these results suggest that LTP may be a manifestation of a rather general form of associative information storage - activity-dependent neuromodulation.

51.7 PRESYNAPTIC MECHANISM OF LONG TERM FACILITATION AT THE CRAYFISH NEUROMUSCULAR JUNCTION INVOLVES SODIUM IONS. J.M. Wojtowicz and H.L. Atwood, Department of Physiology, University of Toronto, Toronto, Ontario, Canada. M5S 1A8

Repetitive stimulation of the excitatory axon innervating the opener muscle of the crayfish claw delivered at 10-20 Hz, for 15-30 minutes results in a gradual increase in amplitude of the excitatory potentials (EPSPs). This enhancement can persist for minutes and sometimes hours after the stimulation is discontinued. The mechanism of such long term facilitation (LTF) was studied by simultaneous presynaptic and postsynaptic intracellular recording. The presynaptic axon was penetrated near the terminal branches, so that the amplitude of the action potentials (AP), the membrane potential (E_M) and the input resistance in the terminal arborization could be measured. During LTF, the amplitude of the AP was reduced by 5-10mV while its width was increased. E_M became more negative by about 5 mV without a detectable change in the input resistance. The hyperpolarization was blocked by ouabain (0.5-1.0 mM) and is thought therefore to be caused by an electrogenic sodium pump. Ouabain also reduced the AP amplitude and enhanced the EPSP.

Together the reduction of the amplitude of the AP and the activation of the sodium pump suggest an accumulation of sodium ions in the terminal axonal branches during the LTF. The time course of the recovery of the AP was followed by the recovery of the LTF.

A causal role for sodium in LTF was confirmed by intra-axonal injections of this ion via the recording micropipettes. Such injections resulted in reductions of the APs and a prolonged enhancement of the EPSPs.

Supported by an operating grant from the Medical Research Council of Canada.

51.8 ASSOCIATIVE CONDITIONING ANALOG IN APLYSIA TAIL SENSORY NEURONS SELECTIVELY INCREASES cAMP CONTENT. K.A. Ocorr, E.T. Walters, and J.H. Byrne, Dept. of Physiol. and Cell Biol. Univ. of Texas Medical School, Houston, Texas 77025.

Sensory neurons involved in the tail withdrawal reflex of Aplysia are located in symmetrical clusters in each pleural ganglion. These cells exhibit heterosynaptic facilitation in response to noxious tail stimulation which is mimicked by bath application of serotonin (5-HT) (Walters et al, 1983). Application of a classical conditioning paradigm to individual sensory neurons produces a temporally specific amplification of the heterosynaptic facilitation when tail stimulation (the US) is paired with intracellular depolarization (the CS) (Walters and Byrne, 1983 and this volume). The present study was designed to examine the role of cAMP in the associative conditioning of these neurons.

We first examined whether brief (60 sec) exposure to 5-HT could increase levels of cAMP in surgically isolated clusters of sensory neuron somata, as well as in the remnants of the pleural ganglia and in the abdominal ganglia. In all cases exposure to 5-HT resulted in an elevation of the cAMP levels compared to controls (see also Cedar and Schwartz, 1972; Pollock et al, 1982). The cAMP levels (in the presence of 10^{-4}M RO 20-1724) were determined by radioimmunoassay and normalized to protein content.

The differential conditioning paradigm of Walters & Byrne was mimicked in vitro by a 5 sec application of high K^+ ASW (CS) followed by a 15 sec exposure to 5×10^{-6}M 5-HT (US). In the paired treatment (CS+) exposure to high K^+ ASW immediately preceded 5-HT exposure; in the unpaired treatment (CS-) 5-HT exposure was delayed for 2.5 min. Each CS+ treatment had a CS- control in the contralateral isolated cluster from the same animal. In all cases 10^{-4} M RO 20-1724 was included in the bath. After the application of 5-HT the samples were immediately shelf frozen. Paired application (CS+) of high K^+ ASW and 5-HT resulted in a significant elevation of cAMP levels in the sensory neuron clusters compared to the unpaired application (CS-) ($p<.025$, N=15, double blind). In contrast a similar pairing procedure produced no significant differences in cAMP levels of the abdominal ganglia (N=15) or the pleural ganglia remnants (N=14).

These results indicate that the activity-dependent neuromodulation underlying associative conditioning of the tail sensory neurons produces pairing specific increases in cAMP levels and supports the possibility that such increases may be a biochemical substrate for associative learning. The temporal constraints on these associative effects and the possible involvement of Ca^{++} in amplifying the cAMP response are currently under investigation.

51.9 PERSISTENT ACTIVATION OF ADENYLATE CYCLASE UNDERLIES THE TIME COURSE OF SHORT-TERM SENSITIZATION IN APLYSIA. Vincent F. Castellucci, Lise Bernier*, James H. Schwartz and Eric R. Kandel. Center for Neurobiology and Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

Sensitization of the gill and siphon withdrawal reflex results from presynaptic facilitation at the excitatory synapses made by sensory neurons on gill and siphon neurons. The facilitation is accompanied by a depression of a K^+ current in sensory neurons which produces an increased influx of Ca^{++} and an increased release of transmitter. Serotonin simulates the natural presynaptic facilitation and produces depression of the K^+ current by means of a cAMP-dependent protein phosphorylation. By using a specific protein inhibitor of the cAMP-dependent protein kinase isolated from muscle, we had previously found that the time course of short-term sensitization does not reside in a persistent phosphorylation of the substrate protein(s), but requires a kinase kept active by the persistent elevation of cAMP. Furthermore, this elevation of cAMP in sensory neurons was found to be mostly free in the cytosol, not bound to protein and thereby unprotected from degradation by phosphodiesterases.

We next inquired: What keeps the level of cAMP elevated? Is it the persistent activity of the adenylate cyclase? To explore this question, first we triggered the memory for short-term sensitization (broadening the action potential with serotonin, 10^{-6} or 10^{-4}M), and then we pressure-injected sensory neuron somata with GDPβS (10^{-6}M), an analog of GDP which is an inhibitor of the cyclase. We found that GDPβS rapidly shortened the duration of the action potential (N=16), indicating that the memory requires a persistent activation of adenylate cyclase. The action potential could again be broadened by intracellular injection of cAMP. Forskolin applied at a concentration of 10^{-4}M (N=5) partially reverses the inhibition by GDPβS.

These results and our earlier data indicate that there are two separate molecular steps for the readout and the time course of memory. The memory is read out (or retrieved) when the substrate protein is in the phosphorylated state. This readout is determined by an active kinase. By contrast, the duration of the memory is determined by a sustained elevation of intracellular cAMP which is caused by the persistent activity of an adenylate cyclase.

51.10 ASSOCIATIVE LEARNING IN AN IN VITRO APLYSIA PREPARATION: FACILITATION AT A SENSORY MOTOR NEURON SYNAPSE. Ken Lukowiak* (SPON: G. Mptisos). Dept. of Medical Physiology, Faculty of Medicine, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

The in vitro siphon, mantle, gill and abdominal ganglion preparation of Aplysia can be classically conditioned using as a CS a weak tactile stimulus to the siphon and a stronger tactile stimulus to the gill as a UCS (Lukowiak, 1982). With classical conditioning training, the CS comes to elicit a gill withdrawal reflex (GWR) which was initially evoked by only the UCS. Presentation of the CS alone; the presentation of the UCS at some random internal following the UCS; and backward conditioning all do not lead to the CS evoking a GWR.

Intracellular recordings were made from central gill motor neurons L_7, LDG_1, and L_9 during the course of training. Initially, the CS evoked only a few AP's in these neurons; however, as training progressed the CS came to evoke a larger neuronal response. In control group preparations the CS evoked fewer AP's in subsequent trials.

An analysis was therefore made of the monosynaptic sensory motor neuron synapse to determine if the efficacy of this synapse was altered by the conditioning. Because of the nature of the UCS and the GWR it evokes, it was not possible to record simultaneously from an LE sensory neuron and a follower motor neuron during the course of the training. Synaptic transmission was initially evaluated by measuring the EPSPs evoked by a single LE AP separated by 20 min; there was no change in EPSP amplitude. The amplitude of the EPSP ranged between 0.8-2.2 mv in the 30 preparations tested. The electrodes were then withdrawn from the cells which were visually recognizable by their position (etc.). The preparation was then given classical conditioning training (n=10), the CS alone (n=10), or the random control procedure (n=10). Following the presentation of 40 trials (ITI 2 min) the LE sensory neuron and the follower motor neuron were reimpaled and synaptic transmission re-evaluated. It was found that the EPSP amplitude increase 2-3 fold in the classical conditioning group; sometimes even evoking APs in the follower cell. In the control groups EPSP amplitude decreased by at least 50%. Further in 2 of the 10 preparations which received classical conditioning training, there was no evidence of conditioning. In these 2 preparations EPSP amplitude also decreased. Pre or post-synaptic loci which could account for the change in synaptic efficacy have not yet been examined. Thus, it appears that classical conditioning training leads to an increase in the synaptic efficacy at the sensory-motor neuron synapse. This could be a neural mechanism which underlies associative learning.

Supported by MRC (Canada).

51.11 MEMORY OF OLFACTORY CONDITIONING MUTANTS OF DROSOPHILA FOLLOWING PROLONGED TRAINING RESEMBLES THAT OF NORMAL FLIES AFTER BRIEF TRAINING. Yadin Dudai, Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Several X-linked mutants of Drosophila,that fail in an olfactory conditioned avoidance paradigm, have been shown to be capable of displaying a very brief memory of odorant avoidance responses (Dudai, J. Comp. Physiol. 130, 271 (1979); Neurosci. Abstr. 7, 643 (1981)). This implies that the mutations do not abolish acquisition but interfere with memory storage or expression. We have set out to test whether alterations in the training schedule of the olfactory avoidance conditioning paradigm can improve the memory of two of the mutants, $dunce^1$ (Dudai et al., PNAS 73, 1684 (1976)) and rutabaga (Duerr and Quinn, PNAS 79, 3646 (1982)). In the paradigm employed, the flies are presented with an odorant associated with electric shock (or with intense vibration) and are later tested for their choice between the shock-associated and a control odorant in a T-maze (the experimental design controls for odor-bias and non-specific sensitization). Each 30 sec presentation with a shock-odorant comprises a training session. A short-lived acquired selective avoidance of odorants is detected in the mutants already after a single training session. We have previously demonstrated that for both mutants, following 3 training cycles, selective avoidance of the odorant presented during training is 60-70% of normal when tested 30 sec after training, but only 2-8% of normal when tested 7 min after training. We have now tested several training schedules and found that increasing the number of training sessions to 6 (presented successively, with 30 sec rest periods between sessions, and with no presentation of non-shock odorant in between), markedly improves memory ($P<0.001$, to about 40% of normal after 7 min). However, intensifying the training by various procedures does not improve the performance of the mutants immediately after training (maximum obtained was 70% of normal at 30 sec). Moreover, the immediate memory decay of the mutants following 6 training cycles resembles that of normal flies following a single training session in the same paradigm. Thus, by differentially varying the intensity of the single-odorant training paradigm, one can equate the performance of normal and mutants when tested several minutes after training. The results suggest that the machinery required to store and retrieve the selective avoidance memory for periods longer than tens of seconds does exist in the mutants, but the threshold for its activation is higher than normal. (Supported by a grant from the US-Israel Binational Science Foundation, Jerusalem.)

PEPTIDES: RECEPTORS I

52.1 RECEPTORS FOR NEUROPEPTIDE Y IN RAT BRAIN A. Unden*, K. Tatemoto*+and T. Bartfai*(SPON:B. Hedlund)
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Neuropeptide Y, NPY, recently isolated, 36 amino acids residue long peptide with tyrosine amide C terminal from porcine brain containing 5 tyrosine (Tatemoto et.al., 1982; Nature 296, 659) was labeled with ^{125}I by the chloramine-T method. The iodinated peptide (specific activity 1000 Ci/mmole) was found to bind in a saturable and reversible manner to membranes from various regions of the rat brain. The binding sites were sensitive to treatment of the membranes with proteolytic enzymes or to heat denaturation. Unlabeled NPY competed for the binding sites with an IC_{50} value of 0.58 nM. Secretin, VIP, Substance P and PHI at 50 nM concentrations did not inhibit binding while a related peptide, pancreatic peptide, gave 25 % inhibition at the same concentration. A sharp pH optimum for the binding was detected between pH 7 and pH 7.7. The apparent B_{max} of ^{125}I-NPY was dependent on the concentration of $Mg^{2+}$and Ca^{2+} (in the range of 0-10 mM). The highest concentrations of binding sites were found in the cerebral cortex (0.37 pmoles/mg protein), hypothalamus and hippicampus, while the cerebellum had the lowest concentration with 28 % of that of cerebral cortex. Specific binding of ^{125}I-NPY was present in all subcellular fractions except the myelin fraction.

Immunoreactive substances related to these peptides were found in noradrenergic neurons (Hökfelt et.al., 1983; Acta Phys. Scand. 117, 315-318). In view of this it is noteworthy preincubation of membranes with β-agonists,at 37°C for 30 minutes, did prevent the decline in the binding of ^{125}I-NPY normally associated with preincubations under these conditions.

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52.2 DESENSITIZATION OF SOMATOSTATIN RECEPTORS NEGATIVELY COUPLED TO ADENYLATE CYCLASE. T. Reisine. Sec. on Pharmacol., Lab. Clinical Sci., NIMH, Bethesda, MD 20205.

Somatostatin (SRIF) is a 14-amino acid containing peptide known to inhibit hormone release from the anterior pituitary. Addition of SRIF to tumor cells of the mouse anterior pituitary (AtT-20/D16-16) blocks corticotropin releasing factor (CRF)-stimulated adrenocorticotropin hormone (ACTH) secretion. In addition, SRIF prevents CRF and forskolin from stimulating cyclic AMP accumulation in these cells suggesting that SRIF receptors are negatively coupled to adenylate cyclase. Pretreatment of AtT-20 cells with SRIF induces two compensatory changes: a reduced ability of SRIF to antagonize stimulated cyclic AMP formation and ACTH secretion and an increase in forskolin-stimulated cyclic AMP synthesis and adenylate cyclase activity. SRIF desensitization is time and dose-dependent, reversible and induced by SRIF analogues. The cyclic AMP analogue 8-bromo-cyclic AMP stimulates ACTH release through a mechanism bypassing adenylate cyclase and it's effects are blocked by SRIF. However, SRIF pretreatment does not alter SRIF's inhibition of 8-bromo-cyclic AMP's effects suggesting that the SRIF desensitization either involves an uncoupling of SRIF receptors from adenylate cyclase or a loss of those receptors. The enhanced cyclic AMP response to forskolin is also time and dose-dependent. Hormone-stimulated cyclic AMP accumulation increases following SRIF pretreatment although longer periods are required than for the increase in forskolin's effects. SRIF pretreatment increases NaF and $Gpp(NH)_p$-stimulated adenylate cyclase activity but does not affect basal adenylate cyclase activity, cyclic AMP synthesis, phosphodiesterase activity or cyclic GMP levels. The increased forskolin response is blocked by cycloheximide suggesting that SRIF treatment either induces new adenylate cyclase molecules or proteins necessary for adenylate cyclase activation.

52.3 IN VITRO BIOCHEMICAL CHARACTERIZATION AND AUTORADIOGRAPHIC DISTRIBUTION OF ^{3}H-THYROTROPIN RELEASING HORMONE (TRH) RECEPTORS IN RAT BRAIN SECTIONS. W.H. Rostene, J.L. Morgat, M. Dussaillant, T.C. Rainbow and A. Sarrieau. INSERM U.55, Hopital St Antoine, 75012 Paris, France.

TRH, originally isolated from the hypothalamus, has been identified in other regions of the CNS where it exerts behavioral and physiological effects unrelated to its neuroendocrine role on the pituitary-thyroid axis. TRH receptors which may mediate these effects have only been characterized with membrane preparations. In the present study, we describe the biochemical characteristics and the autoradiographic distribution of TRH receptors in the rat CNS after incubation of brain slices with ^{3}H-TRH (55 Ci/mmole , CEN Saclay, France) in 50 mM Tris-HCl buffer (pH 7.6) containing 5 mM $MgCl_2$, 0.2% bovine serum albumin and 20 uM bacitracin at 4°C. After incubation, sections were washed in Tris-HCl buffer at 4°C. The specific to non-specific ratio (obtained with 100-fold higher concentrations of unlabelled TRH) was 3:1. Under these conditions, the equilibrium of ^{3}H-TRH specific binding was reached by 90 min of incubation. Increasing concentrations of unlabelled TRH produced a dose-dependent inhibition of ^{3}H-TRH binding with a maximum obtained with 0.5 uM unlabelled TRH, which represents 70% of the total binding. Scatchard analysis showed that, in the range of concentrations tested (0.7-35 nM), ^{3}H-TRH bound to a single class of receptors with an apparent dissociation constant of 2 nM and the number of binding sites was estimated to be 20 fmoles/mg protein. The only analogue as potent as TRH to displace ^{3}H-TRH binding was 3-ME-TRH, whereas I-ME-TRH and TRH free-acid were ineffective. Neither LHRH, neurotensin, somatostatin, D-Ala-Met enkephalin nor VIP showed any significant affinity for TRH binding sites. Autoradiograms obtained by apposition of LKB ^{3}H-Ultrofilm showed that the highest concentrations of ^{3}H-TRH binding sites were found in the amygdala (medial, cortical and basolateral nuclei), the external layer of the cingulate cortex, the paraventricular nucleus of the thalamus and in the hippocampal formation in both dorsal and ventral subiculum and dentate gyrus. The biochemical characterization of ^{3}H-TRH binding in brain sections is in good agreement with previous reports on membrane preparations and the autoradiographic localization of the binding sites provides anatomical support for the effects of TRH in the CNS.

52.4 INHIBITION OF TRH RECEPTOR BINDING BY BENZODIAZEPINES: PHARMACOLOGY AND DISTRIBUTION. N. A. Sharif* and D. R. Burt. Department of Pharmacolology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Maryland 21201

Biochemical, pharmacological and behavioral studies suggest a neurotransmitter role for the tripeptide, thyrotropin-releasing hormone (TRH), in the vertebrate CNS and pituitary. TRH antagonizes many effects of barbiturates and benzodiazepines (BZ), but like chlordiazepoxide (CDE), it attenuates conflict behavior (J.P.E.T., 193, 11; 212, 153). These and other observations led us to study the possible interactions between TRH and a variety of psychoactive drugs using radioreceptor binding techniques. Washed resuspensions (50 mg ww/ml in 50 µl) of rat nervous tissues were incubated with 1 nM [^{3}H](3-Me-His^2)TRH ([^{3}H]MeTRH) at 0°C for 5 h and filtered under vacuum. Specific binding was 70-80% of total with 10 µM TRH as blank. Although 47 neuroactive drugs (antidepressants, amino acids, Ca^{2+}-antagonists, nucleotides) were inactive against [^{3}H]MeTRH binding (IC_{50} > 1 mM), numerous BZs were potent competitive inhibitors, though less active than TRH and MeTRH. The profile of activity (CDE > diazepam (DZM) > flurazepam > lurazepam > flunitrezepam (Flu)) was almost identical for pituitary (Pit), retina (Ret) and amygdala (Amyg); corresponding IC_{50} values for Amyg were (µM): 0.5 ± 0.05, 4 ± 0.5, 24 ± 3, 108 ± 21, 420 ± 64 respectively (n=3-5). However, apparent reduced potency, but the same rank order, was seen in spinal cord, hippocampus and hypothalamus. Scatchard analyses of competition experiments in all 6 regions, run in the presence of IC_{50} concentrations of CDE, revealed it to selectively reduce TRH receptor affinity (≃ 3-fold, $P < 0.02$) without affecting receptor number. These results supported the competitive mechanism of action of BZs suggested previously by inhibition curves parallel to those of TRH and MeTRH. In kinetic experiments, CDE (100 µM) induced a rapid biphasic dissociation of [^{3}H]MeTRH binding from amygdala membranes ($t_{1/2}$ of fast component = 2.5 min). In contrast, TRH (10^{-9}-10^{-5} M) did not compete with either [^{3}H]DZM or [^{3}H]Flu binding to forebrain membranes. However, since high- and low-affinity BZ binding sites exceed those for TRH (16-6500 times), it may not be possible to detect displacement of [^{3}H]BZs by TRH even though the BZs appear to interact with TRH receptors. Our results may be of importance since clinical doses of BZs produce plasma/brain levels > 50 µM and because TRH and BZs interact to influence physiology and behavior. These studies were supported in part by NSF grant BNS 8025469, USPHS grant MH-29671 and U.S. Army Research and Development Command contract DAMD-17-81-C1279.

52.5 **USE OF A SELECTIVE SUPERAGONIST OF CENTRAL SP_2 RECEPTORS TO STIMULATE CENTRAL SITES FOLLOWING PERIPHERAL INJECTIONS.** M.F.Piercey, M.W.Moon*, F.J.Einspahr* and J.R.Blinn*, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

When directly injected into the intrathecal spaces of the mouse spinal cord, very low doses of substance P (SP) elicit a very intense two-sided biting and scratching syndrome, presumably through stimulation of SP_2 receptors (Reg. Peptides 3:337, 1982) located in nociceptive pathways of the dorsal horn (Science 214:1061, 1981). However, when SP is injected intravenously, even high doses fail to elicit this dramatic behavioral syndrome. Thus, systemically-administered SP does not reach centrally located SP_2 receptors either because it is rapidly degraded or it lacks the ability to penetrate the blood-brain barrier. We have now found that $pGlu^6Pro^9SP_{6-11}$ can stimulate central SP_2 receptors following peripheral administration. When directly injected into the cord, $pGlu^6Pro^9SP_{6-11}$ was 15 times more potent than SP in eliciting scratching and biting. In contrast, $pGlu^6Pro^9SP_{6-11}$ only very weakly stimulated SP_1 receptors of the guinea pig ileum and rat colon. To confirm the SP_2 superagonist properties for $pGlu^6Pro^9SP_{6-11}$, the compound was microiontophoresed onto single neurons of the nucleus caudalis of the rat trigeminal complex. Multibarrelled microelectrodes were used with at least one barrel containing $pGlu^6Pro^9SP_{6-11}$ and at least one containing SP. Trigeminal location was verified by histological identification of dye spots ejected at the end of the experiment. Every cell excited by SP was also excited by $pGlu^6Pro^9SP_{6-11}$. For the same ejection currents, $pGlu^6Pro^9SP_{6-11}$ increased cell firing rates more than did SP and the response to $pGlu^6Pro^9SP_{6-11}$ was also much more prolonged. In mice, intravenously injected $pGlu^6Pro^9SP_{6-11}$ elicited a scratching and biting syndrome identical in appearance to that observed with intrathecal injections of SP and $pGlu^6Pro^9SP_{6-11}$. For $pGlu^6Pro^9SP_{6-11}$, the ED_{50} for scratching was 2 ng i.t. and 30 mg/kg i.v.

52.6 IN VITRO CHARACTERIZATION AND AUTORADIOGRAPHIC LOCALIZATION OF SUBSTANCE P RECEPTORS IN RAT BRAIN. C. W. Shults,* R. Quiron, T. L. O'Donohue and T. N. Chase (SPON: S. Rapisardi). Laboratory of Experimental Therapeutics Branch, National Institutes of Health, Bethesda, MD 20205.

A method has been developed for in vitro binding of both ^{125}I-substance P (SP) (Peptides 3:1073-1075, 1982) and ^{3}H SP (Nature, in press) to brain slices. Using this technique we have studied the pharmacology of the binding site in rat brain slices which contained primarily striatum and have mapped the SP binding sites in the rat brain. Saturation curves and Scatchard analysis indicate that ^{3}H SP binds to an apparently saturable single class of sites with a dissociation constant (Kd) of 0.52 nM_3 and a number of sites (B_5max) of 21.6 fmol/mg protein. The IC_{50}'s for ^{3}H SP (2.2×10^{-9}) and ^{125}I SP (1.3×10^{-9}) were similar. The relative potencies of various SP fragments and analogs for displacement of ^{3}H SP of ^{125}I SP were also similar.

	^{3}H SP	^{125}I SP
SP	100	100
physalaemin	16	14
nona SD	3.1	7.6
eledoisin	0.8	2.6
hepta SP	0.3	1.9
hexa SP		.8
$(D\text{-}Pro^2,DTrp^{7,9})SP$	0.01	.03
SP COOH	0.01	.01
penta SP	0.02	.001

The amide group on the C terminal appears to be critical for binding as is shown by the weak displacement by SP-COOH. Also C-terminal fragments of fewer than six amino acids have relatively little affinity for the receptor.

Areas of highest binding are the olfactory bulb, olfactory tubercle, pyriform cortex, suprachiasmatic n., certain amygdaloid n., hippocampus, dentate gyrus, superior colliculus, locus coeruleus, dorsal para brachial n., n. of the tractis solitarius, n. of X, n. of XII, inferior olive, n. ambiguus.

Moderate density of binding was seen in the cingulate cortex, n. accumbens, striatum, globus pallidus, medial septal n., periventricular n. of thalamus, dorsomedial hypothalamic n., periaqueductal gray, dorsal raphe n., interpedunuclear n., cuneiform n., deep cerebellar vermis, and lamina I and II of the dorsal horn of the spinal cord. Surprisingly little binding could be demonstrated in the substantia nigra pars reticulata, an area of high SP concentration.

Although in many areas there is good correlation between SP concentration and amount of binding, in other areas there is disparity between SP concentration and amount of binding. These disparities may suggest new aspects of modulation of synaptic transmission or new functions of neuropeptides.

52.7 AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ RECEPTORS IN THE PYLORIC SPHINCTER OF THE RAT. Gregory T. Smith*, T. H. Moran*, J. T. Coyle, M. J. Kuhar, T. L. O'Donohue and P. R. McHugh. Departments of Psychiatry and Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, NINCDS, NIH, Bethesda, MD 20205.

Systemic infusions of cholecystokinin (CCK) in physiological dosages produce an inhibition of gastric emptying and result in behaviors associated with satiety. The effect of gastric inhibition has been proposed as the mechanism for CCK-induced satiety. CCK receptors have been previously shown to be transported distally along the vagus nerve and selective gastric vagotomy has been shown to abolish the satiety effects of systemic CCK infusions. In an effort to elucidate target sites within the gastrointestinal (GI) tract through which CCK may directly exert its effects, the distribution of CCK receptors was mapped in the stomach and small bowel by in vitro autoradiography.

Receptor sites for CCK were labelled with ^{125}I-Bolton Hunter CCK$_{33}$ (^{125}I-CCK$_{33}$). Binding was carried out on 25 μ slide-mounted serial cross-sections sampling the esophagus, cardia, fundus, corpus, antrum, pyloric canal, pyloric sphincter, proximal duodenum, and terminal ileum. Binding conditions for GI sections were the same as those demonstrated to provide saturable, reversible, and high affinity binding (K_d = 0.45nM) in brain sections with specific binding of 80-90%. Tissue sections were 1) preincubated in 50 mM Tris HCl (pH 7.4) containing 0.5% bovine serum albumin (BSA) for 20 min at 24°C; 2) incubated in 50 mM Tris HCl (pH 6.5) containing 0.5% BSA, 0.025% Bacitracin, 130 mM NaCl, 4.7 mM KCl, 5mM $MgCl_2$, 1mM EGTA and 400 pM ^{125}I-CCK$_{33}$ for 2 hours at 24°C and 3) washed in 50mM Tris HCl (pH 7.4) for 90 min at 4°C. Autoradiographs were then obtained on LKB-Ultrafilm from dried sections.

Specific CCK receptor binding was localized to the circular smooth muscle layer of the pyloric sphincter. Receptors were concentrated in discrete circumferential banding patterns in the distal most portion of the pyloric sphincter. Negligible specific binding was observed in oblique, circular or longitudinal muscle layers at the other GI levels sampled. Moderate non-specific binding confined to the mucosa was observed in all gastric sections. The restriction of CCK receptors to the circular muscle layer of the pyloric sphincter suggests this location as the site through which CCK inhibits gastric emptying. The gastric distention secondary to this inhibition of gastric emptying may be the mechanism for CCK-induced satiety.
(Supported by NIH grant 2-R01-AM19302).

52.8 BRADYKININ RECEPTOR BINDING AND BIOLOGICAL ACTIVITY IN MURINE NEUROBLASTOMA CLONE N1E-115 CELLS. R. Michael Snider and Elliott Richelson. Departments of Psychiatry and Pharmacology, Mayo Clinic and Foundation, Rochester, MN 55905.

Injection of the nonapeptide bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg) into specific regions of the brain elicits pronounced systemic effects. Specifically, microinjection of bradykinin into the lateral septum (or lateral ventricles), periaqueductal gray, and medial or lateral hypothalmus elicits hypertension, analgesia and hyperthermia, respectively. Moreover, bradykinin-like immunoreactivity has been localized in these same areas in the rat brain suggesting a possible neurotransmitter role for this polypeptide. (Correa et al. P.N.A.S. 76:1489, 1979). In spite of these findings, very little concerning the specific biological effects of bradykinin on nerve cells has as yet been reported.

Incubation of bradykinin with intact neuroblastoma cells caused a concentration-dependent, Ca^{++}-dependent stimulation of cyclic GMP synthesis. The response peaked at 45 sec - 1 min and returned to basal levels by 5 min. The EC50 for this response averaged (+ S.E.M.) 1.1 + 0.2 nM (range 0.63 - 1.39 nM) and the cyclic GMP levels increased by 5.2 - 13.5 fold over basal levels.

The binding of [^{3}H]bradykinin to intact neuroblastoma cells was carried out at 25°C in phosphate-buffered saline solution containing 140 µg/ml bacitracin, 1 mM 1,10-phenanthroline and 0.1% bovine serum albumin (crystalized, lyophilized). Free [^{3}H]bradykinin was separated from bound by filtration over Whatman GF/B filters pretreated with 0.1% aqueous polyethyleneimine which was determined to decrease binding of bradykinin to filters. Saturation experiments and Scatchard analysis by computer fit of the data revealed three classes of binding sites on intact cells with K_D of 0.83 pM, 1.0 nM and 4.9 nM with Bmax's for these sites of 11.6, 158 and 253 fmoles/10^6 cells, respectively. For the cyclic GMP response, it appears that the 1.0 nM binding site is physiologically relevant.

These data support the hypothesis that bradykinin has physiologically important effects on nerve cells and substantiates the possibility of a neurotransmitter role for bradykinin. We are currently attempting to evaluate other bradykinin analogues for similar activity in this preparation. (Supported by the Mayo Foundation and Grants MH 27692 and MH 08823 from the U.S.P.H.S.).

52.9 NIGRA NEUROTENSIN RECEPTORS: EFFECTS OF CHRONIC NEUROLEPTIC TREATMENT. G. R. Uhl and M. J. Kuhar. Depts. of Neuroscience, and Neurology, Johns Hopkins University, Sch. Med., Baltimore, MD 21205 and Dept. of Neurology, Massachusetts General Hospital, Boston, Massachusetts 02114

Anatomic studies suggest potentials for interaction between the peptide neurotransmitter candidate neurotensin and dopamine-containing systems at several loci in the neuraxis. Neurotensin may influence dopaminergic function in the substantia nigra. Dense nigral neurotensin receptors are depleted from human brain by Parkinson's disease and from rat brain by 6-hydroxydopamine lesions suggesting peptide receptor localization to dopaminergic cells. Microiontophoresed neurotensin excites nigral cells, suggesting that these receptors are physiologically relevant.

Effects of neurotensin on dopaminergic systems may be reciprocated. In a case of neuroleptic-induced Parkinsonism, we found apparent increases in nigral neurotensin receptors when compared to control brains. We have, therefore, examined the influence of chronic dopamine receptor blockade on neurotensin receptor binding in rats.

Rats were treated daily for one month with 0.5 mg/kg haloperidol or vehicle, subcutaneously. This regimen has been shown to increase dopamine receptor binding. Brains were processed for neurotensin receptor autoradiography, and specific grain densities determined by subtracting parallel values obtained from adjacent "blank" sections with 5 x 10^{-6} M unlabeled neurotensin added to the primary incubation. Haloperidol-treated rats showed increased specific neurotensin binding in the substantia nigra (ca. 200% of control values, $p < .005$). There was no significant difference between receptor densities in the cerebral cortex in the same sections.

Could these changes represent direct influences of haloperidol on neurotensin binding? High concentrations of haloperidol added directly to binding assays have shown no influence on binding. Conceivably, these alterations could represent homeostatic adaptations to chronic dopamine receptor blockade. It is possible that such increased receptivity to excitatory peptide influences could contribute to some of the motor sequelae of long-term neuroleptic use, such as tardive dyskinesia or to other dopamine-related diseases.

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52.10 DISTRIBUTION OF ANGIOTENSIN II RECEPTORS IN RAT BRAIN. F.A.O. Mendelsohn[1]‡ R. Quirion[2], J.M. Saavedra[3], G. Aguilera[1]‡ and K.J. Catt[1]* (SPON: G. Stanton). [1]Endocrinology and Reproduction Research Branch, NICHD; [2]Section on Brain Biochemistry, NIMH; and [3]Laboratory of Clinical Science, NIMH, National Institutes of Health, Bethesda, MD 20205, U.S.A

Angiotensin II (AII) has important CNS actions including stimulation of thirst, salt appetite, autonomic activity, blood pressure and pituitary hormone release. AII-containing nerve terminals in the CNS have been demonstrated by immunohistochemical methods (Fuxe et al, Neurosci. Lett. 2:229,1976) and binding sites for AII have been identified in homogenates of various brain regions (Bennett and Snyder, J.Biol.Chem. 251:7423, 1976; Sirett et al, Brain Res. 122: 299,1977). To determine the precise anatomical distribution of brain AII receptors, we performed in vitro autoradiographic localization of AII binding sites in brain sections. For this purpose, 25 µm sections were cut from snap frozen rat brain, applied to gelatin-coated slides and dehydrated in a vacuum dessicator at 4°C. The slide-mounted sections were incubated with ^{125}I-labeled [Sar^1]AII, washed, dried and exposed to LKB Ultrofilm. The films were processed and grain density quantitated by computerized densitometry.

The AII binding sites were characterized in membranes prepared from fresh brain tissue, incubated with ^{125}I-[Sar^1]AII in the presence of peptidase inhibitors, with separation of free and bound radioactivity by filtration through glass fibre discs. Saturable high-affinity (Ka 2.0 x 10^9 M^{-1}) binding sites for AII were detected at a concentration of ~13 fmol/mg protein. The relative potencies of AII analogs in displacing ^{125}I-AII from the binding sites were as follows: [Sar^1]AII, 1.00; AII, 0.17; des-Asp^1-AII, 0.12; AI, 0.0017. Autoradiographic visualization of ^{125}I-[Sar^1]AII binding distribution shows a very high density of sites in the subfornical organ, periventricular and paraventricular nuclei of the hypothalamus, lateral olfactory tract and nucleus of the tractus solitarius. Moderate levels of receptors are present in the superior colliculus, suprachiasmatic nucleus, lateral septum, habenula, subiculum and inferior olivary nuclei. Low densities of receptors are found in most of the remaining areas.

These studies provide the first demonstration of the anatomical distribution of AII receptors in brain. Specific receptors are concentrated in a number of physiologically relevant sites suggesting multiple roles for angiotensin II in the CNS.

52.11 SECRETIN RECEPTORS ON NEUROBLASTOMA CELL MEMBRANES. Bryan L. Roth*, Margery C. Beinfeld* and Allyn C. Howlett* (SPON: N. Connors), Dept. of Pharmacology, St. Louis Univ. Med. School, 1402 S. Grand Blvd., St. Louis, MO 63104.

Secretin (Sec) is a 27-amino acid peptide known to be present in the gut and in the central nervous system. Although Sec's role in the regulation of pancreatic secretion is well established, the function of Sec in the brain remains obscure. Recent studies utilizing cultured astrocytes and neuroblastoma X glioma hybrids indicated the presence of a Sec-stimulated adenylate cyclase. These studies suggested to us that there might be Sec receptors in neuronally-derived tissue.

Initial studies with neuroblastoma cells (N18TG2) indicated the presence of a peptide-activated adenylate cyclase. In general, Sec elicited an 8-10 fold elevation of cAMP in both intact cells and in a plasma membrane fraction. Interestingly, Vasoactive Intestinal Peptide (VIP) also activated adenylate cyclase although it had about one-tenth the affinity for the cyclase (K_{act}=300 nM for VIP and 30 nM for Sec). For a series of peptides, the rank-order potency was Sec>VIP>PHI>>GIP, Glucagon, hGRF.

We also analyzed the binding of labelled Sec to the peptide receptor. We found that (^{125}I)Secretin bound to both intact cells and plasma membranes with high affinity (k_D=1.06±0.6 nM, B_{max}= 3200±800 fmole/mg protein). Kinetic analysis indicated that the binding was time-dependent and reversible at 25°C. Competition binding assays revealed that the rank-order potency for inhibition of Sec binding was similar to that obtained in the adenylate cyclase assays.

We wondered whether there might be a distinct VIP receptor, but direct binding studies with labelled VIP did not reveal any specific binding. This suggested that VIP and Sec might be binding to the same site. Indeed, computerized analysis of the competition binding data revealed a single site to which both VIP and Sec bound. Analysis of the data by a two-site model did not improve the fit. Further, the Hill Coefficients for activation of the adenylate cyclase approximated 1.0--consistent with the single site model.

Finally, we examined the sensitivity of the Sec receptor to nucleotides. As is seen in other systems, Gpp(NH)p inhibited Sec binding by accelerating the rate of dissociation. Other nucleotides were ineffective.

In conclusion, we have demonstrated a novel peptide binding site in a neuronally derived cell line. The Sec receptor appears to be coupled to adenylate cyclase in a positive manner in a guanine-nucleotide-sensitive fashion. N18TG2 cells thus provide a model system for analyzing the role of Sec in the nervous system. Supported by NS-16513 (AH), NS-18335 (MCB) and a grant from the American Parkinson Disease Foundation (MCB).

52.12 BRAIN INSULIN BINDING AND BRAIN INSULIN CONTENT IN THE HYPERINSULINEMIC ZUCKER (fa/fa) RAT. D.P. Figlewicz*, D. Dorsa*, H. Ikeda*, L.J. Stein*, D. Baskin*, S.C. Woods and D. Porte, Jr.* (SPON: W. Catterall). Depts. of Psychology, Pharmacology, and Medicine, Univ. of Washington, Seattle, WA 98195.

We examined the brain insulin content and the regulation of insulin receptors in the hyperinsulinemic Zucker fa/fa rat. Fasting plasma and CSF insulin was measured in 4-mo. old female fa/fa (fatty) and lean (Fa/fa and Fa/Fa) littermates and age-matched Wistars. Plasma insulin was significantly elevated in the fa/fa's (201±35 µU/ml; n = 9) as compared to either the Fa/fa (18±2.4 µU/ml; n = 7) or the Wistar (12±2.4; n = 7). CSF insulin was also significantly elevated in the fa/fa's (1.59±0.19 vs. 0.23±0.11 and 0.20±0.13 µU/ml). However, insulin content in the olfactory bulb (OB), cerebral cortex (CC), and hypothalamus (H) was significantly lower in the fa/fa's (2.1±0.4, 1.8±0.6, and 2.1±0.5 µU/gm, respectively) as compared to control (10.3±1.0, 5.6±0.6, and 4.9±0.4 µU/gm). To determine whether lowered insulin content could be explained by decreased insulin binding, we measured liver and brain insulin receptors in 8 mo. old Zucker and Wistar rats. Membrane particles were prepared from liver and 4 brain regions. Binding of 0.1 nM ^{125}I-insulin (alone or in the presence of 0.1 nM to 10 µM unlabeled insulin) was measured at 25°C, pH 8, for 2 hr.

Insulin binding in the livers of hyperinsulinemic fa/fa rats was lower (67.64% of the dose of trace ^{125}I-insulin) than binding in controls (84.92% and 85.09% in Fa/Fa's and Wistars); binding in the presence of 10 µM insulin ("non-specific") was between 6 and 8% in all groups. In contrast, insulin binding by OB membranes in fa/fa, Fa/fa, Fa/Fa, and Wistar rats was not different (33%, 26%, 26%, and 27% of a dose of trace ^{125}I-insulin/mg protein). In addition, binding in CC, medial H, and lateral H was not different in fa/fa's vs. control rats. **Conclusions:** Liver insulin receptors in the hyperinsulinemic fa/fa rat were decreased; brain insulin receptors did not "down-regulate." We conclude that reduced receptor numbers cannot account for decreased brain insulin in these animals.

REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS I

53.1 SEROTONIN RECEPTORS (S_1) IN THE SPINAL CORD: CHARACTERIZATION IN THE RAT DURING DEVELOPMENT AND MATURITY. D. Martínez-Fong*, M.P. Fillion+, G. Fillion+, G. Chagoya*, J. Hernández R. , Lab. Neurontogenia, Depto. Neurociencias, Centro de Investigación I.P.N., Ap. Postal 14-740, México, D. F. 07000*. Lab. Neuropharmacologie, Inst Pasteur, 28 Rue du Dr. Roux, F-75724, Paris Cedex 15 France+.

A system of serotonin receptors has been described in the brain of various animal species, characterized as being post-synaptic specific and of high affinity. It is possibly coupled to a specific adenylate-cyclase and GTP-dependent, classified as S_1, in contrast to other sites known as S_2, which recognize specifically |^{3}H|-spiperone. We have measured the specific binding of |^{3}H|5-HT (serotonin) to neuronal membranes of the spinal cord, in the presence of 10 µM GTP, 2 to 30 nM |^{3}H|5-HT, and 10 µM unlabeled 5-HT. Bound radioactive ligand was separated by filtration. The related affinity constant and maximal binding were measured at various ages after birth. In the adult spinal cord |^{3}H|5-HT binding was linear up to 1.5 mg of tissue protein. Specific binding showed a typical association and dissociation kinetics reaching half maximal value at one minute and equilibrium at 10 minutes. The rate association constant (K_1) was 1.955 x 10^{-5} M^{-1} $seconds^{-1}$ and rate dissociation constant (K_{-1}) 2.663 x 10^{-3} $seconds^{-1}$. K_D calculated from these constants was 13.6 nM which is similar to that obtained from saturation curves and Hill plots. Bound ligand was dissociated by an excess of cold 5-HT. 5-HT agonists like Quipazine displace bound |^{3}H|5-HT with IC_{50} from 5 to 30 nM. 5-Hydroxyindole-acetic acid did not displace bound |^{3}H|5-HT, indicating a specific binding. 5-HT antagonists methysergide and cyproheptadine, were less potent to displace bound |^{3}H|5-HT with IC_{50} of 300 to 500 nM. The system is saturable with the following constants: K_D 19 ± 9 nM spinal cord synaptosomes, and 17 ± 7 nM in homogenates: Bmax of 0.1 ± 0.04 pmol/mg protein, and 1.26 ± 0.06 pmol/sp. cord in the homogenate, and 0.15 ± 0.05 pmol/mg protein in synaptosomes. At 0 days (day of birth) |^{3}H|5-HT binding was present with a K_D of 11.5 nM, and Bmax of 0.24 pmol/mg protein or 0.117 ± 0.029 pmol/sp. cord. Bmax increased with age, being about four times as much on day 10 and eight times on day 21 when it reached adult values. Affinity did not change with age. The activity of tryptophan-5-hydroxylase (T5-H), a presynaptic marker of 5-HT pathways was determined at different ages. The developmental pattern of T5-H was parallel to postsynaptic receptors. Our results establish the presence of a system of specific |^{3}H|5-HT recognizing sites in the spinal cord of the adult rat, saturable, reversible, GTP dependent, with a high affinity, possibly belonging to the S_1 group of sites. These receptors are already present at birth in a mature state. Previous results from our laboratory showed that the developmental pattern of |^{3}H|5-HT sites in the brain is similar to that observed in the spinal cord.

53.2 MUSCARINIC ANTAGONIST BINDING SITE HETEROGENEITY AS EVIDENCED BY AUTORADIOGRAPHY AFTER DIRECT LABELING WITH [^{3}H]-QNB and [^{3}H]-PIRENZEPINE. D. R. Gehlert, J. K. Wamsley, W. R. Roeske* and H. I. Yamamura . (SPON: B. Grosser.) Dept. of Psychiatry, University of Utah, Salt Lake City, UT 84132 and Dept. of Pharmacology, University of Arizona, Tucson, AZ 85724.

The binding of tritiated forms of "classical" muscarinic antagonists are consistent with the law of mass action indicating a single class of independent receptors (Hulme et al., Mol. Pharmacol. 14:737-750, 1978). However, pirenzepine, a novel muscarinic antagonist, appears to recognize subpopulations of muscarinic receptors (Hammer et al., Nature 283:90-92, 1980). Recently, a tritiated form of pirenzepine ([^{3}H]-PZ) was prepared and used to directly identify a population of muscarinic receptors in the brain which bound the ligand with high affinity (Watson et al., Life Sci. 31:2019-2023, 1982). Receptor autoradiographic techniques utilizing [^{3}H]-PZ and [^{3}H]-QNB have now been employed to microscopically differentiate the location of the binding sites for these two muscarinic antagonists.

Slide mounted sections of rat brain were pre-incubated for 20 minutes at room temperature in Krebs phosphate buffer, pH 7.4. This was followed by a 60 minute room temperature incubation in the same buffer plus 20nM [^{3}H]-PZ and a subsequent 5 minute rinse in fresh buffer at 0-4°C. Adjacent sections were labeled using 1nM concentrations of [^{3}H]-QNB with a 120 minute room temperature incubation followed by two 5 minute rinses in fresh ice cold Krebs phosphate buffer. The labeled sections were then quickly dried and apposed to sheets of LKB Ultrofilm in X-ray cassettes. After an appropriate exposure period, the films were developed and the autoradiograms were analyzed by computer assisted microdensitometry.

While most areas were labeled equally with both ligands, there were several distinct brain regions heavily labeled with [^{3}H]-QNB and not labeled with [^{3}H]-PZ. These sites include the ventral horn of the spinal cord, hypoglossal nucleus, cerebellum, nucleus tractus solitarius, nucleus of the facial nerve, superficial layer of the superior colliculus, diagonal tract of Broca and lamina IV of the cerebral cortex.

Thus, [^{3}H]-PZ recognizes only a subset of the muscarinic receptors labeled with [^{3}H]-QNB. Interestingly, the sites not labeled with [^{3}H]-PZ are those which have a large percentage of high affinity agonist sites as evidenced by agonist displacement of [^{3}H]-antagonist binding (Wamsley et al., Brain Res. 200:1-12, 1980) and by direct [^{3}H]-agonist binding (Wamsley et al., this meeting). [^{3}H]-PZ thus labels nuclei where a large concentration of low affinity agonist sites exist.

53.3 QUANTITATIVE AUTORADIOGRAPHY OF B_1 AND B_2 ADRENERGIC RECEPTOR SUBTYPES IN RAT BRAIN. Thomas C. Rainbow*, Bruce Parsons and Barry B. Wolfe. Department of Pharmacology, University of Pennsylvania Medical School, Phila. PA 19104

Beta (B) adrenergic receptors can be divided into B_1 and B_2 subtypes on the basis of different affinities for drugs. While it is known that B_1 receptors predominate in the rat forebrain, and that B_2 receptors are more numerous in the cerebellum, more detailed information is lacking about their anatomical distribution. In this study, we have combined the recent LKB film method of autoradiography with computer-iterative methods of receptor analysis to quantify B_1 and B_2 receptors in discrete regions of rat brain.

Our procedure was to label 32u thick frozen sections of rat brain in vitro with 150 pM ^{125}I pindolol (IP:2200 Ci/mmol), a non-selective beta antagonist. Sections were incubated with the ligand for 70 min at 23°. The relative amounts of B receptor subtypes were determined by co-incubating the sections with 10^{-11} to 10^{-6} M of ICI 118,551, a selective B_2 antagonist. The sections were washed 3 x 20 min in 4° buffer, dried on a 60°slide warmer and applied against LKB Ultrofilm for 4.5 hr to generate autoradiograms. Optical densities of the autoradiograms were converted into fm ligand/mg protein using ^{125}I brain-mash standards. Using readings from autoradiograms generated in the presence of 18-25 different concentrations of ICI 118,551, the relative proportions of B_1 and B_2 receptors were determined for a variety of brain regions via computerized analysis of inhibition curves. Non-specific binding was determined separately for each brain region via computerized curve-fitting.

Scatchard analysis of density readings from the caudate-putamen indicated that the binding of IP was saturable, specific and of a high-affinity (Bmax = 400fm/mg P, Kd = 60-90 pM). In contrast to the inhibition curve for l-propranolol, which computer analysis modeled to a single affinity binding site, the inhibition of IP binding by ICI 118,551 was biphasic and corresponded with high significance with predictions made for a two-site model. Computer analysis of optical density readings indicated that high levels of B_2 receptors were found throughout the molecular, purkinje and granular layers of the cerebellum, with high levels of B_1 receptors occurring in the reticular nucleus of the brainstem and such regions of rat forebrain as the lateral septum and layer I of frontal cortex. White matter regions of both forebrain and cerebellum exhibited mixed ratios of B_1 and B_2 receptors. Our results provide the first quantitative map of B_1 and B_2 receptors in rat brain and demonstrate the feasiblity of using the LKB film method to localize receptor subtypes via inhibition analysis.

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53.4 QUANTITATIVE AUTORADIOGRAPHY OF SODIUM-DEPENDENT*[35]S-CYSTEIC ACID BINDING SITES IN RAT BRAIN. Bruce Parsons and Thomas C. Rainbow(Spon: S.D. Erulkar) Dept. of Pharmacology, Univ. of Pennsylvania Medical School, Philadelphia PA 19104.

Cysteine sulfinic acid (CSA) has been shown to be present in rat brain, to be transported into synaptosomes by a Na-dependent high-affinity system, and to exert strong excitatory effects on the spinal cord. In this study, we have employed the LKB-film method of autoradiography to quantify in rat brain Na-dependent (and presumptive re-uptake) binding sites for [35]S-cysteic acid (CA), an analog of CSA.

Our procedure was to preincubate frozen brain sections (32u) for 20 min in ice-cold 50 mM Tris buffer containing 300 mM NaCl, pH 7.4. Binding sites were labeled for 20 min at 4C with 300 ul buffer containing 20 or 150 nM [35]S-CA (41.4 Ci/mmol), with or without 1mM unlabeled CSA. A range of concentrations of unlabeled CA or concentrations (10^{-7}-10^{-3}M) of unlabeled compounds were included in some experiments. After incubation, sections were washed twice for 5 min in 4°C buffer, and removed for scintillation counting or apposed against LKB film for 4 h. Optical density readings were converted into pm/mg protein using [35]S standards made from rat brain mash.

Na-dependent binding of [35]S-CA was saturable, specific and of high-affinity. Scatchard analysis indicated a single population of sites (K_d=0.4uM; Bmax=3 pmol/mg P). CSA was the most potent inhibitor of [35]S-CA (IC_{50}=0.5uM at 20 nM CA); while L-aspartate, D-asparate, L-glutamate and CA were 5-8 times less potent. Very high levels (4-5 pmol/mg P) of CA binding were seen only in the molecular layer of the cerebellar cortex. Moderate levels (0.5-2 pmol/mg P) were observed in nearly all other brain regions, including all layers of the cortex, all subfields and strata of the hippocampus, and all amygdaloid, thalamic and hypothalamic nuclei. The anatomical distribution of [35]S-CA differs from that of the glutamate/aspartate marker, D-aspartate (Parsons and Rainbow, N. Lett. 36:9, 1983), and appears to represent a re-uptake site for CSA.

To test this hypothesis, we performed quantitative autoradiography on "staggerer" mice, mutants known to be deficient in excitatory amino acid re-uptake. We found that cerebellums from "staggerer" mice showed a 40% decrease in [35]S-CA binding sites relative to controls. We also observed a 55-60% decrease in [35]S-CA transport in synaptosome preparations from "staggerer" cerebellums. These results suggest a homology between Na-dependent [35]S-CA binding sites and CSA re-uptake sites, and are consistent with the hypothesis that CSA may act as an excitatory transmitter in the CNS.

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53.5 DIFFERENTIAL LOCALIZATION OF TYPE 1 AND TYPE 2 BENZODIAZEPINE (BZ) RECEPTOR SITES IN THE SUBSTANTIA NIGRA. M.M.S. Lo[1], D.L. Niehoff[2], M.J. Kuhar[1] and S.H. Snyder[1]. [1]Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205. [2] MRC Neuroimmunology Res. Proj. Uni. College, Dept. of Zoology, London, England.

Differential regional localizations of type 1 and type 2 BZ receptors have been reported from one of our laboratories using light microscopic autoradiography of ^{3}H-flunitrazepam (^{3}H-FNZ) binding in the presence and absence of CL218872 (a type 1 selective drug). We have now reexamined the location of type 1 and 2 receptors using (1) displacement of ^{3}H-FNZ by CL218872, (2) ^{3}H-ß-carboline (a type 1 selective drug) and (3) ^{3}H-FNZ binding to detergent treated brain section (this treatment selectively solubilizes type 2 sites) in conjunction with quantitative autoradiography with tritium sensitive film.

In the substantia nigra after unilateral lesions of the striatonigral pathway, shows increases of about 50°/° occur for total GABA and BZ receptor binding. However, differential analysis of type 1 and 2 BZ receptors shows that the lesion elicits a 100-150 °/° increase of type 1 sites and a 50-70°/° reduction in type 2 sites. Conversely, lesions of the substantia nigra evoke a >50°/° reduction in type 1 sites but no change in type 2 sites. In conclusion, our observations are consistent with a postsynaptic location of type 1 sites, whereas type 2 sites are located on axons or terminals of the striatonigral pathway. Other studies in our laboratory consistent with this idea will also be discussed.

53.6 FURTHER CHARACTERIZATION OF THE SPECIFIC CNS ^{3}H.PHENYTOIN BINDING SITE. D.Kafka* and L.Spero* (SPON:J.F.MacDonald).Dept. Pharmacology, University of Toronto, Toronto, Ontario, Canada.

In an attempt to determine the relationship between the phenytoin site (1) and the GABA-chloride ionophore-benzodiazepine complex we have examined the effect of chloride ions on phenytoin binding.Binding to both the high and low affinity sites is shifted to the left, and the magnitude of the high affinity site is increased. This shift is independant of the cation used (Na,NH_4,or K). The IC_{50}'s of a number of anticonvulsant barbiturates for the phenytoin site are also left shifted, and in the presence of 100mM chloride, there is a good correlation between anticonvulsant potency and competition for the phenytoin site. This should be compared with binding to the picrotoxinin site where there is a correlation between binding and sedative potency but not anti-convulsant potency (2).

We have determined that diazepam and flunitrazepam both enhance ^{3}H.phenytoin binding, but that other benzodiazepines tested did not.The benzodiazepine antagonist Ro 15-1788 had no effect on enhancement, which would therefore appear to have the characteristics of the "peripheral" type benzodiazepine receptor. We will test the effects of the "peripheral" benzo-diazepine antagonist Ro5-4864.These finding are in conflict with Bowling & De Lorenzo (3) who demonstrated competition between benzodiazepines and phenytoin, and Shah et al (4) who found that all benzodiazepines they tested enhanced phenytoin binding.

The distribution of the ^{3}H.phenytoin binding site in human brain has been examined. Scatchard analysis reveals two sites, K_{d1} 30-50nm, and K_{d2} 200-500nM. In the rat K_{d1} was 10nM, and K_{d2} was 700nm. The highest binding (B_{max}) was observed in amygdala (700 fmoles/mg), in the motor cortex (500 fmoles/mg) with lesser amounts in the hippocampus (230 fmoles/mg) and cerebellar cortex (150 fmoles/mg).

Using irradiation inactivation analysis of freeze dried synaptosomal membrane the two sites in both man and rat were shown to have different apparent molecular weights, MW^1 approx 72,000,MW^2 approx 113,000.This work was supported by MRC grant MA 7119.

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53.7 AUTORADIOGRAPHY OF SUBSTANCE P RECEPTORS USING ^{125}I-PHYSALAEMIN. S. Wolf*, T. Moody, R. Quirion and T. O'Donohue. (SPON: B. Tempel). Dept. Biochem., GWU Med. Sch., Washington, D.C. 20037 and Expt. Ther. Branch, NINCDS, Bethesda, MD 20205.

Substance P may function as a neurotransmitter in the CNS. While endogenous substance P is discretely distributed in certain brain nuclei, the regional distribution of substance P receptors has only been described recently (Shults, C. et al., Peptides, 3: 1073, 1982). Here the pharmacology and discrete regional distribution of central substance P receptors was investigated using rat brain slices and ^{125}I-physalaemin, a potent structural analogue of substance P.

Binding studies were conducted using 12 µm coronal slices of unfixed rat brain (Wolf, S. et al., Eur. J. Pharm., 87: 163,1983). Forebrain slices bound ^{125}I-physalaemin reversibly, with high affinity (Kd, 0.3 nM) to a single class of sites (Bmax, 22 fmol/mg protein). The ratio of specific to nonspecific binding was 15 to 1. Pharmacology studies indicated that only peptides structurally related to physalaemin such as substance P and eledoisin competed for high affinity ^{125}I-physalaemin binding; peptides structurally unrelated to substance P such as bombesin, β-endorphin, α-MSH, neurotensin or VIP did not compete for specific ^{125}I-physalaemin binding sites.

Autoradiographic studies were conducted using the method of Palacios et al. (Neurosci. Lett., 25: 101, 1981). Highest grain densities were present in the olfactory bulb, dentate gyrus, medial amygdala, superior colliculus, ventral subiculum, as well as the dorsal parabrachial and dorsal tegmental nuclei, locus coeruleus and dorsal horn of the spinal cord. Moderate grain densities were present in the nucleus accumbens, striatum, hippocampus, olfactory tubercle and periaquaductal gray of the midbrain. Low grain densities were present in most thalamic nuclei, the substantia nigra and cerebellum. The corpus callosum and controls treated with 1 µM unlabeled physalaemin had negligible levels of binding. The unique pharmacological and regional distribution data obtained suggest that ^{125}I-physalaemin may serve as a valuable probe for central substance P receptors.

53.8 AUTORADIOGRAPHIC LOCALIZATION OF ANGIOTENSIN II RECOGNITION SITES IN THE DOG CNS. R.C. Speth, C.L. Chernicky, D.R. Gehlert* and J.K. Wamsley. Cleveland Clinic, Res. Div., Cleveland, OH 44106 and Dept. of Psychiatry, Univ. Utah, Salt Lake City, UT 84132

The existence of angiotensin II (Ang II) receptor recognition sites in the CNS of bovine and rodent species is well established. Our radioligand binding studies using ^{125}I Ang II have established their existence in discrete microdissected regions of the canine CNS. Due to the limitations of homogenate binding techniques for precise anatomical localization, it is essential to employ the method of in vitro receptor autoradiography for a better resolution of Ang II recognition sites in the dog CNS. Frozen sections of selected regions of the brain from a dog perfused with 0.01% formalin were cut at 20 µM in a cryostat and mounted onto subbed microscope slides. After a dessication period in a frost free freezer, the sections were thawed and preincubated in a sodium phosphate buffer medium containing dithiothreitol, EGTA, NaCl and $MgCl_2$ with or without 1 µM Ang II for 30 min at 22°C. The sections were then transferred into jars containing the same medium plus 1 nM ^{125}I-Ang II and 0.4% bovine serum albumin with or without 1 µM Ang II, for 45 min at 22°C. These conditions have previously been used to demonstrate specific ^{125}I-Ang II recognition sites in homogenates of the dog CNS. The sections were rinsed for 2-3 min in fresh buffer at 4°C and dried with a cooled-dessicated stream of air. The sections were placed in X-ray cassettes in apposition to an autoradiographic film (Ultrofilm, LKB Instruments) for a 2 to 8 day exposure. The autoradiograms revealed several areas of dense, highly specific ^{125}I-Ang II recognition sites; the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, the anterior pituitary, and the anteroventral third ventricle (AV3V) region. Moderate densities of specific ^{125}I-Ang II binding sites were also observed in the septum, the medial basal hypothalamus and cerebellar cortex. Little specific binding was observed in other brain regions examined. Further autoradiographic examination of the ^{125}I-Ang II recognition sites in dog CNS areas is in progress. Supported by NIH HL-27568 and HL-6835.

53.9 AUTORADIOGRAPHY OF ^{3}H-β-ENDORPHIN BINDING IN RAT BRAIN: EVIDENCE FOR EPSILON SITES. R.R. Goodman, R.A. Houghten* and G.W. Pasternak. Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, N.Y.,N.Y. 10021 and Dept. Immunopathology, Scripps Clinic Research Foundation, La Jolla, CA 92037.

The autoradiographic regional localization of ^{3}H-β-endorphin binding in rat brain differed from that of either ^{3}H-dihydromorphine or ^{3}H-D-ala-D-leu-enkephalin (DADL), determined by the comparison of sequential sections through three regions of rat brain: striatum, hypothalamus/thalamus, and brain stem. ^{3}H-β-Endorphin labeled some clusters as well as the subcallosal streak in the striatum, the nucleus accumbens, Lamina IV of the cortex, medial thalamus, hippocampus, hypothalamus, inferior colliculus, dorsal raphe, median raphe and pontine nuclei. White matter regions had little, in any, binding. Although many of these structures were also labeled by ^{3}H-dihydromorphine and ^{3}H-DADL, the overall pattern of ^{3}H-β-endorphin was unique, consistent with the proposal of central epsilon receptors. Detailed displacement experiments in homogenate binding assays suggested a site selective for β- endorphin, corresponding, perhaps, to the previously proposed epsilon receptor. Saturation studies studies demonstrated a high affinity binding component (K_D 0.4-0.6 nM) as well as a lower affinity component (K_D approximately 15 nM). The higher affinity component consisted of more than one type of site. Approximately half of the high affinity component was eliminated by naloxonazine treatment, suggesting that the lost binding corresponded to mu_1 sites. This high affinity binding of β-endorphin to mu_1 sites was interesting in view of the ability of naloxazone and naloxonazine to antagonize β-endorphin analgesia and catalepsy. The remaining high affinity binding component on Scatchard analysis probably represented epsilon binding. The affinity of β-endorphin for other sites, such as mu_2 and delta was much less. Thus, the lower affinity component probably represented cross-binding between β-endorphin and other classes of opioid binding sites.

53.10 TEMPERATURE-SENSITIVE REVERSIBLE LOSS OF ^{3}H-SEROTONIN BINDING SITES. K. Blaschuk* and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, CANADA, M5T 1R8

Dramatic reversible changes in ^{3}H-serotonin (^{3}H-5HT) binding in rat cerebral cortex crude homogenates can be induced by temperature. With 10 µM serotonin to define non-specific binding, ^{3}H-5HT binding at 37° C for 10 minutes with immediate filtration yielded a monophasic Scatchard profile, with K_d of 4nM and B_{max} of 255 fmoles/mg. protein. The Scatchard plot became clearly non-linear if the incubation tubes were immediately immersed in ice-water for 30 to 60 minutes before filtration after the 37° C - 10 minute incubation. Computer analysis revealed two binding sites with K_d values of 0.3 and 12nM and B_{max} values of 190 and 360 fmoles/mg. protein, respectively.

Temperature stability curves varied with the concentration of ^{3}H-5HT used. At low concentration (eg. 0.40 nM), when the incubation tubes were immersed in ice-water following the normal 37° C - 10 minute incubation, ^{3}H-5HT binding reached a maximum of about 350% of binding at starting point (before immersion into ice-water) within 20 minutes, and remained stable for at least two hours. When the temperature of the incubation tubes was reduced to room temperature after the 37° C - 10 minute incubation, ^{3}H-5HT also increased but reached a maximum of 250% instead of 350% described above. The increase was stable up to 40 minutes. Binding of ^{3}H-5HT maintained at 37° C was not stable. Progressive loss of binding occurred and only 40% of binding remained after 2 hours. Part of the loss (50%) was recoverable when the temperature was reduced to 0° C by immersion in ice-water.

Similar experiments using higher concentrations of ^{3}H-5HT (eg. 7.5nM) showed that the increase in binding was only half of that observed with lower concentrations. When the temperature of the incubation tubes was reduced to either 0° C or room temperature, maximum increases of 180% and 130% respectively were observed. Binding maintained at 37° C beyond the 10 minute incubation was more stable than with lower concentration of ^{3}H-5HT. After 60 minutes, 25% of binding was lost but completely recoverable if the temperature was reduced to 0° C. Two hours at 37° C resulted in a 50% loss of binding, about 70% of which was recoverable.

Our results demonstrate the existence of at least two ^{3}H-5HT binding sites in rat crude cerebral cortex homogenate, the high affinity site of which is highly temperature-sensitive. Our results thus explain the varied K_d and B_{max} reported in the literature. The high affinity site being highly temperature-sensitive, may have been frequently missed under the previous assay conditions reported.

53.11 UPTAKE OF PUTATIVE NEUROTRANSMITTERS BY ISOLATED CATFISH HORIZONTAL CELLS. M. Christensen* and B. N. Christensen. Dept. of Physiology & Biophysics, Univ. of Texas Med. Branch, Galveston, TX 77550.

We have recently been using preparations of horizontal cells enzymatically isolated from intact retina to examine the ionic basis of their physiological properties. These cells maintain many of the electrophysiological properties expected of excitable cells in spite of the enzymatic treatment. However, we have been interested in showing that other properties of these cells remain intact after dissociation. To this end, we have compared the ability of isolated horizontal cells to transport putative neurotransmitters when applied in the extracellular medium. Horizontal cells in the intact catfish retina have a high affinity uptake mechanism for GABA (Lam et al. Proc. Natl. Acad. Sci., 1978) and glutamate and glycine are taken up in only small amounts. The experiments reported on here were done to determine whether the high affinity uptake mechanism for GABA is still intact following enzymatic dissociation.

Freshly dissociated horizontal cells have large resting membrane potentials ranging from -55 to -70 mV and when activated by passing a depolarizing current pulse through an intracellular electrode produce a sodium and calcium dependent action potential (Shingai and Christensen, Neuroscience, in press). These cells also respond to glutamate when this substance is pressure ejected from a pipette. The reversal potential for glutamate is about +5 mV (Shingai and Christensen, abstr. Society for Neuroscience, 1983).

Horizontal cells were enzymatically dissociated from excised catfish retina and incubated in several putative radioactive neurotransmitters. Usually the contralateral retina was left intact and incubated in the same radioactive medium in order to compare results from the dissociated cells with intact retina. The isolated cells and intact retina were fixed, embedded in plastic, and processed for light microscopic autoradiography. Our results show that enzymatically dissociated horizontal cells retain high affinity uptake mechanisms for GABA and that glycine and glutamate are taken up in much smaller quantities by both isolated horizontal cells and cells in the intact retina. To exclude the possibility that isolated horizontal cells have leaky nonselective membranes, we incubated cells in radioactive sucrose. This substance was not taken up by either the isolated cells or the intact retina. We conclude that enzymatically isolated horizontal cells are able to maintain a membrane which retains its ionic conductances and high affinity uptake mechanisms. Supported by grant EY-01897.

53.12 MODULATION OF NEURONAL SEROTONIN (5-HT) UPTAKE BY ^{3}H-IMIPRAMINE RECOGNITION SITES: EVIDENCE FOR AN ENDOGENOUS LIGAND. M. L. Barbaccia,* D. M. Chuang,* and E. Costa. Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Specific, high affinity binding sites for ^{3}H-imipramine located on serotonergic (5-HT) axons have been characterized in crude synaptic membrane preparations of mammalian brains. These sites are located in serotonergic axons and appear to be functionally associated with a regulation of the serotonergic reuptake mechanisms. Though 5-HT is a weak displacer of ^{3}H-imipramine, the regulatory site for 5-HT uptake where imipramine binds is not the regulatory site for 5-HT of the reuptake mechanisms. The number of ^{3}H-imipramine recognition sites located in various rat brain structures is decreased by two daily injections of imipramine or desmethylimipramine (DMI) repeated for 3 weeks. In hippocampal minces when the Bmax of ^{3}H-imipramine binding is decreased the Vmax of the neuronal reuptake of 5-HT is enhanced (+40%). Moreover when the number of ^{3}H-imipramine recognition sites is decreased the efficiency of imipramine as blocker of the 5-HT uptake is attenuated. All these observations taken together suggest that the high affinity ^{3}H-imipramine recognition sites may have physiological significance as a site where an endogenous effector may be operative in modulating 5-HT uptake. Such an effector was extracted and partially purified from whole rat brain. It inhibits in a dose dependent manner the uptake of ^{3}H-5-HT and it displaces ^{3}H-imipramine specifically bound to crude synaptic membrane preparations in a concentration dependent manner. This endogenous ligand for ^{3}H-imipramine regulatory sites failed to displace ^{3}H-flunitrazepam, ^{3}H-mianserin and ^{3}H-dihydrolprendol specifically bound to rat brain membranes. The endogenous effector does not appear to be a peptide or a lipid. The inhibitory activity either on ^{3}H-imipramine binding or on ^{3}H-5HT uptake could be eluted quantitatively from a AG-50Wx8 (0.7x4cm) eluted column with 2 M HCl, this acid fails to elute ^{3}H-5-HT added to the tissue as an internal standard. This finding suggests that the biological activity detected in the brain extract was not due to endogenous 5-HT. Experiments are now in progress to characterize the chemical nature of this endogenous ligand of ^{3}H-imipramine recognition site. This ligand might become a very important tool for the study of the biochemical markers of affective disorders in man.

53.13 **DOPAMINE RECEPTORS: ELECTRON MICROSCOPIC LOCALIZATION IN MOUSE CORPUS STRIATUM.** A.C. Church, J.E. Kleinman,* and R.J. Wyatt. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

The dopamine systems of the mammalian brain have been implicated in a number of disease states. In fact, the clinical potency of the drugs presently used to treat schizophrenia closely parallels their potency in blocking the so-called dopamine type-2 (D-2) receptors. Although these D-2 receptors have been localized regionally using autoradiographic techniques, their organization at the cellular or subcellular level has remained unknown. In order to study D-2 receptors with the electron microscope, an immunocytochemical approach was taken.

Lisuride (Schering AG) is an agonist with a high affinity for the D-2 receptor (K_D=5 x 10^{10}M). Antibodies to lisuride (Anti-L) were raised in rabbits by injection of a lisuride-protein conjugate. Vibratome sections of mouse corpus striatum were incubated in lisuride and then anti-L serum. Anti-L's were stained using the peroxidase-anti-peroxidase technique with diaminobenzidine as the chromophore. Corpus striatum tissue was embedded in plastic and sectioned for electron microscopy.

Electron-dense immunoreactivity was found to be localized at synaptic junctions in the striatal neuropil. The lisuride immunoreactivity appeared to be organized in pre- and postsynaptic pairs, with stain on the synaptic faces of both an axon terminal and its apposed dendritic process. Control material was prepared by incubating tissue in the presence of lisuride and excess sulpiride. The sulpiride treatment effectively eliminated most of the synaptic stain. Our results agree in large measure with binding studies of others who have suggested that about 50% of D-2 receptors in the striatum are on extrinsic axon terminals while the remainder are located on cells intrinsic to this region. The results suggest a novel synaptic arrangement for D-2 transmission in the corpus striatum.

54.1 PASSIVE MOVEMENT SENSITIVITY AT THE INTERPHALANGEAL JOINT OF THE INDEX FINGER. H.L. Cohen and W.S. Battersby* Dept. of Physiol., Downstate Med. Ctr., Bklyn, N.Y. 11203 and Dept. of Psych., Queens College, Flushing, N.Y. 11367

Two experiments were conducted in order to parametrically evaluate passive movement sensitivity in normal, young adults. A highly precise electromechanical system for the delivery of kinesthetic stimulation and for the measurement of angular displacement was used to assess sensitivity at the proximal IP joint of the right index finger. In Expt. 1, stimuli consisted of random presentations of all combinations of seven velocities (.15 - 11.0°/sec.) and two directions (up, down), blocked across three starting positions of the joint (15°, 25° and 35° from the vertical). Movement detection was responded to by a keypress which terminated the movement and by verbal identification of direction. Percent correct detection (PCD) was a monotonically, increasing function of log angular velocity, while response time (RT) was a negatively accelerating, decreasing function. Repeated measures ANOVAs indicated a significant effect of velocity, but not of starting position or direction. In Expt. 2, subjects received automatically timed movement presentations, in contrast to the subject terminated movements in Expt. 1. Stimuli consisted of random presentations of all combinations of six velocities (.05 - 1.5°/sec), six durations (1 - 10 sec) and two directions (up, down). Starting position was kept constant at 25° from the vertical. Movement detection and direction identification were indicated by a verbal response. The results showed that when stimulus duration was kept constant PCD increased as a function of log angular velocity. As stimulus duration increased, both slope and PCD also increased. When angular velocity was kept constant PCD increased as a function of stimulus duration. As velocity increased both slope and PCD also increased, but more gradually than when duration was increased. Repeated measures ANOVAs indicated significant effects of velocity, duration and direction, as well as the velocity by duration interaction. Each velocity-duration combination was then multiplied in order to obtain a range of angular displacements. PCD was shown to be a monotonically, increasing function of log angular displacement. These behavioral results complement both the velocity and displacement sensitive properties of peripheral (muscle, joint and cutaneous receptors) and central (thalamic and cortical) kinesthetic structures defined by unit recordings.

54.2 INTERMOVEMENT SENSORIMOTOR ACTIONS IN THE CONTROL OF COORDINATED FINGER-THUMB MOVEMENTS. Kelly J. Cole,* Vincent L. Gracco,* and James H. Abbs* (SPON: G. Celesia). Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53706.

Most investigations of sensorimotor mechanisms have focused on analyses of movements around a single joint, often involving the use of unanticipated perturbations to reveal underlying control processes. By virtue of the experimental paradigm, these investigations of single joint control have primarily emphasized autogenic sensorimotor mechanisms. In the present investigation, unanticipated loads were applied to the distal joint of the thumb in human subjects during a coordinated thumb-finger "pinching" movement. Subjects were trained to produce this movement rapidly with a controlled thumb-contact pressure in response to a tone. Using a DC brushless torque motor under force feedback control, loads were introduced randomly on 10-15 percent of the movement trials at varying times prior to and following the onset of thumb flexor EMG. Thumb and finger movement, along with EMG from Flexor pollicis longus (FPL) and Flexor digitorum superficialis (FDS), were recorded simultaneously.

Analyses of thumb EMG and movement revealed autogenic compensatory responses in the thumb flexor muscle, as reported by several previous investigators. However, index finger movement and FDS EMG revealed compensatory changes as well. Finger compensatory responses were reflected in increased displacement and velocity of the movement as well as corresponding increases in FDS. The latencies of the FDS responses were as short as 45 msec, but varied with the timing of the thumb load relative to EMG onset. Compensatory changes in the finger movement and FDS appeared the first time a thumb perturbation was introduced. Moreover, subjects' comments indicated that this was not a typical reaction time response; viz., the finger compensation was not initiated intentionally.

The finger compensatory responses appear to suggest a sensorimotor control process that is open-loop. That is, the finger compensatory responses are disassociated from the thumb disturbance and further, this sensorimotor action does not act to correct the error induced in the thumb. In this regard, these results are comparable to those obtained in our earlier investigations where unanticipated perturbations were applied to the lower lip during a combined upper lip-lower lip movement [Abbs and Gracco, Soc. Neuroscience Abstracts, 1982]. In that study, open-loop responses were observed in the upper lip to the lower lip loads. Results of these parallel experiments suggest that intermovement sensorimotor actions, operating open-loop, may play a role in the coordination of multiple movements.

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54.3 EFFECT OF UNILATERAL NEOSTRIATAL LESION ON PHASIC LIMB MOVEMENT AND ASSOCIATED POSTURAL ADJUSTMENT IN THE STANDING CAT. R.P. Di Fabio. Physical Therapy Res. Labs.,Univ. of Iowa,College of Medicine, Iowa City,IA 52242, USA.

During normal limb movement,body posture is coordinated with limb displacement so that potential disturbances in equilibrium are counter-balanced by anticipatory postural adjustments. The basal ganglia (BG) were previously thought to serve a regulating function for preparatory response sets (Buchwald et al.,Growth & Develop. Brain,1975). BG influence on postural strategies prior to initiation of movement is unknown. The purpose of this study was to investigate the effect of unilateral neostriatal lesion on goal-oriented movement and on postural adjustments associated with task performance.

Five cats were conditioned to stand,unsupported,with each paw placed on a corresponding foot-plate dynamometer. Each dynamometer measured the vertical reaction forces during quiet standing and task execution. The task involved pressing a bar spontaneously with the left(phasic) forelimb and returning to full standing (one movement cycle). Baseline EMG and force-plate data were collected during the first 500ms of a 1.5s interval preceding a change in phasic forelimb weight-bearing force. Subsequent changes in EMG activity of 6 postural muscles were referenced to their mean baseline values. Animals were evaluated in control and lesion conditions. Small electrolytic lesions were made in the caudate nucleus with a monopolar lesioning electrode 0.3mm diameter at 2.5ma/25s and were verified histologically.

Perseveration and spontaneous turning to the lesioned side were observed interrupting task performance. Mean movement cycle time increased after partial neostriatal exclusion($p < .01$) and bar press frequency was reduced ($p < .01$). These findings paralled previously reported deficits with unilateral ablation of the caudate nucleus (Olmstead et al.,Exp. Neurol. 53:670,1976). In contrast, when symmetrical pre-lift weight-bearing criteria were met,associated postural responses showed no deficit. There were no significant differences in onset and duration of soleus, rectus femoris,and triceps brachii ipsilateral to the phasic limb or lumbar paraspinal,triceps,and biceps brachii contralateral to the phasic extremity. Peak stabilizing force,rate of loadbearing, and preparatory movement time were also unchanged.

Partial exclusion of the neostriatum affected sequencing of phasic limb movement. However,when stance was symmetrical (prior to initiation of lift),criterion measures of preparatory postural adjustments did not differ from control values. A theoretical model localizing anticipatory balance behavior to a lower closed-loop neural circuit would be supported by the current study.

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54.4 POSTURAL RESPONSES TO INDUCED SWAY IN NORMALS AND PATIENTS WITH ACUTE UNILATERAL VESTIBULAR LESIONS. J.H.J. Allum & C.R. Pfaltz * ORL-Department, University Hospital CH-4031 BASEL, Switzerland.

Coordination of muscle activity in postural responses following direct ankle dorsi-flexion of standing subjects was compared between normals, and patients with either bilateral or acute unilateral peripheral vestibular lesions. EMG activity was recorded with surface electrodes bilaterally from the soleus (SOL) and tibialis anterior muscles (TA) as well as over neck muscles pitching the head backwards. Previous research on normals (Brain Res. 1983, 264,p.297) showed that unexpected dorsiflexions averaging 36^{0}/s over 3^{0}result in a segmental reflex excitation of SOL and inhibition of TA, followed by sway stabizing responses in TA at ca. 80 and 130 ms from the onset of ankle rotation. A weak coactivation of SOL commenced after 130 ms. Head angular accelerations exceeded the threshold of $5^{0}/s^{2}$ for vertical nystagmus within 20ms and thereafter consisted of accelerations due to the perturbation. triceps surae reflex induced sway and forwards sway produced by TA activity. Maximum head accelerations occurred during forward sway and equalled, on average, $220^{0}/s^{2}$.

A marked reduction of both TA stabilising responses was noted in patients with bilateral lesions. Their neck EMG activity was enhanced with respect to normals, always increasing during angular accelerations pitching the head forwards.

Dramatic discoordination of stabilising responses occurred in patients with unilateral lesions. An asymmetry in the early TA responses was always observed (mean ratio of EMG area 1.9 ± 1.0 (s.d.), mean delay difference 4 ms; c.f. area 1.0 ± 0.2 and delay 1 ms in normals) and in 9 of 11 cases the response was larger in area and earlier contralateral to the lesion. Segmental reflex responses in SOL were, on average, unaffected, though in patients with the largest TA asymmetry, a SOL asymmetry was also observed; the largest and earlier response being ipsilateral to the lesion. While vision (eyes open v. closed) had no consistent effect on the TA responses at 130 ms in normals, in 5 patients, vision produced a significant improvement in the later TA response asymmetry. Head stabilizing performance was strongly affected, head accelerations exceeded $400^{0}/s^{2}$ during forward sway and was characterised by a failure of neck EMG activity rotating the head backwards to be coincident with TA activity rotating the body forwards. Instead, neck EMG activity commenced once the head rotated forwards with the body.

Thus patients with acute unilateral lesions experience instability not only because their ankle muscle responses tend to rotate the body towards the side of the lesion but also because centres coordinating neck muscle activity with reflex activity in TA malfunction. Presumably an imbalance of vertical semicircular canal input underlies this malfunction.

54.5 DOES THE HUMAN POSTURAL CONTROL SYSTEM ADAPT TO REPEATED PSEUDORANDOM STIMULI? C. Wall III and L.M. Nashner. Dept. of Otolaryng., Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15213, and Neurological Sciences Institute, Portland, Oregon 97210.

We tested the hypothesis that the human postural control system, which displays response adaptation to simple predictable stimuli (repeated 0.2 sec 50 deg/sec anterior-posterior velocity pulses) given at random intervals, would also display response adaptation to a second stimulus that was mathematically equivalent but possibly less predictable. A pseudorandom binary sequence (PRBS) velocity stimulus of bandwidth 0.04 to 3.33 Hz, having a repeat period of 25.5 seconds, was used as the less predictable stimulus. This stimulus was presented for six stimulus repetition periods and the response was averaged to increase signal to noise ratio.

It is possible to calculate an impulse response to the PRBS stimulus that is directly comparable to the actual velocity pulse response under certain assumptions. For both stimuli, A-P sway measured at the pelvis was the measured response. As expected, all subjects demonstrated rapid response adaptation to the time pulses which showed up as a decrease in the magnitude of the oscillatory aftercomponent of the response that occurred when the pulse was presented for the second or third time. By contrast, the PRBS impulse response averaged for six stimulus repetition periods showed no such adaptation and resembled the response to the first time pulse. In addition, the computed six individual impulse responses, although noisier than the average response, showed no systematic trends that would indicate response adaptation. There was considerable intersubject variability as indicated by the degree of "ringing" of the nonadapted time pulse response, but the degree of "ringing" for each subject correlated well between the time and PRBS responses.

In addition, the PRBS impulse response in three out of four subjects demonstrated a "small signal" linear range with respect to the fast dynamics, but a progressively greater amount of damping which occurred with increasing stimulus. Thus, it appears that the postural control system must have a simple, predictable stimulus to which to adapt and that a pseudorandom stimulus, although it is repeated several times, cannot be predicted and it is therefore useful in the study of the unadapted state of the postural control system.

54.6 AMPLITUDE AND TIMING OF POSTURAL AND TASK MUSCLES IN UNILATERAL SHOULDER FLEXION IN STANDING HUMANS. W.A. Lee, T.S.Buchanan*. Northwestern Univ. Dept. of Physiol. and Rehab. Inst. of Chicago, Chicao, IL 60611. Supported by the Coleman, Hearts, J.M.Joyce, Regenstein, Searle Foundations, and the L. Lavins.

In recent motor control studies, invariant temporal ordering of muscle patterns used to produce intentional actions has been emphasized. This study tested whether electromyographic (EMG) patterns in postural (hamstring:HM; erector spinae: ES) and task (anterior deltoid: AD) muscles demonstated invariant temporal order for 90 degree flexions made over an eight-fold range of speeds.

Ten standing subjects performed 15 trials at 5 target speeds (40, 80, 160, 320°/s and 'as fast as possible') in a unilateral shoulder flexion task. An oscilloscope displayed a constant velocity target to subjects. A shoulder potentiometer measured joint angle and provided feedback about the match between target and actual speeds. EMG activity from HM, ES, and AD muscles ipsilateral to the arm moved were also recorded. Trials at each movement speed were identified as showing either 'early' (pre-movement onset) or 'late' (post-movement) onset of HM, ES, and AD activity; these trials were averaged for estimation of EMG latencies.

Analysis of individual trials showed that proportions of trials with 'late' HM or ES onsets were greatest for slowest movements (25-100% of trials for all subjects). As movement speeds increased, proportions of these late trials decreased; for movement speeds greater than 90 degrees/sec, all trials showed HM, ES, and AD before movement onset. With respect to EMG phase relations, ES onset was invariably later than both HM and AD, regardless of movement speed. Order of HM and AD varied with movement speed. At low average speeds, HM followed AD onset (lag of 300 to 20 ms); at higher speeds (> 100 /sec), HM tended to precede AD onset (lead of 5 to 15 ms).

For 3 subjects, the relationships between EMG (latency, integrated values over the 100 ms after EMG onset) and average acceleration (first 100 ms of movement) and peak velocity were analyzed from individual trials. For slow movements, latencies of HM varied greatly (-200 to 2000 ms); for faster movements, AD and HM latencies were constant (-200 to -60ms), except for one subject showed an exponential decrease of AD onset with speed. Integrated AD and HM values increased linearly (r=.67-.92) with acceleration and peak velocity for all 3 subjects. Latencies and IEMG values for HM and AD were significantly correlated, suggesting commonality of drive to the two muscles.

54.7 INTRAMUSCULAR PARTITIONING OF THE FACIAL MUSCLES DURING SPEECH MOVEMENTS: POSSIBLE CORTICAL CORRELATES. J. H. Abbs,* V. L. Gracco,* and C. A. Blair. Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53706.

Most previous studies of functional intramuscular partitioning have focused upon the nature of these subdivisions at subcortical levels. The present studies were aimed at discerning whether functional muscle partitioning is evident also in "voluntary" behaviors requiring motor cortical actions. The activity of intramuscular subdivisions of facial muscles in human subjects was examined during select speech motor tasks. The perioral/facial muscles were chosen because these multifunctional muscles (1) participate in generating several different three-dimensional positions and shapes of the lips for speech (f, p, b, sh, u), (2) receive monosynaptic projections from the face area of the motor cortex, and (3) do not appear to be influenced significantly by lower brainstem perioral reflex pathways during voluntary speech movements; compensatory response latencies to unanticipated lip loads indicate long-loop sensorimotor pathways [Gracco and Abbs, Soc. Neuroscience Abstracts, 1982]. Collectively, these considerations suggest that if task-dependent partitioning is present in the facial muscles, these functional subdivisions may reflect motor cortex organization.

Two sets of data were obtained to address this hypothesis. Both experiments involved recording from multiple sites in three facial muscles (Orbicularis oris inferior, Orbicularis oris superior, and Mentalis) using restricted field, intramuscular, bipolar electrodes. In the first experiment, observations were made for several static speech positions of the lips and for some simple speech movements. Analyses revealed task-dependent intramuscular differences in the magnitude and timing of the multiple site EMG signals. In a second experiment, unanticipated loads were applied to the lower lip during several different speech movement tasks (p, b, f). The multiple intramuscular sites were observed to respond differentially to these perturbations with latencies that implicated long-loop pathways. The results from these experiments, taken together, indicate functional, task-dependent, intramuscular partitioning of the facial muscles and likely representation of these subdivisions at cortical levels. These results raise an interesting question concerning whether multiple motor cortical representations are of whole muscles, or rather reflect independent, task-dependent muscle subdivisions. Research supported by NIH grants NS-13274-07 and 5-P30-HD-03352-14.

54.8 DIFFERENTIAL SENSORIMOTOR ACTIONS FOR MOTOR PROGRAMMING AND CONTROL OF SPEECH MOVEMENTS. V. L. Gracco* and J. H. Abbs* (SPON: C. Welt). Speech Motor Control Labs., Waisman Center, University of Wisconsin, Madison, WI 53706.

Complex coordinated motor behaviors appear to involve moment-to-moment variations in the contribution of sensorimotor processes. Our results on perturbations applied to lower lip movements during speech similarly indicated temporal variations in sensorimotor actions [Gracco and Abbs, Soc. Neuroscience Abstracts, 1982]. In that study, compensations occurred in different combinations of multiple synergistic muscles of both the upper and lower lips with variable latencies (22-85 msec). In general, these compensatory responses were influenced by the timing between the load perturbation onset and the initiation of movement-related muscle activation.

In the present study, we investigated these compensatory response variations further in relation to systematic changes in the velocity and displacement parameters of the lower lip loads. Inferiorly directed loads (35-50 gms) were delivered to the lower lip via a DC brushless torque motor.

Perturbation displacement and velocity parameters influenced lip muscle compensatory responses differentially as a function of load onset time. Perturbations occurring 20-60 msec prior to muscle activation resulted in lower lip muscle compensation proportional to the displacement of the perturbation. Compensations to these early loads were predominately in the lower lip muscles; upper lip muscle compensations were not sensitive to displacement. By contrast, later loads introduced immediately prior to or during muscle activation resulted in compensatory responses that were (1) related to the perturbation velocity and (2) most prominent in the upper lip muscles. These data appear to reflect differential sensorimotor processes operating prior to and during motor execution, respectively. Indeed, compensatory responses to perturbations occurring during the pre-execution interval resulted in modification of the motor program with latencies corresponding to a rapid reaction time (65-100 msec). By contrast, responses to loads introduced later during the execution process were more suggestive of feedforward, sensorimotor actions; viz., velocity information from the lower lip load was used in an open-loop manner to adjust the motor output in the upper lip. Latencies of these latter responses were usually less than even a rapid reaction time (22-60 msec).

We interpret these results to suggest that peripheral afferent inputs interact with central neuromotor centers initially to set up the motor program, and once execution is initiated, to shape the details of the action based upon the kinematics of the evolving movement. Research supported by NIH grants NS-13274-07 and 5-P30-HD-03352-14.

54.9 PERIPHERAL REGULATION OF MUSCLE OUTPUT: INTERPLAY BETWEEN MOTOR UNIT RECRUITMENT AND FIRING RATE. H. Broman*, B. Mambrito* and C. J. De Luca (SPON: J. J. Bouyer). NeuroMuscular Res. Lab., Dept. of Orthopaedic Surgery, Children's Hosp. Med. Ctr., Harvard Medical School, Boston, MA 02115; and Liberty Mutual Res. Ctr., Hopkinton, MA 01748

The modulated output of a muscle is relatively smooth because the active motor units generally discharge asynchronously and with different contraction times. In such a scheme, the force transition through a level where a motor unit is recruited or decruited could conceivably be associated with quantal transitions. Such force behavior has rarely (if ever) been observed. Hence, an investigation was performed to observe the behavior of the firing rates of previously activated motor units when a new motor unit is recruited during a gradual increase in the force output of a muscle. This investigation was made possible by our recently developed myoelectrical decomposition technique which provides a verifiably accurate separation of every action potential of several simultaneously active motor units (LeFever and De Luca, IEEE BME 29:149-157, 1982). Experiments were performed on three normal, adult subjects. The interpulse intervals of 49 motor units in 23 records from the first dorsal interosseous, and tibialis anterior muscles were obtained while the subject gradually increased the force output of the monitored muscle. Force outputs ranged from 3 to 60% of the maximal level.

In all 15 records from the tibialis anterior muscle and 5 of 8 records from the first dorsal interosseous muscle, the firing rate of one or more of the previously activated motor units **decreased** after the recruitment of an additional motor unit. The firing rate decrease was affected by the characteristics of the force developed by the newly recruited motor unit: In cases of recruitment associated with a smooth output force, the previously activated motor units decreased their firing rates gradually over one or several seconds. If the newly recruited motor unit was associated with a twitch contribution to the output force, the firing rates of the previously activated motor units decreased in a step-wise fashion. Similar effects have been noted in data from the deltoid, flexor pollicis longus and extensor pollicis longus muscles.

Our data indicates that more than one mechanism is responsible for the observed inhibitory effect of a newly recruited motor unit on the firing rates of those already active. Among the possible candidates, recurrent inhibition and stretch reflex mechanisms originating from the newly recruited motor unit are the most likely.

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54.10 MOTOR CONTROL OF ANTAGONIST MUSCLES, B. Mambrito*, C.J. De Luca and H. Broman*. NeuroMuscular Res. Lab., Dept. of Orthopaedic Surgery, Children's Hosp. Med. Ctr., Harvard Med. School Boston, MA 02115; and Liberty Mutual Res. Ctr., Hopkinton, MA

The electrical activity of several concurrently active motor units (MU) has been simultaneously recorded from the human Flexor Pollicis Longus (FPL) and Extensor Pollicis Longus (EPL) muscles, respectively sole flexor and sole extensor of the distal phalanx of the thumb. The detected multichannel myoelectric signals have been decomposed into their constituent motor unit action potential trains (MUAPT) using a computer assisted algorithm (LeFever and De Luca, IEEE BME 29: 149-157, 1982), allowing accurate calculation of the firing times of up to 8 concurrently active motor units.

The experimental protocol, designed to promote different ME behavioral modes in the two antagonist muscles included the following isometric contractions: constant force, triangular force-varying, force tracking of unpredictable trajectories, and voluntarily initiated and sustained co-contraction of FPL and EPL with negligible net torque output at the joint (joint stiffening).

To date 11 contractions have been analyzed, including 56 decomposed MUAPTs. The following observation have been made:

MUs in the FPL and EPL are recruited in an orderly fashion (size principle) as already reported for other human muscles (De Luca et. al. J. Physiol. 329: 113-128, 1982)

During triangular force-varying isometric contractions, some MUs in the antagonist muscle may be recruited at the "apex" of the triangle when the slope of the force signal from positive (force increasing) becomes negative (force decreasing). This phenomenon is invariably evident when the subject has no prior knowledge of the force reversal.

When tracking unpredictable force trajectories having small oscillations (less the 10% of maximal voluntary exerted force) about the zero force level, co-activation of FPL and EPL is observed, but the firing rates of MUs in the two different muscles are "out of phase" i.e. when the firing rate of a MU in the FPL is increasing (together with the firing rates of the other active units in the same muscle), the firing rates of the observed MUs in the EPL are decreasing and vice-versa.

During voluntarily exerted FPL and EPL co-contraction (joint stiffening), firing rates of MUs in the two different muscles may fluctuate "in phase". This common behavior of firing rates of simultaneously active MUs has been observed within one muscle at a time (De Luca et. al. J. Physiol., 329: 129-142, 1982) and described in terms of common drive the motor-neuron pool. However, previously it had never been observed among firing rates of simultaneously active MUs belonging to different muscles.

The preliminary results indicate that reciprocal inhibition exists between antagonist muscles during voluntary contraction, but the control strategy is altered to co-contraction under specific, identifiable conditions. These results provide basic examples indicating that two antagonist muscles acting on the same joint can be controlled by different strategies while generating similar external joint torque. **(Supported by Liberty Mutual Insurance Company).**

54.11 MOTOR EFFECT OF RUBROSPINAL AND PYRAMIDAL TRACT ACTIVITY IN AWAKE CATS DEPENDS ON BEHAVIORAL CONTEXT. V.E. Amassian and D.E. Batson. Dept. of Physiol., SUNY, Downstate Med. Ctr., Bklyn, N.Y. 11203.

In the intact awake cat, flexors may be activated by stimulating forepaw hairs only if the limb hangs unsupported, a precondition for tactile placing. Possibly, depending on the behavioral context, spinal motor relays respond variably to a given descending discharge from the higher motor control centers. We tested this hypothesis as follows: 125 um nichrome wires insulated to the tip were implanted in the contralateral bulbar pyramid (PT), or ipsilateral rubrospinal tract (RT) in the medulla. Trains of 3-9 rectangular pulses, each 100 us in duration and with a frequency of 500-1,000 Hz were delivered to the PT or RT, usually through bipolar electrodes. Alternatively, the contralateral red n. was stimulated through a tungsten microelectrode, with monitoring of the decussated RT response; prior destruction of the bulbar pyramid ipsilateral to red n. stimulation did not change the results described below.

When the stimulus intensity to RT, red n. or PT was adjusted to evoke a short latency (8-10 msec) biceps EMG response in the hanging, unsupported forelimb, standing the limb on a solid abolished or markedly diminished the biceps response. Laying the forearm down so that the ventral surface was supported was less effective in diminishing the biceps response; in this position, the elbow was most flexed. If the stimulus train was triggered when the unsupported paw was brought gently into contact with a placing apparatus, the biceps response was greatly enhanced and its latency reduced to, e.g., 6 msec with PT and 5 msec with RT stimulation. Such stimulus trains often delayed subsequent placing of the forelimb. Contact alone did not elicit a biceps response at the latency of the response to the unconditioned stimulus train.

In general, the above changes in amplitude of biceps response were not paralleled by changes in the level of resting biceps activity immediately preceding the stimulus train, except when the train was delivered during a spontaneous movement. Such lack of coherence of resting and stimulated biceps activity implies that behavioral context affects the motor consequences of PT or RT activity not through interaction directly at the flexor motoneuron, but at the level of the spinal interneuronal relays.

Furthermore, both PT and RT activity elicited at short latency by tactile input would have its motor effect enhanced by tactile input locally relayed through spinal interneurons.

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54.12 CONTROL OF MULTI-JOINT ARM POSTURE. S. Hocherman*, F. A. Mussa-Ivaldi*, E. Bizzi and N. Hogan*. Dept. of Psychol., MIT, Cambridge, MA 02139.

The purpose of these experiments is to understand some of the neural and biomechanical factors underlying multi-joint arm posture in monkeys and humans. It is known that, under static conditions, the force generated by a muscle is a function of length and the level of alpha and gamma activation. The combined spring-like behavior of all of the arm muscles results in a stiffness field defining force at the hand as a function of displacement. This field has a shape, an orientation and magnitude. Controlling the magnitude, the shape and the orientation of the stiffness field of the hand in different portions of the work space is one of the important ways in which the central nervous system may control posture and interactions with the environment.

The goals of these experiments have been: (1) to explore whether arm movements converging on a location of the work space from different directions set up stiffness fields which differ in shape and orientation and (2) to investigate the time varying properties of the stiffness field after a given posture was reached. Subjects and monkeys grasped the vertical handle of a light-weight hand-position transducer and moved the handle to each of a series of visual targets. The arm was restricted to move in a horizontal plane and the wrist was braced. When the hand reached zero velocity, a perturbing force of known magnitude and direction was applied to the handle resulting in a hand displacement. We measured the amplitude, peak velocity and direction of the displacement. We also varied the time of the application of the perturbing force.

The results indicate that in both humans and monkeys, the direction of the preceding movement does not influence the shape and orientation of the stiffness field at the final position. These results were obtained for six targets placed in different locations of the work space. With respect to the question of the time varying properties of the stiffness field, we have found no significant variation in the shape and orientation of the field even with delays up to 850 msec from the onset of postural stability.

In conclusion, arm movements converging upon the same point of the work space from different directions set up stiffness fields which do not differ in any significant way in shape and orientation. This unexpected result represents a contribution to our understanding of the processes occurring during the transition between the end of the trajectory and the beginning of postural stability. (Research supported by NIH grants AM27610 and NS09343 and by NASA grant NAG2-126.)

55.1 INHIBITION OF SYMPATHETIC PREGANGLIONIC NEURONS DURING HEMORRHAGIC HYPOTENSION IN THE ANESTHETIZED CAT. Paul S. Blum and James A. Spath, Jr.*. Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107.

Experiments were performed to investigate the interaction of serotonin (5-HT) and opiate pathways on mean arterial blood pressure (MABP) and preganglionic sympathetic neurons in hypotensive cats. Groups of animals were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), paralyzed with gallamine, and prepared for recording splanchnic nerve (SPN) activity and MABP. Positive pressure respiration was adjusted to maintain normal blood gas values. Seven animals were pretreated with para-chlorophenylalanine (p-CPA, 300 mg/kg) 48 hr before the experiment. In these cats, 5-HT in the medulla and spinal cord was reduced more than 85%. Following baseline measurements, cats were hemorrhaged to a MABP of 40 ± 1 mm Hg for 1 hr. At that time, 12 intact cats and the cats pretreated with p-CPA were given the opiate antagonist naloxone (NAL, 2 mg/kg bolus followed by 2 mg/kg/hr). An additional 8 cats were given saline. The MABP and SPN activity are summarized below for 60 min, 65 min (5 min post NAL), and 80 min (20 min post NAL).

Minutes of Oligemia

	60 min		65 min		80 min	
Groups	MABP	SPN*	MABP	SPN	MABP	SPN
Saline	39 ± 1	75 ± 6	38 ± 1	71 ± 6	33 ± 1	70 ± 7
NAL	41 ± 1	67 ± 4	51 ± 2+	69 ± 6	47 ± 3	70 ± 8
p-CPA/NAL	40 ± 1	53 ± 13	60 ± 4+	58 ± 13+	56 ± 5+	66 ± 7+

*Activity expressed as percent of maximum.

+ $p<0.05$ paired t test, compared to 60 min.

These data suggest that preganglionic sympathetic neurons were inhibited by an opiate-mediated pathway and a 5-HT mediated pathway during hemorrhagic hypotension. When both pathways were blocked (p-CPA/NAL group), there was a sustained increase in MABP with a concomitant increase in SPN activity. Administration of NAL in intact animals resulted in a small pressor effect at 5 min but SPN activity was unchanged and the pressor effect was not maintained. The pressor effect in the NAL group, therefore, must be at a nonsympathetic site, perhaps in the periphery. (Supported in part by NIH grant #GM 30473, the American Heart Association of Delaware, and Endo Laboratories.)

55.2 PNEUMOTAXIC CENTER INFLUENCES ON THE RESPIRATORY PERIODICITY OF SYMPATHETIC NERVES. C. A. Connelly and R. D. Wurster. Dept. of Physiology, Loyola University Medical Center, Maywood, IL 60153.

Pneumotaxic center stimulation causes excitation or inhibition of phrenic activity, while ablation results in an apneustic breathing pattern in anesthetized, vagotomized cats. Pneumotaxic center stimulation also elicits cardiovascular pressor responses. (Mraovitch, Kumada and Reis, *Brain Res.*, 232:57-75, 1982). The present study was conducted to 1) investigate effects of ipsi- and contralateral pneumotaxic center stimulations on the respiratory modulation of sympathetic activity, and 2) determine the effect of the apneustic breathing pattern, resulting from bilateral pneumotaxic lesions, on the rhythmic activity of sympathetic nerves. Phrenic and inferior cardiac sympathetic nerve activities were recorded in alpha-chloralose anesthetized, paralyzed, ventilated, vagotomized cats. pH, blood gases, end-tidal CO_2, and rectal temperature were regulated within normal limits. Both ipsi- and contralateral stimulations (10-100 μA.) of the parabrachial areas by stereotaxically positioned coaxial electrodes evoked activation (60 msec delay) followed by inhibition of sympathetic nerve activity. Evoked activity predominated over the intrinsic respiratory modulation of sympathetic activity, irrespective of the phase of respiration. Following direct current (DC) lesions of the stimulated areas, evoked responses were eliminated. An apneustic breathing pattern was acquired when lesions were optimally extensive. All lesions were histologically verified. During the apneustic phrenic pattern, sympathetic activity was enhanced at the onset and inhibited at the termination of inspiration. When blood pressure oscillations were coordinated with sympathetic activation during apneusis, slow rhythmic waves (9-15/min) were superimposed on the phrenic-linked activation and inhibition of sympathetic activity. In conclusion, parabrachial areas have strong biphasic bilateral influences on sympathetic activity. Further studies are necessary to assess the nature of the slow sympathetic rhythms superimposed on the respiratory related sympathetic pattern during apneusis. (Supported by NIH Grant HL 27612)

55.3 IDENTIFICATION OF SPINAL SYMPATHETIC INTERNEURONS IN BARORECEPTOR-DENERVATED CATS. Susan M. Barman, Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

Do bulbospinal neurons mediate their effects directly on preganglionic sympathetic neurons (PSNs) or indirectly via spinal interneurons (INs)? Regarding this question, two types of neurons with cardiac-related activity were identified in the vicinity of the intermediolateral nucleus (IML) of the upper thoracic spinal cord of the cat by Gebber and McCall (Am. J. Physiol. 231: 722-733, 1976). Type 1 neurons were classified as PSNs since they were antidromically activated by electrical stimulation of their axons in the cervical sympathetic nerve. Type 2 neurons could not be antidromically activated by stimulation of the cervical sympathetic nerve and, thus, were considered to be INs in a spinal sympathetic network (i.e., sympathetic INs). That is, the possibility was raised that the cardiac-related activity in sympathetic nerves is transmitted from the brain stem to PSNs via a pathway that contains INs in IML. This interesting proposal requires further testing for two reasons. First, type 2 neurons may have been elements of a nonsympathetic network that shared baroreceptor input with PSNs. Second, these neurons may have been PSNs whose axons were not distributed to the cervical sympathetic nerve. Thus, this study was initiated to evaluate more critically the proposal that the IML region contains sympathetic INs. The second thoracic IML was searched for neurons whose spontaneous discharges were correlated to 2-6 c/s slow wave activity in the inferior cardiac postganglionic sympathetic nerve of baroreceptor-denervated cats. Bilateral section of the carotid sinus, aortic depressor and vagus nerves eliminated the coupling of sympathetic and nonsympathetic networks via shared baroreceptor input. Spike-triggered averaging revealed two types of neurons in the IML region with activity synchronized to the 2-6 c/s sympathetic nerve slow wave. PSNs (n=13) were identified antidromically by activation of their axons in the T_2 white ramus. PSNs exhibited minimum interspike intervals of 126±31 ms and mean firing rates of 0.9±0.2 Hz. The second group of neurons with sympathetic nerve-related activity (n=13) had minimum interspike intervals of 27±7 ms and mean firing rates of 2.8±0.5 Hz. These neurons could not be antidromically activated by stimulation of the segmental preganglionic nerve. Thus, these neurons were classified as sympathetic INs antecedent to PSNs. Furthermore, the observation that the discharges of both PSNs and INs were synchronized to a point near the peak of the 2-6 c/s inferior cardiac nerve slow wave supports the view that the INs subserve a sympathoexcitatory function. (Supported by a Michigan Heart Association Grant-in-Aid and PHS Grant HL-13187.)

55.4 MEDULLARY RAPHE NEURONS WITH SYMPATHETIC NERVE-RELATED ACTIVITY: BARORECEPTOR INPUT AND SPINAL CONNECTIONS. S.F. Morrison and G.L. Gebber. Depts. of Pharmacol./Toxicol. and Physiol./Biophys., Michigan State Univ., East Lansing, MI 48824.

We have previously used crosscorrelation analysis to identify single neurons in the cat medullary raphe nuclei with spontaneous activity related to inferior cardiac postganglionic sympathetic nerve discharge (SND). In the present study, we have classified these neurons into 3 groups on the bases of their spinal connections and responses to baroreceptor reflex activation.

Group 1 neurons were excited during 5 s periods of elevated carotid sinus pressure (i.e., baroreceptor reflex activation). Critically time-controlled collision tests demonstrated that these neurons could be driven antidromically by stimuli applied to the lateral funiculus of the cervical spinal cord and intermediolateral nucleus (IML) of the thoracic spinal cord. Threshold current for antidromic activation was as low as 20 μA. Axonal conduction velocity of group 1 neurons (n=34) was 1.9±0.7 m/s. These observations lead to the conclusion that group 1 neurons are involved in mediating spinal sympathoinhibition of baroreceptor reflex origin. Group 2 neurons also were excited during baroreceptor reflex activation. However, group 2 neurons could not be driven antidromically from the spinal cord. Thus, these neurons may be involved in mediating supraspinal sympathoinhibition of baroreceptor reflex origin. Nine of 28 group 2 neurons were orthodromically activated with constant onset latencies by stimuli applied to regions of the cervical spinal cord from which group 1 neurons could be antidromically driven. The onset latencies of antidromic and orthodromic responses of group 1 and 2 neurons, respectively, were indistinguishable. These observations raise the possibility that group 1 and 2 neurons are synaptically interconnected.

The discharges of group 1 and 2 neurons were synchronized to the 2-6 c/s rhythm in inferior cardiac nerve activity when blood pressure was lowered so as to disrupt the phase relations between SND and the cardiac cycle (i.e., functional baroreceptor denervation). This observation indicates that raphe neurons in the baroreceptor reflex pathway also are contained in or receive input from the brain stem oscillator responsible for the 2-6 c/s rhythm in basal SND. Thus, these neurons mediate sympathoinhibition of non-baroreceptor as well as baroreceptor origin.

Group 3 neurons were inhibited during periods of elevated carotid sinus pressure. These neurons could be driven antidromically from the lateral funiculus of the cervical spinal cord but not from the thoracic IML. Axonal conduction velocity of Group 3 neurons (n=18) was 1.8±0.6 m/s. Whether these neurons subserve sympathoexcitatory function remains to be determined. (Supported by PHS Grants HL-13187 and NS-06693.)

55.5 NEURONS IN THE VENTROLATERAL MEDULLA PROJECTING DIRECTLY TO THE INTERMEDIOLATERAL NUCLEUS RECEIVE BARORECEPTOR AND CHEMORECEPTOR AFFERENT INPUTS. M. M. Caverson, J. Ciriello and F. R. Calaresu. Department of Physiology, The University of Western Ontario, London, Canada, N6A 5C1.

It has been shown that neurons in the ventrolateral medulla (VLM) projecting directly to the region of the intermediolateral nucleus (IML) of the thoracic cord respond to electrical stimulation of the carotid sinus and aortic depressor nerves and of pressor sites in the paraventricular nucleus of the hypothalamus (Soc. Neurosci. Abstr. 8:722, 1982). The present study was done to determine the response of VLM units that project directly to the region of the IML to selective activation of peripheral baroreceptors or chemoreceptors. Experiments were done in four chloralosed, paralyzed and artificially ventilated cats. Silent and spontaneously active single units were identified in the reticular formation of the VLM by antidromic excitation to stimulation of functionally and histologically verified sites in the region of the IML at the level of T_2. Antidromically identified units were further tested for their response to activation of baroreceptors (2 µg/kg phenylephrine in 0.5 ml saline, i.v.) and chemoreceptors (20-60 µg sodium cyanide/0.1-0.3 ml saline via a cannula in the medial thyroid artery). Sixty-three units were antidromically excited in the VLM, of which 53 were spontaneously active and 10 silent. The firing frequency of 67% (42/63) of the units tested was altered by selective activation of either baroreceptors or chemoreceptors and 21 did not respond. Of the 42 responsive units, 27 increased their frequency of firing during chemoreceptor activation and also either decreased their firing frequency (n=5), increased their firing frequency (n=3), or were not affected (n=19) by stimulation of baroreceptors. Fourteen of the 42 units decreased their frequency of firing during chemoreceptor activation and also either increased their firing frequency (n=5), decreased their firing frequency (n=1), or were unaffected (n=8) by baroreceptor activation. One unit increased its firing frequency to stimulation of baroreceptors but was unaffected by chemoreceptor activation. These results demonstrate that selective activation of baroreceptors and chemoreceptors alter the activity of VLM neurons which send axons directly to the region of the IML, and suggest that these neurons are involved in complex integrative mechanisms in the control of the circulation.

(Supported by the Ontario Heart Foundation and MRC of Canada).

55.6 RELATIONSHIP BETWEEN ARTERIAL PRESSURE (AP) AND ACTIVITY OF SPINALLY-PROJECTING NEURONS IN THE VENTROLATERAL MEDULLA (VLM) D. Les Brown and Patrice G. Guyenet, Univ. of Virginia, School of Medicine, Dept. of Pharmacology, Charlottesville, VA 22908

The intermediate portion of the VLM contains a population of adrenergic neurons which project to the lateral horn and are thought to be involved in regulation of the sympathetic nervous system. The present electrophysiological experiments were undertaken to explore the VLM for neurons with spinal projections and with discharge characteristics suggesting a role in the control of AP. Experiments were performed in Sprague-Dawley rats, anesthetized with urethane (1.5-2.0 g/kg), paralyzed and artificially respirated. End-tidal CO_2 was kept at 3.5-4.5%. AP was recorded via the femoral artery. Drugs were administered through the tail vein. Mean AP was 88 mmHg and could be lowered to 55 mmHg with the ganglionic blocker trimethaphan (1.2 mg/kg), suggesting the presence of sympathetic nervous system activity.

An area 0.2 to 1.0 mm posterior to the caudal end of the facial nucleus (identified with field potentials), 0.2 to 0.8 mm above the ventral brain surface, and 1.4 to 2.0 mm lateral to the midline was systematically explored for cells antidromically activated with a stimulating electrode positioned in the cervical or thoracic spinal cord. Recording sites were verified by histological localization of fast green deposits and electrode tracks.

In 9 animals, the activity of 31 cells were studied in detail. Antidromic-orthodromic spike collision was demonstrated in 13 cells. Axonal conduction velocities ranged from 1.8 to 23.2 m/s. One group of cells (n=6) exhibited changes in firing rate opposite to spontaneous and drug-induced fluctuations in AP. Drugs used to modify AP were norepinephrine (50 µg/kg), arg-vasopressin (3 µg/kg), trimethaphan (750 µg/kg), and sodium nitroprusside (75 µg/kg). Five additional cells, which could not be antidromically activated, showed similar responses to changes in AP. Two cells were completely silent at AP above 130 mmHg. Other cells were nearly silent when AP was raised to 90-150 mmHg. The effect of clonidine (20 µg/kg) was tested on four cells. Activity was decreased approximately 50%, based on estimates of expected firing rates during the lower AP which clonidine produced. During clonidine inhibition, activity remained sensitive to changes in AP. A second group of cells (n=4) exhibited changes in firing rate similar in direction to fluctuations in AP. Only one of the cells could not be antidromically activated. Three cells were completely silenced by lowering AP to 40-80 mmHg.

These data show the presence in the VLM of at least two types of spinally-projecting neurons which exhibit prominent changes in activity in response to changes in AP: one of these types may represent the C1 adrenergic neurons. Supported by NIH HL28785.

55.7 ADRENALINE NEURONS OF ROSTRAL VENTROLATERAL MEDULLA MEDIATE BARO- AND CARDIOPULMONARY REFLEXES. A.R. Granata, D.A. Ruggiero, D.H. Park, T.H. Joh and D.J. Reis, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Adrenaline neurons of the C1 group of the rostroventrolateral medulla (RVL) project bilaterally to the intermediolateral column of the spinal cord (Ross et al., Brain Res., in press); they are also directly innervated by an ipsilateral projection from cardiovascular nucleus tractus solitarius (NTS) (Ruggiero et al., Neurosci. Abst., 1982). We sought to determine if the NTS-C1 projection mediates the vasodepressor reflex (VDR) elicited by carotid sinus stretch (CSS) or electrical stimulation of the central end of the vagus nerve (VS). Rats were anesthetized, paralyzed and ventilated. Arterial pressure (AP) and heart rate (HR) was recorded. Lesions were placed electrolytically, and agents were microinjected into the brain in volumes of 0.1 µl, delivered from a micropipette. C1 neurons were localized immunocytochemically with antibody to PNMT. After bilateral electrolytic lesions of C1, MAP and HR fell to (from 99.8 ± 6.2 mmHg and 375.5 ± 30 bpm to 50.1 ± 5.2 mmHg and 223.6 ± 15.0 bpm; n=8, $p<.001$), levels comparable to those produced by spinal cord transection. The VDR to CSS and VS were abolished. In all cases, the lesions destroyed the majority of C1 adrenaline neurons on both sides. To isolate the left NTS-C1 pathway and yet maintain MAP, a lesion was placed in the right (r) NTS. This lesion elevated MAP and reduced the VDR to CSS or VS by only 37% (before -31.7 ± 2.8 mmHg; after lesion -19.9 ± 3.5 mmHg, n=8, $p<0.05$). The combined rNTS plus subsequent left C1 area lesions maintained MAP at normal levels (95.1 ± 3.3 mmHg before lesion 87.4 ± 3.5 mmHg after lesion, n=9, NS). After the lesion of rNTS, a microinjection of tetrodotoxin (TTX) (10 pmoles) or a lesion of the left C1 area abolished the VDR to CSS or VS (before, -30.6 ± 4.1 mmHg; after NTS/C1 lesions, -1.1 ± 0.8 mmHg, n=8, $p<0.001$). A rNTS lesion was followed by a microinjection in the C1 area of kainic acid (400 pmole; n=6) or glutamic acid (200 nmole; n=6) which block neuronal activity by persistant depolarization of neurons but not fibers. Both agents transiently increased AP during the first 15 min after the injection. Both abolished the VDRs 30 min after the injection when AP had returned to control levels. Complete blockade of the VDR was also achieved by lesioning the C1-spinal pathway in the dorsomedial medulla. Lesions avoiding both the C1 pathway and C1 perikarya, i.e. nucleus gigantocellularis pars dorsalis and ventralis (n=4), lateral or ventral aspect of the lateral tegmental field (n=3), spinal trigeminal nucleus (n=3), vestibular complex (n=2), inferior olive (n=3), adjacent medullary serotonergic nuclei (n=3), or A1 area (n=3) did not alter VDR. The results are consistent with the hypothesis that: (a) C1 neurons mediate the vasodepressor responses to stimulation of cardiopulmonary and arterial baroreceptors. (b) Afferent information reaches C1 by an ipsilateral projection from NTS. (Supported by Grant HL18974).

55.8 ELECTROPHYSIOLOGICAL IDENTIFICATION OF NEURONS IN THE VENTROLATERAL MEDULLA THAT SEND COLLATERAL AXONS TO THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI AND RECEIVE CARDIOVASCULAR AFFERENT INPUTS IN THE CAT. J. Ciriello and M. M. Caverson. Department of Physiology, The University of Western Ontario, London, Canada N6A 5C1.

Direct projections from neurons in the ventrolateral medulla (VLM) to the paraventricular (PVH) and supraoptic (SON) nuclei of the hypothalamus have recently been demonstrated both anatomically and electrophysiologically. The finding in these previous studies that the anatomical distribution of neurons in the VLM projecting directly to the PVH and SON overlapped, suggested the possibility that some of these VLM neurons may send collateral axons to both hypothalamic structures. This possibility was investigated in a series of experiments done in chloralosed, paralyzed and artificially ventilated cats. The region of the VLM was initially explored for single units antidromically activated by stimulation of histologically verified sites in the SON. Antidromically identified units were then tested for antidromic activation to stimulation of histologically verified sites in the PVH. Single units antidromically activated by stimulation of both sites were then tested for their orthodromic response to stimulation of the carotid sinus (CSN) and aortic depressor (ADN) nerves. Twenty-one units were identified in the VLM which were antidromically activated by stimulation of both the PVH and SON. Each unit followed similar frequencies of stimulation and had similar conduction velocities to stimulation of the PVH and SON. In addition, the antidromic spike evoked by stimulation of the PVH was always cancelled by the previously evoked spike to stimulation of the SON. These units responded with latencies corresponding to conduction velocities of 1.4-11.4 m/s. Of the 21 antidromically identified units, 4 responded orthodromically by excitation only to stimulation of the CSN (mean latency, 9.0 ± 2.1 ms) and 5 units were excited orthodromically to stimulation of only the ADN (mean latency, 11.2 ± 2.8 ms). The remaining 12 units did not respond to either buffer nerve input. These data provide electrophysiological evidence of neurons in the VLM sending collateral axons to both the PVH and SON, and suggest that these neurons are involved in the release of vasopressin by SON and PVH magnocellular neurosecretory neurons during activation of cardiovascular afferent fibers.

(Supported by the Ontario Heart Foundation).

55.9 RELEASE OF SUBSTANCE P FROM RAT SPINAL CORD AFTER KAINIC ACID INJECTIONS INTO THE VENTRAL MEDULLA. Y. TAKANO*, J.E. MARTIN, S. LEEMAN†, A.D. LOEWY. Depts. of Anatomy & Neurobiology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110 and †Dept. Physiology, University of Massachusetts Medical School, Worcester, MA 01605

The ventral medulla plays a critical role in maintaining blood pressure by providing tonic excitation of sympathetic vasomotor neurons. Both serotonergic-like (Brain Res. 211:146, 1981) and substance P-like (Brain Res. 243:147, 1982) neurons of the ventral medulla project mainly to the intermediolateral cell column (and to a lesser degree to the ventral horn).

In the present study, we have measured the release of endogenous substance P or a substance P-like peptide (SP) from rat spinal cord after activating the ventral medulla with a unilateral 1-µl microinjection of 40 mM kainic acid. These injections were centered on the area of the nucleus interfacicularis hypoglossi but spread approximately 1 mm rostrally and caudally. The spinal cords (the T1-L1 spinal segments) of rats anesthetized with sodium pentobarbital (35 mg/kg) were superfused in vivo at a rate of 50 µl/min with phosphate buffered saline (Physiol Behav. 17:1031, 1976). Every 10 minutes, a fraction was collected, freeze-dried, and subsequently assayed for SP by radioimmunoassay. Assay sensitivity was 6.5-9.0 pg/tube. Kainic acid injections caused an almost immediate elevation in mean arterial pressure which reached a maximum after 10-20 min. This is mediated by a descending pathway (Brain Res. 243:147, 1982) which excites the sympathetic preganglionic neurons. This response can be blocked by intravenous injections of a ganglionic blocker, hexamethonium (2 mg/kg). Concomitant with this rise in blood pressure is a 2-fold increase in the amount of SP in the superfusion samples which lasts for 20 minutes. In order to measure basal levels of SP, 10-min fractions from two rats had to be pooled (~10 pg/10 min). After kainic acid, the SP levels reached approximately 20 pg/10 min.

There are several possible neural pathways that could be the source of the increased SP-immunoreactivity that appears in the cerebrospinal fluid. The most likely pathway is the descending SP-like pathway from the ventral medulla to the sympathetic preganglionic cell column and ventral horn (Brain Res. 243:147, 1982). However, it is possible that intrinsic SP interneurons of the spinal cord could be activated by descending medullary pathways (Soc. Neurosci. Abstr. 8:584, 1982). (Supported by USPHS Grants HL 25449 and AM 29876.)

55.10 CARDIOVASCULAR EFFECTS OF ELEVATED VASOPRESSIN AND NOREPINEPHRINE AFTER VENTROLATERAL BRAINSTEM LESIONS IN THE RABBIT. J.M. Elliott,* M.J. West and J.P. Chalmers.* Centre for Neuroscience, Flinders University, South Australia, 5042.

Electrolytic lesions of the ventrolateral medulla coinciding with the A1 group of norepinephrine cells (A1 lesions) produce acute hypertension, bradycardia and in some animals pulmonary oedema. Since A1 neurons project to regions of the hypothalamus rich in vasopressin (AVP)-synthesizing cells, and since they were also able to demonstrate an increase in plasma AVP after A1 lesions, Blessing et al (Science 217, 661, 1982) suggested that the increase in blood pressure after A1 lesions is dependent on the release of AVP into the systemic circulation. In this study we have examined the effects of the vasopressin antagonist (VA; $(CH_2)_5$Tyr(Me)AVP) on the cardiovascular response to A1 lesions and compared the responses after A1 lesions with those produced by infusions of AVP.

Arterial pressure, heart rate and cardiac output (Doppler flow velocity) were monitored in conscious animals before and for one hour following A1 lesions or infusions of AVP (2.0 ng/kg/min). In some animals VA was given prior to A1 lesions (40 µg/kg iv) or to AVP infusions (10 µg/kg iv). Other animals were pretreated with propranolol and scopolamine so as to produce cardiac autonomic block. Blood samples were taken to measure plasma AVP and norepinephrine (NE) levels.

After typical A1 lesions the animals developed the usual hypertension and bradycardia with a 25% fall in cardiac output and a 100% increase in peripheral resistance; in these animals plasma NE levels doubled and plasma AVP levels rose 10-20 fold. In other animals with less complete lesions, which developed the usual bradycardia without any hypertension, plasma NE did not rise but plasma AVP again rose 10-20 fold. Administration of VA before A1 lesions had no effect on the hypertensive response but reduced the bradycardia by 50%.

Other rabbits not subjected to A1 lesions received infusions of AVP which raised the plasma concentration of AVP some 10-20 fold to levels seen after A1 lesions. The infusions depressed the heart rate and cardiac output by 10%, but did not elevate arterial pressure though peripheral resistance rose by 15%. These effects of AVP infusions were blocked by pretreatment with VA but were unaffected by administration of propranolol plus scopolamine.

Increased levels of plasma NE after A1 lesions probably reflect increased activity of peripheral sympathetic nerves contributing to the vasoconstriction and hypertension. In contrast, the increased AVP levels may be more important in relation to the bradycardia than the hypertension and may be exerting a direct depressant effect on the heart.

55.11 LESIONS OF A1 AREA OF RAT VENTROLATERAL MEDULLA INCREASE CARDIAC OUTPUT AND ARTERIAL PRESSURE WITHOUT MODIFYING MECHANORECEPTOR REFLEXES. T. Imaizumi*, A.R. Granata, A.F. Sved, E. Benarroch* and D.J.Reis, (SPON. JOANNE CARROLL) Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

In rabbits and rats, bilateral electrolytic lesions of the area of the caudal ventrolateral medulla containing A1 noradrenergic neurons (A1 area) produces fulminating hypertension (A1 hypertension) dependent, in part, on release of vasopressin (Blessing et al., Science, 1983; Imaizumi et al., Fed. Proc., 1983). We sought to characterize the hemodynamics of A1 hypertension and to determine if the area is involved in the mediating baro- and cardiopulmonary mechanoreceptor reflexes. Rats were anesthetized with halothane and the femoral artery and right jugular vein cannulated. A thermoprobe (YSI 520) was introduced from the right carotid artery to the aortic root. Cardiac output (CO) was measured by the thermodilution method. Basal values of mean arterial pressure (MAP), HR and CO were measured 30 minutes after discontinuation of halothane in the awake animal (control). Rats were then re-anesthetized, paralyzed and mechanically ventilated. Bilateral electrolytic lesions were placed in the A1 area or nucleus tractus solitarius (NTS). Halothane and mechanical ventilation were discontinued. All measurements were done 60 min after lesion in the awake animal. After A1 lesions (n=5), MAP increased from 118 ± 5 to 158 ± 3 mmHg ($p<0.005$) while HR did not change (424 ± 19 to 374 ± 10 bpm; ns). CO increased from 4.13 ± 2.1 to 4.99 ± 2.8 (ml/min/100g); $p<0.001$) consequent to increased stroke volume (SV) (1.12 ± 0.07x10^{-1} to 1.35 ± 0.11x1^{-1} (ml/beat/100 g; $p<0.025$). Vascular resistance (VR) remained unchanged (0.74 ± 0.05 to 0.83 ± 0.11; ns). The vasodepressor response elicited by supermaximal electrical stimulation of the central end of the transected vagus nerve (.8MA 2 msec pulse, 10 sec train) was examined in four anesthetized rats. Before A1 lesions, vagal stimulation decreased MAP by -33 ± 7 mmHg and after lesions -32 ± 5 mmHg (ns). We compared the cardiodynamics of A1 lesions with those produced by lesions of the NTS. In NTS hypertension (n=5), MAP increased from 118 ± 3 to 176 ± 2 mmHg ($p<0.005$); HR from 398 ± 11 to 456 ± 14 bpm ($p<0.025$); CO decreased from 39.0 ± 2.0 to 24. 8 ± 2.6 (ml/min/100g) ($p<0.005$) and SV from 0.92 to 0.04x10^{-1} to 0.55 ± 0.06x1^{-1} (ml/beat/100g) ($p<0.005$). VR increased from 0.78 ± 0.04 to 1.88 ± 0.20 (units) ($p<0.005$). We conclude that: (a) A1 hypertension is largely due to increased CO in contrast to NTS hypertension in which CO is decreased while peripheral resistance is enhanced; (b) A1 hypertension is not the result of impairment of vasodepressor reflexes. (Supported by Grant HL18974).

55.12 NEURAL PROJECTIONS FROM PARAVENTRICULAR NUCLEUS RESPONSIBLE FOR CARDIOVASCULAR FUNCTION PASS THROUGH THE VENTROLATERAL MEDULLA. J.P. Porter* and M.J. Brody. Dept of Pharmacology and the Cardiovascular Center, University of Iowa, Iowa City, IA. 52242.

Previous studies in our laboratory showed that the cardiovascular response produced by right PVN stimulation was not affected by a medullary knife cut designed to interrupt the neural connections of the right NTS. These data suggested that the neural pathways from the PVN which are responsible for cardiovascular control pass through the ventral medulla. Since these experiments were performed unilaterally, the possibility remains that crossing fibers mediated the effect. In the present investigation, studies were carried out to identify more clearly the pathway taken by projections from the PVN which subserve cardiovascular function by 1) eliminating crossing fibers with a medullary knife cut and 2) directly blocking neural conduction by injecting a local anesthetic into the proposed projection site; ventrolateral medulla.

Rats were instrumented with miniaturized pulsed Doppler flow probes and a femoral arterial catheter. Changes in arterial pressure and mesenteric, renal, and hindquarter resistances were determined during right PVN stimulation before and after a total left medullary hemisection and after an additional right NTS undercut. The undercut was made by lowering an L-shaped knife 1.5 mm below calamus scriptorius on the midline and rotating it 180° to the right. Stimulation of the right PVN increased arterial pressure 15 ± 4 mmHg, mesenteric and renal resistances 261 ± 40 and 69 ± 22% respectively, and decreased hindquarter resistance 29 ± 8%. Total left medullary hemisection lowered pressure from 94 ± 3 to 69 ± 4 mmHg but the response to PVN stimulation was unaffected. Right NTS undercut, in addition to the left medullary hemisection, did not affect baseline pressure and the response to right PVN stimulation was essentially intact except for a reduction in mesenteric constriction.

In separate experiments, the left medulla was transected using the ventral approach. Injection of lidocaine (200 nl of 4% solution) into the right ventrolateral medulla reduced pressure and the response to PVN stimulation was significantly attenuated. Twenty min after lidocaine the response returned to control values.

These data support our original observation that the projections from PVN which subserve vasomotor functions pass through the ventrolateral medulla. Monosynaptic projections from PVN to intermediolateral column of the spinal cord have been demonstrated to pass through ventrolateral medulla. We hypothesize that this monosynaptic projection subserves cardiovascular functions.

55.13 SELECTIVE ACTIVATION OF VASOCONSTRICTION AND VASODILATION BY MICROELECTRODE STIMULATION OF DISCRETE NUCLEI WITHIN THE AV3V REGION. M.J. Brody and M.L. Mangiapane, Dept. of Pharmacol. and the Cardiovasc. Center, U. of Iowa, Iowa City, IA 52242.

Electrical stimulation of the AV3V region with constant voltage and large bipolar electrodes results in a complex pattern of hemodynamic responses: a small decrease in arterial pressure, hindquarter vasodilation, and mesenteric and renal vasoconstriction. The overall decrease in arterial pressure is not consistent with the protective effects of AV3V lesions against many forms of experimental hypertension. However, since separate constrictor and dilator projections could be simultaneously activated by AV3V stimulation, the purpose of the present experiments was to determine the regional blood flow and arterial pressure responses stemming from microelectrode stimulation of discrete nuclei within the AV3V region. Urethane-anesthetized rats were instrumented with femoral arterial catheters and with pulsed Doppler flow probes on the renal and superior mesenteric arteries and on the abdominal aorta. Constant-current stimulation (100 µamps) was delivered to the dorsal (DNM), anterior (ANM), and ventral (VNM) nucleus medianus, periventricular preoptic nucleus (POP), and organum vasculosum-lamina terminalis (OVLT) region using stereotaxically-placed tungsten microelectrodes. The following table summarizes the changes in hindquarter (HQ R), mesenteric (M R), and renal (R R) vascular resistance and arterial pressure (AP) produced by stimulation of these nuclei (NS = no significant change):

		PERCENT CHANGE		
	AP(mmHG)	HQ R	R R	M R
POP	-13	-30	NS	NS
VNM	-12	-33	NS	NS
ANM	-13	-29	NS	NS
DNM	+9	+16	+15	+15
OVLT	+13	NS	NS	+120

These results indicate that the OVLT area of the AV3V region can generate an increase in arterial pressure. This is consistent with the findings that AV3V lesions attenuate or eliminate many forms of experimental hypertension. In addition, the finding that DMN stimulation elicits a pressor response and uniform vasoconstriction shows that stimulation of DNM evokes responses qualitatively similar to those elicited by subfornical organ (SFO) stimulation. This suggests that efferents of the SFO are activated by DNM stimulation, since these efferents are known to pass through the DNM en route to the AV3V and more caudal hypothalamic regions. Finally, the data indicate that vasodilator responses can be activated selectively within the AV3V region and that these effects are responsible for the overall depressor response elicited by AV3V stimulation.

55.14 MECHANISMS OF THE HEMODYNAMIC RESPONSES TO ELECTRICAL STIMULATION OF SUBFORNICAL ORGAN (SFO) IN NORMAL AND SINOAORTIC-DENERVATED RATS. M.L. Mangiapane and M.J. Brody, Dept. of Pharmacology and the Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242. SPON: Phillip G. Schmid, M.D.

Along with other circumventricular organs, the SFO has been implicated in the mediation of pressor responses to circulating angiotensin II. We recently described increases in arterial pressure and regional vasoconstrictor responses produced by electrical stimulation (ES) of the SFO. The purpose of the present experiments was to investigate the mechanisms and central pathways involved in the pressor and regional hemodynamic responses to SFO-ES in normal rats and in rats with sinoaortic baroreceptor denervation. Rats were anesthetized with urethane and instrumented with femoral artery catheters and with pulsed Doppler flow probes on the superior mesenteric and renal arteries and on the abdominal aorta. Constant-current stimulation (200 µamps) was delivered to the SFO via stereotaxically placed tungsten microelectrodes. SFO-ES resulted in frequency-dependent pressor responses and vasoconstriction in all vascular beds tested. At 32 hz, these responses were: a 14 mmHg increase in arterial pressure and resistance increases of 26% in the hindquarters, 33% in the mesenteric, and 13% in the renal bed. Movement of the electrode away from the SFO produced significantly smaller responses. Intravenous administration of a specific antagonist of the pressor activity of vasopressin significantly attenuated the responses to SFO-ES, while intravenous administration of the ganglionic blocker chlorisondamine completely eliminated the responses. Coronal knife cuts placed in the area of the lateral hypothalamus-medial forebrain bundle also attenuated the responses, while dorsally placed control cuts had no effect. Rats given sinoaortic denervation one week before the experiment had resting arterial pressure under urethane anesthesia which was similar to normal rats but yielded pressor and regional vasoconstrictor resonses more than twice as large. In addition, these sinoaortic-denervated rats responded to current levels as low as 30 µamps. These results indicate that (1) electrical stimulation of the SFO elicits uniform vasoconstriction which is most pronounced in the mesenteric bed; (2) the sympathetic nervous system is responsible for the effects but there may be facilitation of the responses by vasopressin; (3) a significant portion of the response is mediated through the medial forebrain bundle; (4) sinoaortic denervation greatly enhances the responses to SFO stimulation.

FEEDING AND DRINKING: CENTRAL MECHANISMS I

56.1 BOMBESIN: ANOTHER PEPTIDE THAT INHIBITS FEEDING IN MAN. N.E. Muurahainen*, H.R. Kissileff, J. Thornton* and F.X. Pi-Sunyer*, Obesity Research Center, St. Luke's Hosp. and Columbia University Institute of Human Nutrition, New York, N.Y. 10025

Exogenously administered bombesin (BBS) and cholecystokinin (CCK) produce a dose-related inhibition of food intake in animals without overt malaise. However, these neurogastrointestinal peptides are distributed differently and appear to modulate food intake by separate mechanisms, (1).

To determine whether BBS, like CCK, also reduces food intake in man, eight lean healthy men received intravenous infusions of either .15 M saline or BBS (4.0 ng.kg.$^{-1}$min^{-1}) during single course meals. The dosage of the synthetic BBS tetradecapeptide was estimated to be within physiological range. On 6 nonconsecutive days, each subject ate a 300 kcal breakfast 2.5 h before a saline infusion was started. Ten minutes after the saline infusion began, subjects received a 200 kcal preload of crackers and jelly. Seven minutes after the preload a switch was made to a second I.V. reservoir which contained either BBS or saline. On the first 2 days (adaptation) the second reservoir contained saline. On the last four days (test) it contained either BBS or S in a double-blind counterbalanced sequence such that each subject received 2 days of infusion with each. The design enabled measurement of both direct effect and residual effect (effect of treatment on succeeding days, e.g. by learning).

At the time either BBS or S from the second reservoir entered the vein (12 min. after consuming the preload) a liquified test meal of yogurt and fruit (0.91 kcal/g; 18% protein, 65% carbohydrate and 17% fat) was consumed by straw. The infusion continued until 5 min. after the subject voluntarily stopped eating.

For the test days, intake was significantly ($p < .05$) less by 135.5 g ± 34.3 SE (direct effect) during BBS (722.9 g) than during S (858.4 g). Reductions in intake due to residual effect (82.8 g ± 41.4 SE) were not significant ($p > .05$). Meal duration was 1.63 min (±.87) less during BBS (7.45 min) than during S (9.10 min). There were no overt side effects, but in a postmeal questionnaire, the subjects reported a sick sensation in the stomach (where 0=none 1=slightly, 2=moderately, 3=very, and 4=extremely) of greater magnitude (1.0 ± .23 SED) after BBS ($\bar{x}$=1.25) than after saline ($\bar{x}$=0.25). Reduction of food intake was not significantly related to the magnitude of the sick sensation ($r = .47$, $p > .05$). These results are similar to those we observed with CCK (2,3). BBS may be a natural appetite suppressant in man.

(1) Stein and Woods Peptides 2:431, 1981, (2) Kissileff et al Am.J.Clin.Nutr. 34:154, 1981,(3) Pi-Sunyer et al Physiol. and Behav. 29:627, 1982.

56.2 THE ROLE OF DYNORPHIN (1-17) AND KAPPA OPIATE RECEPTORS IN THE REGULATION OF FEEDING. J.E. Morley and A.S. Levine. Neuroendocrine Research Lab, Minneapolis VAMC, Minneapolis, MN, 55417.

A growing body of evidence has suggested a role for endogenous opioid peptides in the modulation of feeding. Dynorphin is the putative endogenous ligand for the kappa opioid receptor. Much evidence has suggested a role for the kappa receptor in the regulation of food intake. We have examined the effect of ICV administration of dynorphin (1-17) and a variety of dynorphin fragments on food intake. Also we examined the effect of the highly selective kappa opioid agonist U-50,488 on food intake and compared its effects to morphine and other opiate agonists. Dynorphin (1-17) ICV (20 µg) is a potent inducer of food intake with a latency of 17.2 ± 3.1 minutes. This effect is highly specific as it is not accompanied by any significant changes in fluid ingestion, or time spent resting, grooming or moving. Dynorphin (1-17) did produce a slight increase in rearing behavior during the first 60 minutes following administration. This effect on feeding is blocked by naloxone. Neither dynorphin (1-8) or (1-9) increased food ingestion. Dynorphin fragments (1-10), (1-11) and (1-13) all increased feeding, but were not as potent as (1-17). Dynorphin (3-13) also increased feeding whereas the effect of (6-17) was not statistically significant. These studies suggest an important role for the non-opioid as well as the opioid moeity of dynorphin inducing feeding. Dynorphin B (rimorphin) also enhances food intake.

U-50,488 increases food intake after subcutaneous administration and its effect is blocked by naloxone in a stepwise manner. The early effects of U-50,488 were more marked than morphine and were produced at lower doses. The other kappa agonists, butorphanol, ketocyclazocine and tifluadom all significantly increased feeding. These studies suggest that the kappa opioid receptor is involved in modulation of food ingestion.

Based on these contradictory findings we suggest that the dynorphin receptor involved in the modulation of feeding consists of two segments (the "double-lock" concept). The non-opioid moeity of dynorphin acts as a key allowing access of the opioid moeity to the kappa-like feeding receptor. These studies confirm the highly specific role of dynorphin (1-17) in the induction of feeding and suggest a central role for kappa opioid receptors in the opioid modulation of feeding.

56.3 BEHAVIORAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF THE FUNCTION OF CHOLECYSTOKININ IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. P.L.Faris*, A.C.Scallet, J.W.Olney, M.A.Della-Fera and C.A.Baile
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The hypothalamic paraventricular nucleus (PVN) has been suggested by several investigators to be a critical site for the control of food intake. Both norepinephrine (NE) and opiate microinjections into this region increase food intake, whereas cholecystokinin (CCK) injected directly into the PVN has been shown to inhibit NE-induced feeding (McCaleb & Meyers, Peptides 1, 1980). Although it is generally believed that CCK does not play an important role in the central control of food intake in the rat, the action of CCK on NE-induced feeding and the fact that CCK immunoreactive cell bodies and fibers are numerous in the PVN prompted us to examine the effect of CCK microinjected into this region on spontaneous feeding. Following recovery from unilateral implantation of chronic indwelling cannulae in PVN, each rat received saline and CCK octapeptide (5 ng/μl) on alternate days in a counterbalanced fashion. Immediately following injection, animals were presented with a wet mash of powdered lab chow. The uneaten food and spillage was weighed one hour later. CCK significantly attenuated feeding ($25\%/p<0.05$) under these circumstances. This finding suggests that either local circuit CCK neurons in PVN or extrinsic CCK inputs to PVN may play a role in the physiological regulation of food intake. Since CCK and opiates have been shown to be physiological antagonists of each other in several systems, including the guinea pig ileum assay, (Jurna & Zetler, Eur J Pharmacol 73, 1981) and opiate analgesia (Itoh & Katsura, Eur J Pharmacol 80, 1983; Faris et al., Science 219, 1983), one intriguing possibility is that the PVN represents a site of integration for opiate-stimulation and CCK-inhibition of food intake. If this were the case, then opiate and CCK neurons might be anatomically connected in this region. Using standard immunohistochemical techniques, we have stained CCK and proopiomelanocortin (POMC) neurons on alternate sections (20 μm) of rat brain through the region of the PVN. In superimposed drawings of these sections made using a drawing tube attachment to a light microscope, POMC nerve fibers arising from the arcuate nucleus appear to abut CCK cell bodies in the PVN. We have also employed double-staining techniques using a PAP-immunofluorescence combination to demonstrate this same neural pattern of interaction between CCK and opiate-containing neurons. More definitive evaluation of POMC/CCK neuroanatomical connections in PVN awaits electron-microscopic application of double labeling methods. Supported in part by DA-00259, NS-20000 and RSA MH-38894 (JWO).

56.4 VENTROMEDIAL HYPOTHALAMIC LESIONS REDUCE BRAIN β-ENDORPHIN IN THE RAT. C.W. Richard III, K.K. Vaswani*, G.A. Tejwani, and J.R. Bianchine. Department of Pharmacology, The Ohio State University, College of Medicine, Columbus, Ohio 43210

Recent research has focused on the role of altered autonomic tone in the ventromedial hypothalamic (VMH) syndrome of obesity. VHM-lesioned animals exhibit increased parasympthetic tone (PT) and decreased sympathetic tone (ST). Deficits in brain neurotransmitter systems after lesioning have not been identified.

Intracerebroventricular (ICV) administration of β-endorphin (BE) has been shown to increase plasma glucose and catecholamines. This increased sympathetic outflow is blocked by chlorisonamine and bilateral adrenal sympathectomy. ICV BE also decreases gastric acid secretion suggesting an inhibitory effect of BE on PT.

BE cell bodies are confined to the medial basal hypothalamus (MBH), immediately ventral to the VMH. BE fibers from this area project dorsally and anteriorally, innervating hypothalamic nuclei and the lateral septum. BE fibers turn caudally at the level of the thalamus, entering the pons mostly ventral to the cerebral aqueduct in midline peri-ventricular structures. Fibers spread laterally to innervate dorsal and lateral regions of the peri-aqueductal gray (PAG), locus coeruleus, n. solitary tract and dorsal motor n. vagus X.

Thus, since exogenous ICV BE is associated with increased ST and decreased PT, and VMH lesions are associated with decreased ST and increased PT, we hypothesize that VMH-lesions causing obesity may destroy BE fibers en passage emanating from the MBH. In another animal model of obesity, injection of monosodium glutamate in neonatal animals causes gross obesity and is associated with destruction of hypothalamic and extrahypothalamic BE and up-regulation of opiate receptors. Ventromedial hypothalamic lesions were placed in five female Sprague-Dawley rats and sham lesions in six control rats. Animals were maintained on regular rat chow ad lib, with 12 hr light dark cycles. At the end of thirty days brains were dissected and the following brain parts assayed for BE by RIA: hypothalamus, PAG, and medulla. BE was reduced in the hypothalamus, PAG and medulla by 64%, 71% and 66%, respectively. No change in pituitary BE was found.

In conclusion, VMH lesions causing hyperphagia and weight gain are associated with reduction in brain BE. This reduction in BE may be causally related to the altered autonomic tone found in this syndrome.

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56.5 RATS RENDERED OBESE BY PARASAGITTAL HYPOTHALAMIC KNIFE-CUTS (KCs) DISPLAY NORMAL BROWN ADIPOSE TISSUE (BAT) RESPONSES TO ACUTE COLD. D.V. Coscina, J.W. Chambers*, S. Hogan* and J. Himms-Hagen*. Sect. of Biopsychol., Clarke Inst. of Psychiatry, Toronto, M5T 1R8; and Dept. of Biochem., Univ. of Ottawa, K1H 8M5; Ontario, Canada.

BAT is recognized as the major metabolic compartment for maintaining body temperature in response to environmental cold. BAT also appears to play a significant role in the thermogenic response which occurs after ingesting high-calorie foods. Recent research from our group suggests that the CNS mediation of these BAT responses may be dissociable. We have found that bilateral heat lesions of the medial hypothalamus (MH) which induce overeating and obesity impair BAT responses to high-calorie foods but not cold. The present experiment was conducted to begin determining if BAT from rats made obese by KCs shows similar responses. Subjects were 48 adult female Holtzman rats housed singly with ad lib access to Purina Lab Chow and water. After 1 wk adaptation, 24 rats received stereotaxically-placed 3x3 mm bilateral KCs located 5-8 mm rostral to the interaural line, 0.7 mm lateral to the midline and 6.5-9.5 mm below the dura. Another 12 rats received sham cuts in which the guide shaft was lowered without the knife extended; another 12 rats served as unoperated controls. Three wks after surgery, groups of 3 KC and 3 control rats were exposed to 4^{o} C for 21 hrs before sacrifice; equivalent numbers remained at 28^{o} C. KC rats ate 50% more food than controls and became very obese (200 + 8 g vs. 40 + 3 g gained by controls). Interscapular BAT weighed 50% more in KC rats, which was largely attributable to increased white fat content. Cold exposure enhanced BAT weight for both KCs and controls. Functional indices of BAT response (protein content, DNA content, cytochrome oxidase activity and mitochondrial GDP binding) indicated that tissue from KC rats was normal at 28^{o} C. Exposure to 4^{o} C elicited the same enhanced responses in both groups. These findings imply that BAT responses to cold are not impaired by KCs, much as we found after MH lesions (Hogan et al., Am. J. Physiol., 243: E338, 1982). However, since both types of hypothalamic injury produced overeating and obesity, it might have been expected that BAT responses would have been enhanced at 28^{o} C. The lack of such increases suggests that BAT responses to high-calorie foods would be impaired. This latter prediction has been substantiated recently for MH-lesioned rats (Himms-Hagen et al., unpublished observations). Given the similar BAT profiles between MH-lesioned and KC rats, we would predict comparable impairments to high-calorie foods after KCs. Taken together, the results of this work show an important role for neural pathways at the level of the hypothalamus in the regulation of BAT responses to diet-induced thermogenesis.

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56.6 PEPTIDERGIC MODULATION OF FEEDING IN THE NORMAL AND DIABETIC CHINESE HAMSTER. C.J. Billington*, J.E. Morley, A.S. Levine and G.C. Gerritsen*. Neuroendocrine Research Laboratory, Minneapolis VAMC, MN and the Upjohn Co., Kalamazoo, MI.

Diabetes mellitus is often associated with hyperphagia. Diabetes has been shown to affect the opiate receptors involved in feeding and pain. Prompted by these observations, we sought to examine the feeding and satiety systems in the Chinese hamster, an animal that spontaneously develops diabetes mellitus in certain inbred strains. We used forty diabetic hamsters that were pair-matched to forty normal animals in these experiments. Quantitation of food eaten in baseline condition revealed that the diabetic animals were hyperphagic, consuming nearly twice as much dry food as the normals. The circadian variation in feeding was maintained, though the diabetics ate more than normals at all times of the day. Both normal and diabetic animals were able to respond to variable degrees of starvation by transiently increasing eating, but this increase was seen principally in the first hour after restoring access to food and the animals do not make up for the degree of food deprivation. Putative satiety agents were examined using feeding driven by the natural peak in hunger augmented by six hours of starvation. In this system, a number of neuropeptides that inhibit feeding in other animals failed to show an effect: cholecystokinin, bombesin, somatostatin, glucagon, and pancreatic polypeptide. Calcitonin suppressed feeding, and was more effective in the diabetics; an effect seen also in the diabetic rat. The dopamine antagonist, haloperidol, also suppressed feeding very effectively, and diabetics were much more sensitive to its effect - both as percent inhibition relative to control and in absolute amount of food eaten. The opioid feeding system was found to be quite different than that found in the rat. Naloxone did not suppress eating in the peak feeding - minimal starvation paradigm described above. Further, the opiate agonist, butorphanol, the most potent opiate feeding inducer in the rat, could not induce feeding. However, normetazocine, a sigma opiate receptor agonist, did provoke feeding in the diabetic but not the normal Chinese hamsters. In vitro opiate receptor studies on brain membranes showed minimal ^{3}H-naloxone and ^{3}H-EKC binding over the 0.1 to 10 nM range.

We conclude that the feeding and satiety systems in the Chinese hamster are markedly different from the well-described rat model and that diabetes appears to produce alterations in the appetite regulation system of these animals. The lack of high affinity opiate binding sites may explain the absence of a classical opioid feeding system in these animals.

56.7 DEFICIENCIES IN HYPOTHALAMIC HYPERPHAGIA, NOREPINEPHRINE-INDUCED FEEDING, AND GLUCOPRIVIC FEEDING IN BRATTLEBORO RATS LACKING VASOPRESSIN. Paul F. Aravich* and Celia D. Sladek (SPON: Gloria E. Hoffman). Departments of Neurology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14642.

The Brattleboro rat is characterized by a complete absence of vasopressin (VP), diabetes insipidus (DI), and several other physiological and behavioral abnormalities. We now report that the DI rat responds abnormally to three treatments that produce feeding in normal rats: parasagittal knife cuts in the perifornical hypothalamus, norepinephrine (NE) injections into the paraventricular hypothalamus (PVH), and glucoprivation induced by systemic injections of 2-deoxy-D-glucose (2DG). Adult female rats (Blue Spruce Animal Farms, Altamont, NY) served as subjects in two experiments. DI rats (age, 19-22 weeks; weight, 227.9 ±3.4 g) and normal Long Evans (LE) rats (age, 13-15 weeks; weight, 266.5 ± 4.8 g) participated in each experiment. Experiment 1 examined the effects of perifornical cuts on food intake and body weight; Experiment 2 evaluated the orexigenic effects of varying doses of NE (injected into the PVH) and 2DG (administered systemically). The maintenance diets in Experiment 1 were a Purina chow diet followed by a 33% fat diet. Purina chow was the maintenance and test diet in Experiment 2. A 1-hr. test period followed the NE injections while a 3-hr. test period followed the 2DG injections. Experiment 1 revealed the following: When maintained on the Purina chow diet and observed for 30 days, the knife cuts failed to increase body weight in the DI rats in contradistinction to their effects in the LE group. The cuts did, however, produce an increase in caloric consumption by the DI group, though this effect was smaller and more transitory than that observed in the LE-cut group. When switched to the fat diet, the DI-cut group, but not the DI-sham group, was virtually indistinguishable from the LE-cut group in terms of weight gain and caloric intake. Experiment 2 found that the DI rat was deficient in NE feeding with respect to percent change from saline levels. A similar trend appears to exist (experiment in progress) with regard to 2DG-induced feeding. Three possible explanations for these findings are: a) VP directly influences feeding behavior perhaps via an interaction with the pituitary-adrenal axis; b) chronic vasopressin deficiency produces secondary changes in adrenal cortical function which, in turn, alter feeding; and/or c) other secondary changes resulting from chronic VP insufficiency (e.g., impaired water balance, excess oxytocin) account for the abnormalities. (Supported by NIMH Fellowship 1-F32-MH08872-01 and NIH Grant R01-AM-1976.)

56.8 THE EFFECT OF SEX STEROID HORMONES ON OPIOID MODULATION OF FEEDING. A.S. Levine, B.A. Gosnell, M. Grace*, J. Kneip*, and J.E. Morley (SPON: S. Hsiao). Neuroendocrine Research Lab, Minneapolis, VAMC, Minneapolis, MN, 55417.

Numerous studies have suggested a role for the endogenous opioid system in the modulation of appetite. Gonadal steroids play a role in food ingestion and weight gain and have been demonstrated to alter the concentrations of endogenous opioids in the CNS and to modulate opioid receptor number. For these reasons we investigated the effect of female gonadal steroids on the opioid feeding system. We used 4 groups of female rats: ovariectomized (ovx); ovx + estradiol 2 μg/kg (ovx + E_2); ovx + E_2 + progesterone 2 mg/kg (ovx + E_2 + P); ovx + P; and sham operated. In comparison to the ovx controls the E_2 treated animals were 20 times less sensitive to naloxone. The shams required twice as much naloxone as the ovx controls to suppress food intake and were 10 times more sensitive to naloxone than the E_2's. The addition of P to the E_2 treatment improved the sensitivity of the animals to naloxone but did not return it to the level of the shams. Ketocyclazocine (KC) increased food intake significantly at 2 hours at both the 35 and the 3.5 μM/kg doses in both the shams and E_2 + P groups without there being a significant effect in the ovx. At 4 hours the shams and the E_2 + P treated animals again showed significantly increased food intake at a lower dose than any of the other groups. At 6 hours, the highest KC dose significantly increased food intake in the ovx controls, shams and E_2 treated animals. In order to further define the reason for the increased sensitivity to naloxone and decreased sensitivity to KC in ovx animals we measured ir-dynorphin in the cortex, striatum, hypothalamus, thalamus and brain stem in all of the animals following sacrifice. Ovx had decreased ir-dynorphin in the cortex compared to the sham and this was partially reversed by E_2 + P. P treatments tended to increase brain stem ir-dynorphin. In view of the finding that ir-dynorphin levels are decreased in ovx animals, it seems that altered receptor sensitivity is the most likely explanation for the observed effects of female sex hormones on opioid modulation of feeding. These studies add to a growing body of literature suggesting a role for the peripheral endocrine system in the modulation of opioid feeding systems. The alterations in the sensitivity of the opioid feeding system appear capable of explaining, in part, some of the effects of sex steroid hormones on feeding.

56.9 BEHAVIORAL COMPONENTS OF AREA POSTREMA LESIONS CAN BE PRODUCED SEPARATELY BY PARABRACHIAL LESIONS. R.C. Ritter and E.E. Ladenheim.* College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

Lesions of the area postrema (AP) and the adjacent nucleus of the solitary tract (NST) produce a constellation of behavioral changes which include loss of feeding in response to 2-deoxyglucose (2DG), overconsumption of highly palatable foods and exaggerated drinking in response to angiotensin II. We reasoned that by making lesions of anatomically distinct projections of the AP and NST, it might be possible to separate the individual behavioral components of lesions involving the AP and NST. The NST and the AP send projections to the parabrachial nuclei. In fact, Ohman and Johnson have reported that lesions which involve the lateral parabrachial nucleus cause rats to overdrink in response to angiotensin II. We compared the behavioral effects of lesions of the lateral (LPN) and the medial (MPN) parabrachial nuclei. We found that lesions of the MPN abolish or attenuate the increase in feeding elicited by 2DG administration. When food intake was monitored for six hours following 2DG injection (200 mg/kg), sham-lesioned rats ate significantly more food (4.0±1.0 g) than they ate following a control injection of saline. Likewise, rats with lesions of the LPN also increased their intake in response to 2DG (3.9±0.8 g). Nevertheless, rats with MPN lesions did not increase their food intake significantly following 2DG injection (0.5±0.3 g). Our lesions of the parabrachial nuclei did not cause rats to overconsume palatable foods, such as cookies. When offered cookies for two hours, the consumption of all three groups was statistically indistinguishable (shams, 6.5±0.3 g; MPN-lesions, 6.8±1.1 g; LPN-lesions, 7.1±0.8 g). When injected subcutaneously with angiotensin II (0.5 mg/kg), sham-lesioned and rats with MPN lesions drank 5.2 ±1.0 g and 8.6±1.1 g, respectively. However, rats with lesions of the LPN drank significantly more (17.6±3.2 g). Drinking by the three groups was indistinguishable following control injections. Our data demonstrate that lesions of discrete portions of the parabrachial nuclei produce some of the behavioral changes observed after lesions of the AP and adjacent NST. The parabrachial nuclei contain ascending projections and fibers of passage from the AP and NST. Therefore, these results suggest that the AP and adjacent NST may exert their effects on ingestive behavior via several different ascending projections.

56.10 AREA POSTREMA LESIONS INCREASE INTAKE OF PALATABLE FOOD IN VAGOTOMIZED RATS. G.L. Edwards and R.C. Ritter. Dept. of VCAPP, College of Veterinary Medicine, Washington State University, Pullman, WA 99164 and WOI Regional Program in Veterinary Medical Education, University of Idaho, Moscow, ID 83843.

We have previously demonstrated that lesions which destroy the area postrema (AP) and damage the adjacent nucleus of the solitary tract (NST) cause rats to overconsume highly palatable foods (Edwards and Ritter, Brain Res., 216: 265-276, 1981). Both the AP and the NST receive primary afferent terminals from the vagus nerve. Therefore, it is possible that overingestion of palatable foods by AP-NST-lesioned rats simply is due to the loss of abdominal vagal afferents. To test this hypothesis, we performed total subdiaphragmatic vagotomies on AP-NST-lesioned rats and on sham-lesioned rats. All of the rats were then tested for their response to a highly palatable solid food (cookies). Vagotomized, AP-NST-lesioned rats consumed significantly more cookies than did vagotomized, sham-lesioned rats ($p<.02$) (see table). Vagotomized, sham-lesioned rats did not consume more than non-vagotomized, sham-lesioned rats. The fact that vagotomized, lesioned rats consumed less than lesioned rats which were not vagotomized may be the result of damage to vagal efferents to the esophagus and stomach.

Consumption of Highly Palatable Food (g)

	AP-NST-Lesioned	Sham-Lesioned
Vagotomized	7.7 ± 0.8	4.7 ± 0.6
Sham-Vagotomized	13.0 ± 0.9	5.8 ± 1.1

Since the satiety effect of CCK-8 is mediated by the gastric vagus, we used the response to CCK-8 as a test for completeness of our vagotomies. Lesioned and sham-lesioned rats with intact vagi significantly reduced their food intake after injection of CCK-8. However, none of the vagotomized rats reduced their intake after CCK-8. This finding, taken together with post-mortem gross and histological examination, indicates that our vagotomies were successful.

Our results demonstrate that overconsumption of palatable foods by rats with lesions of the AP and NST is not due to loss of abdominal vagal afferents. The fact that rats with lesions of the AP and adjacent NST only overconsume highly palatable foods, suggest that this phenomenon results from altered responsiveness to gustatory cues.

57.1 LATERAL HYPOTHALAMIC LESIONS IMPAIR THE FEEDING OF RATS EXPOSED TO CHRONIC COLD. Gretchen L. Snyder*, Edward M. Stricker and Michael J. Zigmond. Psychobiology Program, Dept. of Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Rats given bilateral lesions of the lateral hypothalamic (LH) area become aphagic and adipsic. Although these animals may gradually recover ingestive behaviors, they do not eat normally during acute glucoprivation or drink during acute dehydration. On the other hand, they increase their food intakes as control rats do when exposed to cold (Epstein & Teitelbaum, Am. J. Physiol. 213: 1159, 1967). The present experiments determined whether this response was in fact appropriate to their needs.

Twenty male Sprague-Dawley rats (300-350 g), each given LH lesions (1-2 mAmp, 10-15 sec), were tested 3-4 weeks after their recovery of ingestive behavior. Six weight-matched control rats also were studied. Body weights and intakes of Purina chow and water were monitored daily while animals were maintained in the colony room (22°C) and after they were transferred to the cold (5°C). They were housed individually in stainless steel cages.

The control animals increased caloric intake by 40-50 kcal/day within 7 days and steadily gained body weight (+50 g by Day 20). Fourteen of the 20 rats with LH lesions also became hyperphagic in the cold, as reported previously. However, none of them gained weight normally; six simply maintained body weight while the other eight continually lost weight (-70 g by Day 20). Because their increases in caloric intake were comparable to those of controls, it appears that rats with LH lesions lost more calories as heat than did controls. In this regard, control animals decreased their tail temperatures from 28°C to 17°C when exposed to cold whereas the brain-damaged rats showed smaller and less consistent evidence of vasoconstriction. We conclude that rats with LH lesions have impaired feeding responses to cold stress, as revealed not by comparison with the intakes of control rats but by comparison with their own caloric needs.

The feeding deficits were more pronounced in the other six brain-damaged animals, which included the rats taking the longest period of time to recover ingestive behavior after LH lesions. When placed in the cold, they did not become hyperphagic and lost body weight rapidly (-70 g by 4 days). However, when 5% sucrose solution was available instead of tap water, these animals increased their intake of chow and were able to maintain their body weights. Thus, although LH lesions clearly impair both the physiological and behavioral responses to cold stress, minor adjustments in the testing conditions may allow thermal homeostasis to continue even in rats with large brain lesions.
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57.2 INFLUENCE OF SURGICAL AND CHEMICAL SYMPATHECTOMIES ON THE LATERAL HYPOTHALAMIC LESION SYNDROME - I. ACUTE EFFECTS. Michael G. Tordoff, Carlos V. Grijalva, Kalin A. Robinson,* Larry L. Butcher, Donald Novin,[1] Xavier Pi-Sunyer,*[2] Dennis A. VanderWeele.[3] [1]Dept. Psychology, UCLA, [2]St. Luke's Hosp. Ctr., Columbia Univ., and [3]Dept. Psychology, Occidental College.

Lateral hypothalamic (LH) lesions produce acute symptoms such as hyperthermia, hyperglycemia, hypoinsulinemia, diarrhea, and gastric pathology. The present research attempted to discover if the sympathetic nervous system (SNS) mediates these effects.

In one series of studies, groups of 13-17 adult male Long Evans rats received adrenal demedullation (MDL), adrenalectomy (ADL), splanchnicectomy (SPL), celiac ganglionectomy (GAN) or a sham operation (SHM). Ten days later, following overnight food deprivation, they were given either LH lesions (1.2 mA x 15 sec, anodal current) or control (CON) surgery. Rectal temperatures and symptoms were recorded prior to, 6 hr after, and 24 hr after surgery. Following the last observation, the rats were sacrificed, their stomachs analyzed for gastric pathology, blood taken for glucose and insulin assay, and tissue samples collected for verification of sympathectomy by catecholamine histofluorescence.

The various sympathectomy procedures (MDL, SPL, GAN) had no effect on lesion-induced hyperthermia, gastric pathology, insulin levels or external symptoms. However, lesion-induced hyperglycemia was attenuated by SPL and blocked by GAN. (Glucose $\bar{X}$s (mg/dl): CON: SHM=108.6, MDL=107.2, SPL=106.8, GAN=102.9, LH: SHM=123.5, MDL=123.9, SPL=113.1, GAN=99.0). ADL-LH rats were hypothermic, showed greater gastric pathology and displayed lower levels of plasma insulin and glucose than the other groups. However, these effects were present in some ADL-CON rats, suggesting a general morbidity caused by surgical stress and food deprivation.

In parallel studies, male rats neonatally sympathectomized by treatment with guanethidine (GUA) or given saline control injections (SAL) were subjected to LH lesions when 70-90 days old. In one study, GUA rats displayed lower body temperatures prior to the lesion (GUA=37.3°C, SAL=37.8°C) and less increase in temperature after it (GUA=+1.0°C, SAL=+2.0°C). GUA-LH rats did not differ from SAL-LH rats in gastric pathology. However, glucose and insulin values in both GUA-LH and SAL-LH rats did not differ from GUA-CON and SAL-CON rats. In a second study, 11 of 12 GUA-LH rats died within a few hours of the lesion.

These experiments found no role for circulating catecholamines in acute aspects of the LH syndrome. The SNS innervation of the abdominal viscera, as judged by surgical sympathectic denervation may be important in lesion-induced hyperglycemia. The attenuated hyperthermia and high fatality rate in lesioned rats with extensive denervation by chemical sympathectomy may argue for a role of the entire SNS in adaptation to the effects of the lesion.

57.3 INFLUENCE OF SURGICAL AND CHEMICAL SYMPATHECTOMIES ON THE LATERAL HYPOTHALAMIC LESION SYNDROME - II. CHRONIC EFFECTS. Donald Novin, Michael G. Tordoff, and Carlos Grijalva. Dept. Psychology, UCLA, CA 90024.

To determine if the sympathetic nervous system (SNS) is important in long-term adaptation to the lateral hypothalamic (LH) lesion syndrome, adult male Long Evans rats were given surgical sympathectomies and LH lesions. Rats received adrenal demdullation (MDL), splanchnicectomy (SPL), celiac ganglionectomy (GAN), or a sham operation (SHM). As GAN surgery resulted in a loss of body weight, weight matched controls (GCON) were also included. Ten days later, the rats received either LH lesions (1.2 mA x 15 sec, anodal current) or control surgery (CON). LH rats were initially tube-fed and then fed moist palatable foods to allow them to recover. The duration of each stage of recovery, food intake, and body weight were recorded.

Sympathectomized-LH rats did not differ from SHM-LH rats in rate of recovery, food intake, or body weight during the 90 days postlesion. ($\bar{X}$ body weights (N) on Day 90: CON: SHM=499.7 (9), MDL=504.6 (7), SPL=497.6 (7), GAN=473.7 (6), LH: SHM=279.5 (11), MDL=287.5 (11), SPL=316.2 (9), GAN=307.1 (11), GCON=289.0 (10)).

To test the hypothesis that the impaired responsiveness of LH rats to glucopenia was due to lesion-induced SNS hyperactivity, all subjects received intraperitoneal injections of 2-deoxy-D-glucose (2DG). As some of the LH rats could not maintain themselves on solid chow at this time, all rats were adapted to wet mash for 10 days. They were then given counterbalanced injections of 2DG (150, 300, and 450 mg/kg) or saline as a control. Food intake was recorded 30, 60, 120, and 180 min after each injection. Despite a large suppression of 2DG-induced eating in LH animals, no differences were present between sympathectomized and SHM LH groups at any time.

Although surgical sympathectomy had little effect on body weight or food intake, it might be argued that insufficient SNS destruction was present to produce noticeable effects. As an attempt to test this possibility some of the rats in the SHM and MDL groups received 25 40-mg/kg injections of the adrenergic neurotoxin guanethidine (GUA) over a 5-week period. The rest received saline (SAL) injections. Both LH and CON rats treated with GUA displayed severe diarrhea during, and for several weeks after, the treatment. Despite relatively high mash intakes, GUA-treated animals lost weight and most LH rats died, even when given access to a variety of palatable foods. The few LH rats that survived GUA treatment eventually regained weight to that of SAL-LH rats, but did not surpass it.

In summary, no evidence was found to suggest that the SNS is involved in chronic aspects of the LH-lesion syndrome.

57.4 FAILURE TO ALTER BODY WEIGHT AND FAT, LINEAR GROWTH AND CALORIC INTAKE AND EPIDIDYMAL AND BROWN (BAT) ADIPOSE TISSUE WEIGHT BY FEEDING OF JUNK FOOD IN WEANLING RATS WITH VENTROMEDIAL HYPOTHALAMIC LESIONS (VMNL RATS). Lee L. Bernardis and Paul J. Davis*. Div. Endocrinology, VA Med. Ctr., and Dept. of Med., SUNY at Buffalo, Buffalo NY 14215.

Weanling male VMNL rats and sham-operated controls were maintained on lab chow for two post-operative weeks. The VMNL rats became obese (Lee Index) in the presence of normal food intake and body weight gains. The two groups were then divided into two subgroups each: one subgroup continued to receive lab chow, whereas the other subgroup was in addition fed various junk food items for 72 days. Rats from each group were killed weekly, beginning 51 days post-operatively. As expected, VMNL rats showed higher carcass fat, epididymal fat pad weights, BAT lipid, and fat deposited per kcal eaten. They also exhibited reduced linear growth and epididymal and BAT protein. Unexpectedly, they also showed increased body weight and body weight gained per kcal ingested. Whereas no diet effect was noted in body weight, length, fat, caloric intake, epididymal weight and BAT weight, lipid and protein, diet effects were evident in reduced carcass and epididymal pad protein in junk-fed rats as well as fat deposited per kcal eaten. A time effect was noted in body weight and length, carcass fat, total kcal ingested, epididymal weight and protein, BAT protein and fat deposited per kcal eaten. Quite notably, an interaction between diet and lesion effect was noted in only one parameter, i.e., epididymal protein. A diet and time interaction was noted in body weight, total calories eaten, epididymal weight and BAT protein. An effect of lesion x time was seen in body weight and linear growth, carcass fat, epididymal weight and protein and fat deposited per kcal eaten. Finally, an interaction diet x lesion x time was evident in body weight and epididymal weight. Positive correlations were calculated between carcass fat and body weight, total caloric intake and epididymal pad weight for all groups except the junk food-fed controls. Highly significant correlations were also found between carcass fat and Lee Index, but only in the VMNL rats. Negative correlations were observed between carcass fat and BAT protein, again only in the two VMNL groups. The data show that, in contrast to mature rats, weanling VMNL rats will not increase their caloric intake when fed palatable junk foods, will not gain weight and deposit fat in excess of VMNL rats fed lab chow and will not show gross changes in BAT. The efficiency of food utilization appears enhanced in both VMNL groups, as shown by the increased amount of fat deposited per kcal eaten. In contrast to one report, neither intact nor VMNL rats will show an increase in linear growth when fed the junk food diet.

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57.5 HYPOTHALAMIC-HINDBRAIN FEEDING INHIBITORY SYSTEM: AN EXAMINATION UTILIZING ASYMMETRICAL KNIFE CUTS AND HRP HISTOCHEMISTRY.
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Extensive evidence indicates that the hypothalamic hyperphagic-obesity syndrome results from the interruption of a fiber system interconnecting the anteromedial hypothalamus with the caudal brainstem. This system extends caudally from the area of the paraventricular nucleus (PVN) to at least the level of the midbrain. From this region the trajectory of the system has not been determined. In an attempt to extend the mapping of this feeding inhibitory system knife cuts were made in the following groups of female rats: Group UNILATERAL (n=8) received a unilateral parasagittal knife cut through the medial hypothalamus (MH cut); Group PONS (n=8) received a unilateral coronal knife cut through the caudal pons combined with a contralateral MH cut; Group MEDULLA (n=5) received a unilateral coronal knife cut through the rostral medulla combined with a contralateral MH cut; Group SHAM (n=8) received sham surgery. Food intake (high fat diet) and body weight were recorded for 21 days postoperatively.

The rats in the PONS group were hyperphagic following surgery, and their postoperative weight gain exceeded ($p<.05$) that of the UNILATERAL rats, as well as that of the SHAM animals (100 vs. 53 and 22 g/21 days). The rats in the MEDULLA group, on the other hand, failed to overeat or gain reliably more weight than did the SHAM rats (38 vs. 22 g).

To identify the location of the cell bodies whose fibers were severed by the pontine knife cut, similar cuts were made in additional rats using an HRP-coated knife. The results of this experiment implicate PVN efferents as part of the hypothalamic -hindbrain feeding inhibitory system. This is further indicated by the results of a third experiment in which hyperphagia was produced by small pontine knife cuts placed in the trajectory of the PVN efferents.

These findings demonstrate that the hypothalamic feeding -inhibitory system extends to the level of the caudal pons, and follows the path of PVN efferents. The failure of the medullary knife cut, which was only 1 mm caudal to the pontine cut, to produce overeating and obesity indicates that either the feeding inhibitory fibers do not extend into the rostral medulla or that the medullary cut produced additional damage which interfered with the expression of the hyperphagia syndrome. This issue is currently being investigated.

57.6 VENTROMEDIAL HYPOTHALAMIC AREA LESIONS INDUCE GRADUAL CLIMBING OF THE SET-POINT FOR BODY WEIGHT IN RATS.
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We recently reported (Physiol. Behav. 27: 709-713, 1981) that electrolytic (DC) lesions in the area of the ventromedial hypothalamus (VMH) in adult rats produce not one, but two abnormal stages of weight gain; a linear phase of fattening rather than a plateau of body weight was found to follow the dynamic phase. The present experiment supports our previous speculation that the continuous, post-dynamic fattening may reflect a lesion-induced gradual elevation of the set-point for body weight.

In adult female rats maintained on ad lib Purina Lab Chow pellets, bilateral DC or radiofrequency (RF) lesions of the VMH area produced a negatively-accelerated curvilinear phase of weight gain which lasted 10 weeks, followed by a linear phase of fattening which continued at a rate approximately double that of operated control rats of the same age (DC lesions, $p < .001$; RF lesions, $p < .01$). There was no significant correlation ($p > .05$) between the weight gained during the first 10 post-lesion weeks and the rate of weight gain over the 10th to 20th week postlesion period, across either DC or RF-lesioned rats. During the second, linear phase of fattening, we food-restricted the lesioned rats between the 20th and 26th weeks postlesion. Compared to the rate of weight gain in the linear phase prior to food restriction, the rate over the same weight range following release from food restriction was significantly greater ($p < .001$) for both DC and RF-lesioned rats. Furthermore, by the 40th postlesion week, the lesioned rats had approached the weight they would have been if not food-restricted.

These findings suggest that both DC and RF lesions of the VMH area induce a gradual climbing of the set-point for body weight which is independent of actual food intake or body weight, and which either follows or is superimposed on an immediate elevation of the set-point responsible for the initial, curvilinear phase of weight gain. Consistent with two separate effects of such lesions is the lack of a significant correlation between dynamic phase weight gain and post-dynamic rate of weight gain. VMH area lesions may initiate a primary, slowly developing adipocyte proliferation and/or preadipocyte recruitment in at least some fat depots of adult rats. Thus, the steadily climbing set-point for body weight in our lesioned rats may reflect a gradual hyperplasia beginning immediately postlesion. Furthermore, this mechanism may prove to be a useful model for human idiopathic obesity which also reflects a continuous upward shift in the level at which fat stores are regulated. (Supported by M.R.C. of Canada and the C.K. Clarke Psychiatric Research Foundation).

57.7 RELATIONSHIP OF HYPERINSULINEMIA TO HYPERPHAGIA IN RATS WITH VENTROMEDIAL HYPOTHALAMIC LESIONS.
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Animals with lesions of the ventromedial hypothalamus (VMH) are hyperinsulinemic both after a fast and in response to an oral glucose load (King & Frohman, Neurosci. Biobehav. Rev. 6: 205-214, 1982). Basal hyperinsulinemia is abolished by both subdiaphragmatic vagotomy (King, Phelps, & Frohman, Am. J. Physiol. 239: E437-E441, 1980) and adrenalectomy (King, Banta, Tharel, Bruce, and Frohman, Am. J. Physiol., in press), but the hyperphagia, obesity, and postabsorptive hyperinsulinemia persist after the vagal transections and after corticosterone administration in adrenalectomized animals. The present experiment tested the hypothesis that postabsorptive hyperinsulinemia is due in part to pancreatic β-cells made hyperresponsive on the basis of hyperphagia.

Plasma insulin and glucose levels were assayed after a 4-hr fast and 17 minutes after the initiation of a meal (6 ml sweetened milk in 7 minutes) in animals with sham lesions (SVMH, n=8), VMH animals maintained at preoperative body weight by food restriction (LVMH, n=7), and VMH animals fed ad libitum (OVMH, n=6). The OVMH animals displayed a mean weight gain of 8.1 ± 0.7 g/day during the first 12 days after surgery. The LVMH animals displayed a mean weight gain of 9.6 ± 1.0 g/day when fed ad libitum for 10 days at the conclusion of the experiment. The basal and postabsorptive insulin levels of the LVMH animals were significantly lower than in OVMH rats, but significantly greater than in SVMH animals ($P<0.005$). Plasma glucose levels did not significantly differ under the basal condition, but the postabsorptive glucose levels of the OVMH rats were significantly lower than the other two groups ($P<0.05$). It is concluded that the postabsorptive insulin responsiveness, as well as the basal hyperinsulinemia, is the result of both a primary effect of the lesion and of hyperphagia.

57.8 CHANGES IN CONSUMATORY BEHAVIOR OF THE RAT FOLLOWING LESIONS OF THE TRIGEMINAL LEMNISCUS AT THE LEVEL OF THE VENTROBASAL THALAMUS.
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Recent research has produced results differing over the presence of postoperative consumatory deficits following damage to the rat trigeminal lemniscus (LTr). In the present study, placement of lesions at the LTr's terminal coursing within and beneath the thalamic medial ventrobasal complex (VBm) was followed not only by previously employed consumatory measures (body weight, food and water consumption), but also by behavioral observations of feeding. Significant differences occurred between lesion and control groups on postsurgically recorded measures of body weight and water consumption. Differences in the measure of food utilized were non-significant. However, observed aphagia (no feeding) following food deprivation was significantly greater for the lesion group than the control group. These findings imply that lesion-induced food consumption deficits were here hidden by inefficiencies which produce sometimes massive spillage and suggest that terminal LTr lesions within the VBm adversely affect rat consumatory sensory processes.

57.9 ANORECTIC EFFECT OF CALCITONIN: LOCALIZATION TO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS. R. de Beaurepaire*, and W.J. Freed. Preclinical Neurosciences Section, Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

High densities of calcitonin binding sites are found in the hypothalamus (Olgiati et al., Brain Res. 265:209, 1983). Injections of calcitonin (43 ng) into the lateral ventricle of the rat inhibit eating (Freed et al., Science 206:850, 1979). The medial hypothalamus, particularly the paraventricular nucleus, is a major site of regulation of feeding behavior, and infusions of norepinephrine into this area stimulate eating (Leibowitz, Pharmacol. Biochem. Behav. 8:163, 1978). We therefore hypothesized that the paraventricular nucleus is the site at which calcitonin acts to inhibit eating behavior.

Cannulae were implanted into the paraventricular nucleus or surrounding areas and after recovery the animals were trained to eat wet mash on a 24-hour schedule. Synthetic salmon calcitonin (courtesy of Armour Pharmaceutical Company) in a dosage of 15 ng in 0.2 microliter was infused unilaterally and the intake of wet mash was recorded for 30 min starting 30 min after infusion. Injections which were histologically confirmed to be in the paraventricular nucleus inhibited eating markedly, while injections into the lateral hypothalamus were ineffective.

Percentage Change (Means ± S.E.M.) in Eating After Infusion of Calcitonin (Negative Indicates Decrease)

Infusion Site	Percentage Change*	
Paraventricular Nucleus	-82 ± 4	(n=6)
Lateral Hypothalamus	+15 ± 21	(n=5)

*t=4.60, p<0.01, Behrens-Fisher t test

The animals were also satiated and given norepinephrine (40 nmoles in 0.3 microliter) into the same sites. After norepinephrine injections into paraventricular sites the animals ingested of 13 ± 3 grams (mean ± S.E.M.) of food, but infusions into the lateral cannula sites resulted in the ingestion of only 2 ± 1 grams. Norepinephrine is therefore effective in stimulating eating only for those cannulae sites where an inhibition of eating by calcitonin can also be obtained.

We therefore suggest that calcitonin inhibits eating by acting directly on the hypothalamus. It is also possible that an interaction between norepinephrine and calcitonin in this area of the hypothalamus is involved in the regulation of eating behavior.

57.10 ANALYSIS OF PLASMA AND BRAIN CHOLECYSTOKININ LEVELS IN CANCER ANOREXIA. F.M. van Lammeren*, W.T. Chance, M.H. Chen*, W.J. Chen*, R.F. Murphy*, S.N. Joffe* and J.E. Fischer*. Department of Surgery University of Cincinnati Medical Center, Cincinnati, Ohio 45267

Although anorexia is often observed in cancer patients, the etiology of this dysfunction in appetite remains unclear. Recent investigations suggest that several peptides that are common to the gut and brain may be involved in regulating feeding. In particular, cholecystokinin (CCK) has been suggested to function as a satiety signal in several mammalian species. In order to determine whether abnormal concentrations of CCK were contributing to cancer anorexia, we assayed plasma and brain levels of CCK in anorectic tumor-bearing (TB) and control rats. Methylcholanthrene (MCA) sarcomas were induced in 16 adult, male Fischer 344 rats by the sc. injection of 2 x 10^6 viable cells that were obtained as a cell suspension of the solid tumor. An additional 16 rats were injected (sc.) with normal saline (0.2 cc) and served as freely-feeding (FF) and pair-fed (PF) control groups. Body weight, food intake and water intake were monitored daily. Half of the TB rats (TB-16) were sacrificed 2 days after the development of significant anorexia (day 16), while the remaining TB animals (TB-28) were killed during severe anorexia (mean food intake less than 3 g/100 g body wt.). The food allowed to the PF group was further reduced to match their body weights to the individual carcass weights of the TB-28 rats. Levels of CCK were determined by radioimmunoassay (radioiodinated CCK-39) in the plasma as well as in the hypothalamus and cerebral cortex, after homogenization in boiling water. Assay mixtures containing the sample, antiserum solution and CCK-33 standards were incubated in 0.04 M phosphate buffer (pH = 7.4) for 48 hr. After the addition of labelled CCK-39, the mixture was again incubated for 48 hr. Bound and free CCK were separated with dextran coated charcoal. The following table presents the results of these experiments.

Group	Food Intake (g/100g)	Plasma CCK (ng/ml)	Hypothalamic CCK (ng/g)	Cortical CCK (ng/g)
FF	7.38 ± 0.25	1.24 ± 0.19	241 ± 13	526 ± 25
PF	1.59 ± 0.45	1.17 ± 0.19	264 ± 15	516 ± 37
TB-16	5.03 ± 0.40	1.55 ± 0.18	118 ± 11*	428 ± 16*
TB-28	2.91 ± 0.44	1.50 ± 0.16	143 ± 17*	414 ± 26*

Presented values are means ± S.E.M. *p<0.01

These data demonstrate that plasma levels of CCK are not significantly altered in experimental cancer anorexia. Contrary to our hypothesis, concentrations of CCK in the hypothalamus and cerebral cortex were significantly reduced. If brain CCK is involved in satiety, the reductions in immunoreactive CCK in the hypothalamus and cortex may suggest that the TB rat is attempting to compensate for the anorexia by down-regulating CCK satiety mechanisms.

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57.11 INSULIN, TRIIODOTHYRONINE, GROWTH HORMONE AND SOMATOMEDIN CONCENTRATIONS IN GROWTH RETARDED RATS WITH DORSOMEDIAL HYPOTHALAMIC NUCLEI ELECTROLYTIC OR KAINIC ACID LESIONS OR SHAM OPERATIONS. L.L. Bellinger, L.L. Bernardis, R.H. McCusker*, and D.R. Campion*. Dept. Physiol. Baylor College of Dentistry, Dallas, TX 75246; VA Med. Ctr. and Dept. Medicine, SUNY at Buffalo, Buffalo, NY 14214; USDA-ARS, R.B. Russell Agr. Res. Ctr. Athens, GA 30613.

In Experiment (Exp.) 1 rats received bilateral (n=8) kainic acid lesion (1 µg/0.5 µl) or sham operations (n=10) at about 155g body weight [Bellinger et al. Am. J. Physiol. 224:R389, 1983], while in Exp. 2 the rats received bilateral electrolytic (n=12) lesions (1 ma, 10 sec) or sham (n=9) operations at about 70g body weight. Both groups of DMNL rats remained hypophagic (P<0.01) compared to their controls. The animals were sacrificed by decapitation within 20 sec of removal from their cages. At sacrifice the DMNL rats: weighed less (Exp. 1, 256.8±6.8g vs 314.8± 3.9g; Exp. 2, 216.3±7.0g vs 277.5±5.6g, both P<0.01) and were shorter, naso-anal length (Exp. 1, 203.4±2.1mm vs 219.4±4.0mm; Exp. 2, 200.8±1.6mm vs 213.2±1.3mm, both P<0.01) than the sham operated rats but the DMNL rats had normal Lee Obesity Indexes (Exp. 1, 314.6±1.9; Exp. 2, 298.6±1.8). Lesions were confirmed histologically and data were analyzed by parametric and non-parametric (i.e., growth hormone) tests. Insulin, growth hormone and triiodothyronine were determined by R.I.A. and somatomedin in Exp. 2 by bioassay (porcine). In Exp. 1, DMNL vs sham: Insulin, 1.04±0.04 vs 0.99±0.03 ng/ml; growth hormone, 178.9±45.7 vs 49.3± 8.8 ng/ml, P<0.04; triiodothyronine, 1.66±0.16 vs 1.45±0.09 ng/ml, while in Exp. 2: Insulin, 0.84±0.06 vs 0.45±0.05 ng/ml, P<0.01; growth hormone, 138.8±75.9 vs 144.3±56.5 ng/ml; triiodothyronine, 0.59±0.1vs 0.60±0.10 ng/ml; and somatomedin, 0.94±0.05 vs 1.06± 0.05 units. Normal rat serum has a somatomedin value of 1.0 units. The data reveal that hypophagic growth retarded DMNL rats (whether lesioned electrolytically or by kainic acid) show no deficits in the growth promoting hormones analyzed. The data support the concept that DMNL produce a 'reset' of body weight, which is not due to an alteration of classical growth promoting hormones.

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57.12 EFFECTS OF INTRACRANIAL ESTRADIOL ON INGESTIVE BEHAVIORS. P.C. Butera* & J. A. Czaja (SPON: L. J. Pellegrino). Department of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

The present study examined the effects of central estradiol on food intake, water intake, and body weight in ovariectomized guinea pigs. Thirty-five adult females, selected from a heterogeneous stock (Topeka) of English short-hair guinea pigs, were implanted with double-walled bilateral cannulae (21 ga guide, 27 ga inner cannula) stereotaxically aimed at either the ventromedial hypothalamus, paraventricular nucleus (PVN), or preoptic area, and were stimulated unilaterally with cholesterol and estradiol 17-beta. This yielded a total of 70 implant sites.

Across all subjects, estradiol implants significantly reduced feeding, drinking, and body weight relative to implants of cholesterol. The occurrence of vaginal membrane rupture in some animals following central estradiol stimulation indicated that the hormone was leaking into peripheral circulation. The possibility that the behavioral changes observed were due to the peripheral rather than central effects of the implants was evaluated by comparing the results of females with vaginal membrane rupture to the results of females without vaginal membrane rupture. There were no significant differences between these groups on any of the dependent variables studied, indicating that the peripheral changes were neither necessary nor sufficient to account for the behavioral effects.

Estradiol implants in the ventromedial-arcuate region (VM-ARC) and PVN significantly lowered food intake and body weight. Placements in the anterior hypothalamic-preoptic area, thalamus, or lateral ventricles had no significant effects on feeding or body weight. Only implants in the VM-ARC significantly reduced drinking, although this effect was much less robust than the changes in feeding and body weight. Additionally, the changes in water intake following central estradiol were uncorrelated with the changes in food intake.

These findings establish that estradiol applied to the brain of ovariectomized guinea pigs can lower ingestive behaviors and body weight. The results support the hypothesis that estrogen can suppress feeding and body weight by acting in areas outside VMH, possibly by interacting with catecholamine systems involved in the control of feeding behavior.

57.13 CENTRAL CONNECTIONS OF THE TONGUE IN NEONATAL RATS. W. W. Pugh, J. A. Browde, Jr.* and W. G. Hall. N.C. Foundation for Mental Health Research, Raleigh, NC 27611 and Dept. of Psychology, Duke Univ., Durham,NC 27706.

2-10 ul of 1% horseradish peroxidase (HRP) covalently linked to wheat germ agglutinin (WGA) or to cholera toxin (CT) was injected into various antero-posterior and medial-lateral quadrants of the tongue in 6-day-old rat pups using a micropipette coupled to a microsyringe. Survival times ranged from 48 to 96 hours at which time the animal was deeply anesthetized and perfused per procedure II of Rosene and Mesulam (J. Histochem. Cytochem., 26: 28, 1978) for TBM histochemistry. Subsequent sectioning and reacting of the brain with the TMB chromagen revealed both labeled cell bodies (retrograde transport) and labeled terminal fields (transganglionic transport) in the brainstem.

Retrogradely HRP filled perikarya, representing motor neurons having end plates within the tongue were found in n.intercalatus, n. XII, and in the parvocellular reticular formation dorsally adjacent to caudal n.VII. Additionally, HRP-CT extensively labeled the dendrites of these neurons. While n.intercalatus dendrites extended mostly laterally for short distances, some projected dorsally into n.X while others crossed (commissural) to the opposite nucleus. N.XII dendrites only projected ventrally and laterally; however, some long lateral dendritic branches were seen to course nearly to the solitary and trigeminal sensory nuclei. Large and small peroxidase filled neurons in the parvocellular reticular formation had radially oriented dendrites, and again, some of these dendrites could be followed to near the NTS.

Transganglionic transport of conjugated HRP from peripheral sensory receptors in the tongue to the brainstem was seen in dorsal-lateral n.V. Labeling in n.V began caudal to the obex and some labeled terminal fields were seen projecting medially into the solitary complex at this level. This n.V projection pattern continued rostrally to slightly above the genu of the facial nerve in coronal sections. Although it is clear that not all sensory components of the tongue, particularly taste receptors, were labeled in this study, this technique avoids some of the difficulties of interpretation that arise when mixed nerves are dipped in HRP.

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57.14 APHAGIA AFTER UNILATERAL MICROINJECTIONS OF A RETROGRADE FLUORESCENT TRACER IN RAT HYPOTHALAMUS OR GLOBUS PALLIDUS. G.F. Alheid[1], J. Mogensen[2], I. Divac[2], and L. Heimer[1]. 1. Clinical Neurosciences Research Center, Univ. of Va., School of Medicine, Charlottesville, Va. 22908, 2. Inst. Neurophysiology, Panum Inst, Copenhagen 2200 Denmark.

When granular blue (GB), a fluorescent retrograde tracer is injected in the CNS, a central zone of necrosis surrounded by labeled cells unvariably results. We have taken advantage of the fact that GB remains in neurons even after prolonged survival periods (Innocenti, 1981) to study the functional and anatomical consequences of tracer induced lesions. Implanted glass micropipettes were used to deliver GB; this approach results in small (500 µm) injections of regular size, with little or no labeling of the pipette tract (Alheid and Carlsen, 1982).

Unilateral globus pallidus or lateral hypothalamic "injections" of GB were made at several different anterior posterior coordinates of male albino rats. Nine of the 27 animals were aphagic for periods of 24-48 hours (food intake less than 1.0 gm). The most severe effects of GB on body weight were found for rats with injections made at A.P. 6.5 (de Groot). These animals lost 10% of their preoperative weight by day 9, before gaining weight again. Control body weights were never regained during the 24 day survival period. GB injections in the ventral or dorsal pallidum just below or just dorsal and posterior to the anterior commissure resulted in aphagia and weight loss that was similar to that of the anterior hypothalamic injections.

Post sacrifice, (26 µm) cryostat brain sections were examined for retrogradely labeled neurons. With hypothalamic injections, retrograde labeling with GB included the medial part of n. accumbens, amygdala, ventromedial hypothalamic area, substantia nigra, ventral tegmentum and brainstem parabrachial nuclei.

Labeling after dorsal or ventral pallidal injections included neurons in the ventral caudate or n. accumbens respectively, substantia nigra, and ventral tegmentum area. In addition, neurons were labeled in the posterior lateral hypothalamic area just medial to the subthalamic nucleus (see Heimer et al., this meeting).

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57.15 ELECTROMYOGRAPHIC ANALYSIS OF INGESTION AND REJECTION RESPONSES ELICITED BY SAPID STIMULI IN THE RAT. J.B. Travers and R. Norgren. The Rockefeller University, New York, NY 10021.

Intraoral injections of sucrose (S) and QHCL (Q) produce differential oro-facial responses in the rat (Grill and Norgren, '78). Several lines of evidence indicate that these oro-facial responses constitute the observable concomitants of ingestion and rejection, respectively. In order to further specify this relationship we recorded electromyographic (EMG) responses from a subset of the muscles involved in both these behaviors and in swallowing in freely moving rats. The stimulating techniques (intraoral cannula) and behavioral monitoring techniques (videotape) were identical to those described in Grill and Norgren ('78). In addition, activity in up to 4 muscles was recorded simultaneously using chronically implanted fine wire electrodes (60µ NiCr). Responses to S and Q were easily differentiable in both the videotape and EMG records. Intraoral infusions of 50µl of 0.3M sucrose produced low amplitude mouth movements correlated with EMG activity in the anterior digastric (AD), zygomatic (ZYG), and styloglossus (STY) muscles. EMG activity in these muscles consisted of rhythmic contractions (6-8/sec) with each contraction lasting 20-40 msec (AD, ZYG) and 60-80 msec (STY). During these bouts of muscle activity, episodic bursts of synchronous activity in intrinsic laryngeal and pharyngeal muscles indicated the presence of swallows. These bursts occurred regularly during the responses to sucrose. Otherwise, the animal was motionless and no fluid was visible on its lips. In contrast, stimulation with 50 µl of 0.01M QHCl produced large amplitude mouth movements (gapes) at a rate of 3-5/sec. Bursts of EMG activity in the AD, ZYG, STY and pharyngeal muscles were correlated with the gapes. Like the gapes themselves this muscle activity was of considerably longer duration (60-180 msec) than the contractions elicited in response to S. During a bout of gapes, little or no activity was observed in the laryngeal musculature, strongly suggesting the absence of swallowing. Moreover, fluid was invariably seen on the outer surface of the mouth and on the floor of the observation chamber. The behavioral responses elicited by intraoral S and Q apparently constitute the oral motor phase of ingestion and rejection, respectively. Responses to S are interspersed with frequent, regular swallows, while swallows are absent during the behavior accompanying Q stimulation. Many of the EMG responses differentiating S from Q stimulation also occur in a lightly anesthetized preparation in which non-traumatic stereotaxic procedures can be employed. These preparations have allowed us to begin characterizing the central neural activity correlated with the responses of ingestion and rejection in areas previously shown to project to the oral motor nuclei (Travers and Norgren, '83). Supported by grants NS18731 and NS10150).

57.16 HIERARCHICAL INFORMATION PROCESSING OF BLOOD CHEMISTRY BY CHEMOSENSITIVE ELEMENTS IN PERIPHERAL AND CENTRAL NERVOUS SYSTEM, Y. Oomura, Y. Mizuno*, N. Shimizu* and T. Minami*. Dept. of physiology, Fac. of Medicine, Kyushu Univ. 60, Fukuoka 812, Japan.

A few behavioral experiments indicated that there may exist glucose responding neurons in the nucleus tractus solitarious (NTS). Therefore, 162 neurons were tested in rat brain slices containing the NTS. A statistically significant number ($p<0.05$) of glucose-sensitive (GS) neurons were observed in that region. In the caudal region, 25 (28%) GS, / (8%) glucoreceptor (GR), and 57 non responsive neurons (28%) were noted among 89 cells tested. Of 73 cells tested in the rostral part, only a few responded, 8 (11%) GS and 4 (5%) GR. Differences in the left and right side populations were not statistically significant in either the rostral or the caudal parts.

The GS neuron was hyperpolarized in solution at high glucose concentration and depolarized at low glucose concentration (osmolarity, adjusted by sucrose) with a slight membrane conductance increase. The GR neuron was depolarized with a membrane conductance decrease in solution at high glucose concentration and hyperpolarized with a membrane conductance increase at low glucose. In solution with low Ca and high Mg or with 0.1mM Cd, at the condition of a blockage of synaptic inputs, the GR neuron still exhibited the glucose response. This is the first to demonstrate that the GR neuron is depolarized by glucose probably with potassium permeability decrease.

From anatomical and electrophysiological evidences the NTS is a relay point in the blood chemistry analysis pathway, and serves as a caudal counterpart to the more rostral hypothalamus. The NTS is close to ventricle IV as the hypothalamus is close to ventricle III, so the NTS could consolidate peripheral information, including the presence of hepatic, pancreatic, and intestinal glucose or insulin with intraventricle or plasma levels and send the summarized information to one of two directions. It can return caudally as a reflex from the NTS by way of either the dorsal motor vagal nucleus or the intermediolateral column in the spinal cord to produce reflex responses in the appropriate viscera. Integrated information then goes to the hypothalamus. The GS and GR neurons are in the lateral hypothalamic area and the ventromedial nucleus respectively. Recently our group found the GS and GR neurons in the dorsomedial hypothalamic and paraventricular nuclei. Further, the integrated blood information then send to the prefrontal cortex.

The prefrontal cortex are known to have active pathways of communication with the hypothalamus and have been shown to respond in synchronism with the hypothalamus during feeding behavior. Thus, communication with association areas plus further integration of exogenous stimuli may lead to feeding related decisions which could then, in part, return to the visceral organs back over the paths parallel to those along which the afferent signals originally ascended and proceed to the motor system by way of the motor cortex.

57.17 HYPOTHALAMICALLY ELICITED EATING, BUT NOT BITING, IS INHIBITED BY BLOATING IN RATS. Jöel M. Kaplan* and Samuel M. Feldman. Dept. of Psychology, New York University, New York, NY 10003.

Electrical stimulation of the lateral hypothalamus (ESLH) that elicits feeding and/or other oral behaviors also facilitates reflexive biting of a tactile probe applied to the perioral region (Smith, 1972). We have investigated stimulus-bound feeding (SBF) and the perioral response (POR) as functions of ESLH intensity and of the gastric loading that results from SBF. Rats were tested in a cloth harness to which they had been previously adapted. By reducing the range of behavioral options, the use of the harness simplified the detailed analysis of eating and reflexive biting. Videotaping permitted off-line analysis. The receptive field for the POR was studied by repeated application of a tactile probe to 7 loci in the perioral region: 3 on the upper lipline (midpoint and corners) and 2 on each side of the face at different distances from the mouth. Sensitivity of the POR at a given locus was defined as the proportion of bite responses to the probe as a function of current. SBF trials at a given current were run in blocks (with 2 min intertrial intervals) that terminated when a rat resisted eating for two consecutive trials. During 30 sec SBF trials, a dish of wet mash (3:2, water: lab chow) was placed under the rat's mouth. Amount consumed, latency and duration of eating, and bite frequency and pattern were measured for each trial.

Perioral responding and SBF were both ESLH-current sensitive. The receptive field for the POR increased with ESLH intensity. On the earlier trials in SBF blocks, ingestion rate was also an increasing function of current, primarily the result of an increase in the average amount eaten per bite of food. The effect of bloating on reflexive biting was studied by testing the POR before and after SBF blocks. For all currents tested, POR sensitivity was unchanged by gastric loading. Analysis of the biting pattern during SBF showed that the biting of food, like reflexive biting (POR), was not inhibited by gastric loading. In fact, at higher currents, bite frequency markedly increased over trials with ingestion rate either remaining roughly constant or slowly declining over the course of a test session. The cessation of SBF was not accompanied by elimination of ESLH-elicited biting. Rats often bit the edge of the food dish, the cables that suspended the harness within its frame, a wood block (if presented), or, occasionally, the air.

We conclude that the POR and ESLH-elicited biting in general, unlike intracranial self-stimulation supported by the same electrodes that produce SBF (e.g., Hoebel, 1969), are insensitive to gastric loading or bloating.

57.18 Fourth ventricular phlorizin injection stimulates feeding but not hyperglycemia. F.W. Flynn*, H.J. Grill and D. Rooney*, Dept. Psychology and Inst. Neurol. Sci., Univ. of Pennsylvania, Philadelphia, 19104.

Injections of phlorizin, which competitively inhibits glucose transport, stimulate feeding. Previously, Glick and Mayer (Nature, 1968,219,1374) reported that infusions of 100-200ul of phlorizin into the lateral ventricle resulted in hyperphagia. It is not clear from this study the location of the neural mechanisms mediating the feeding behavior. Recent evidence indicates that the neural mechanisms responsive to reductions in glucose availability elicited by intracerebroventricular (ICV) 5-thioglucose (5TG) are in the caudal brainstem, in the vicinity of the fourth ventricle. We report that microinfusions of phlorizin and 5TG into the fourth ventricle of rats cause feeding. However, unlike 5TG, phlorizin does not elicit hyperglycemia.

Rats (n=7) were implanted with cannulae aimed at the fourth ventricle. Food intake was meaured 3 and 24 hours following 5 ul (ICV) injections of saline (0.9%), phlorizin (3 mM and 6 mM) and 5TG (.76 uM and 1.07 uM). Food intake following ICV saline and 3 mM phlorizin was not significantly different. In contrast, three hour food intake following 6 mM phlorizin (3.9±.8g) was significantly greater than that following saline injection (1.0±.5g). In addition, compared to saline injection, 6 mM phlorizin injection caused a small but significant increase (2.1±.8g) in 24 hour food intake. Rats ate significantly more food in the three hour period following the two doses of 5TG compared to that amount ingested following ICV saline. The increase in food intake was similar following the two doses of 5TG and 6 mM phlorizin.

Subsequently, tail blood was collected at 1, 2, 3, and 6 hours following ICV saline, 6 mM phlorizin, and 1.07 uM 5TG injection. Plasma glucose concentrations were comparable following ICV saline and 6 mM phlorizin. Hyperglycemia was elicited in these rats only by ICV 5TG injection (1 hour post saline=133.0±2.6 mg%; 1 hour post 5TG=236.1±30.1 mg%).

To verify that the phlorizin was acting centrally and not at some peripheral site, food intake was measured 3 and 6 hours following 5ul IP injections of saline and 6 mM phlorizin. Food intake did not differ under these conditions. We conclude therefore, that inhibition of glucose transport by phlorizin in tissue in the vicinity of the fourth ventricle is a potent stimulus for feeding but not hyperglycemia.

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57.19 LEVEL OF SATIETY: IN VITRO GABA-SHUNT AND PENTOSE-SHUNT ACTIVITIES IN THREE BRAIN SITES ASSOCIATED WITH FEEDING. T. R. Kasser*, R. B. S. HARRIS* AND R. J. MARTIN (SPON: J. M. Bowen). Dept. Foods and Nutrition, Univ. of Georgia, Athens, GA 30602

Mechanisms by which long-term food intake and energy balance are regulated have not been clarified. Experiments were conducted to determine if the level of satiety (highly positive, normal, or highly negative energy balance) altered specific pathways of glucose oxidation in selected brain areas. The hypothesis addressed was that metabolic activity within specific brain areas may be altered to depict peripheral metabolic status.

Sixty-three female Sprague-Dawley rats (225g) received 150, 100 or 50% of normal intake by gastric intubation for seven days. Body fat at conclusion of tube-feeding was 20.9, 13.6 and 7.4% in 150, 100 and 50% fed rats, respectively. If permitted to return to a free-feeding state, 150%-fed rats would be hypophagic and 50%-fed rats would be hyperphagic, relative to intake of 100%-fed rats, to achieve energy balance. Thus, the incentive for spontaneous feeding would be inhibited in 150%-fed rats (anorectic), stimulated in 50%-fed rats (hungry) and maintained in 100%-fed rats (control).

Cortex, ventrolateral hypothalamus (VLH), ventromedial hypothalamus (VMH) and a site in the caudal brainstem that included the area postrema-nucleus of the solitary tract (AP-NTS) were dissected from coronal sections obtained four hours after the last tube-fed meal. Using the method described by Hothersall et al. (J. Neurochem. 37:1484, 1981), the yield of $^{14}CO_2$ form ^{14}C-2-glucose minus ^{14}C-6-glucose equals GABA-shunt activity and ^{14}C-1-glucose minus ^{14}C-6-glucose equals pentose shunt activity.

Glucose flux through the GABA-shunt was specifically altered in the VLH relative to the level of satiety. GABA-shunt activity in the VLH was 32% lower in hungry rats and 35% higher in anorectic rats relative to control values. No treatment effects were observed for GABA-shunt activity in the VMH, AP-NTS or cortex. Glucose flux through the pentose-shunt was altered in the VMH and AP-NTS relative to satiety. Pentose-shunt activity in the VMH was 111% lower in hungry rats and 152% higher in anorectic rats relative to control values. Pentose-shunt activity in the AP-NTS was 116% lower in hungry rats and 60% higher in anorectic rats relative to control values; however, hungry and anorectic rats had AP-NTS pentose shunt activities that were not different from control values but were different from each other. No treatment effects were observed for pentose-shunt activity in the VLH or cortex. The data demonstrate that within selective brain sites, specific pathways for glucose oxidation are affected by energy intake and may be used by the rat to assess and respond to changes in peripheral energy status.

58.2 ANALYSIS OF MACRONUTRIENT SELECTION FOLLOWING ABLATION OF THE AREA POSTREMA OF THE RAT. Jon N. Kott*, Christine L. Ganfield*, and Nancy J. Kenney. Department of Psychology, University of Washington, Seattle, WA 98195.

Ablation of the area postrema and adjacent caudal-medial nucleus of the solitary tract (AP/cmNTS) results in a transient period of hypophagia and weight loss. While daily intake of laboratory chow returns to control levels within 2 weeks after lesioning, body weight of AP/cmNTS lesioned rats is permanently reduced compared to that of unlesioned controls. This study examines the effect of AP/cmNTS lesions on intake of protein, fat and carbohydrate by rats in a "self-selection" situation.

Ten rats were adapted to the "self selection" procedure for 3 weeks. Based on total caloric intake and on proportion of total intake from each of the macronutrients during the final 5 days of the adaptation period (baseline), rats were divided into 2 matched groups of 5 animals each. One group received thermal lesions of the AP/cmNTS. The other was sham lesioned. After a 4-day recovery period, daily intakes of the 3 macronutrients were analyzed for four consecutive 5-day periods. Body weights were measured daily throughout the experiment.

AP/cmNTS lesioned rats lost more weight (23 + 0.6 g) than did non-lesioned controls (8.6 + 1.9 g) during the first 4 days after surgery. Rates of weight change of lesioned animals did not differ from those of controls throughout the remainder of the experiment. Total caloric intakes of AP/cmNTS lesioned rats were significantly below those of controls during the first 2 postsurgery measurement periods.

Sham-operated animals showed no changes from baseline levels in the number of calories taken as fat, carbohydrate or protein during any of the post-surgery measurement periods. The decrease of total caloric intake of lesioned rats was due entirely to a decrease in the number of calories taken as fat. Fat intake of lesioned rats averaged 42.0 + 15.6 Cal/day during the baseline period compared to 10.2 + 4.3 and 18.2 + 8.0 Cal/day during the first two post-ablation test segments, respectively. Daily intake of carbohydrate and of protein did not change following AP/cmNTS ablation.

Thus, in the "self-selection" situation, the reduction of food intake which immediately follows AP/cmNTS lesions is due to a specific decrease of the the number of calories taken as fat and does not reflect a general suppression of ingestion of all dietary components.

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58.3 DISCRIMINATION BETWEEN EQUIMOLAR NaCl AND LiCl IN RATS: ROLE OF THE AREA POSTREMA. R. Ladowsky* and K.-P. Ossenkopp (SPON: F.V. Szeligo). Dept. of Psychology, University of Western Ontario, London, Ontario, Canada, N6A 5C2.

Area postrema (AP), a circumventricular structure at the caudal end of the fourth ventricle, has been shown to be involved in taste aversion (TA) learning in rats induced by injections of LiCl (Ritter et al., Brain Res., 1980, 201, 501). Rats are also able to form a discrimination between equimolar solutions of NaCl and LiCl when presented simultaneously (Strom et al., Psychon. Sci., 1970, 18, 290), possibly due to the development of a TA to the LiCl. We were interested in delineating any role AP might have in the formation and retention of this discrimination. In the first experiment, rats on a 23 hr/day water deprivation schedule were presented with equimolar (0.12 M) NaCl and LiCl in a two-bottle choice test for 1 hr/day over ten days. All rats learned to discriminate between the two solutions and showed a strong preference for the NaCl solution ($p<.01$). Half of the animals then received thermal cautery lesions of AP and the other rats were sham lesioned. After recovery and re-adaptation to the water deprivation schedule, all rats were again given the two-bottle choice test between NaCl and LiCl (1 hr/day). Both the lesioned and the sham lesioned animals exhibited good retention of the discrimination ($p<.01$), however the AP lesioned group drank more LiCl over the ten days of testing than the sham lesioned group. When given only NaCl or only LiCl on Days 11 and 12 respectively, the AP lesioned rats drank as much NaCl as the sham lesioned rats ($p>.05$), but drank significantly more LiCl than the sham lesioned animals ($p<.01$). In experiment 2 rats received either AP lesions or sham lesions and after recovery were placed on a 23 hr/day water deprivation schedule. All animals were then given a two-bottle choice test (1 hr/day) between equimolar (0.12 M) NaCl and LiCl over the next ten days. Both AP and sham lesioned rats acquired a strong preference for NaCl by the third test day. When challenged with only LiCl on Day 11, AP lesioned rats drank significantly more LiCl than sham lesioned rats ($p<.01$). Thus, AP does not appear to play an essential role in either the acquisition or retention of the Na - Li discrimination, but this brainstem structure does influence the amount of Li rats are willing to ingest.

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58.4 IMPAIRED VASOPRESSIN SECRETION IN RATS WITH ELECTROLYTIC LESIONS OF THE "AV3V AREA" LOCALIZED TO NUCLEUS MEDIANUS. Thomas W. Gardiner*, Joseph G. Verbalis, and Edward M. Stricker. Depts. of Psychology and Medicine, Univ. Pittsburgh, Pittsburgh, PA, 15260

Lesions ablating the periventricular tissue surrounding the anteroventral border of the third cerebral ventricle (the "AV3V" area) in rats produce adipsia and excessive renal fluid losses. Many rats resume drinking after a few days, but remain permanently impaired in their drinking responses to acute dehydration (Buggy & Johnson, Am.J.Physiol., 233:R44, 1977). More discrete lesions within this periventricular area, limited to the ventral (subcommissural) part of nucleus medianus (vNM), also cause adipsia, polyuria, and long-term drinking impairments in rats (Gardiner et al., Soc. Neurosci. Abstr. 7:168, 1981). However, we find that several weeks later they exhibit significant capacity to concentrate their urine in response to both overnight water deprivation and sc injection of hypertonic NaCl. We therefore examined the plasma vasopressin (AVP) response to hypertonicity, 4-6 weeks following vNM lesions in rats.

Two ml of 2M NaCl solution were infused (iv) over a 5 minute period in each of 12 rats with vNM lesions and 7 controls (BWt= 450-560 g). Thirty minutes later 1.2-1.5 ml of blood were remotely withdrawn via the same catheter. Food and water were withheld during testing. Plasma AVP was measured by specific radioimmmunoassay. In 9 of the 12 brain-damaged rats, AVP levels were only 2.0-11.1 uU/ml (M + SE= 5.23 + 1.0 uU/ml) despite plasma sodium concentrations of 151-166 mEq/l, whereas all control rats had AVP levels greater than 14.5 uU/ml (22.5 + 2.5 uU/ml) despite plasma sodium concentrations that did not exceed 153 mEq/l. Thus, it would appear that vNM lesions impair the rapid vasopressin secretion of rats in response to acute osmotic stimuli. These findings may be related to the fact that the brain damaged animals also do not drink rapidly in response to dehydration. However, most rats initiate drinking after a delay of 5-8 hr, when the dark period begins (Gardiner & Stricker, Soc. Neurosci. Abstr. 8:601, 1982), whereas their urine osmolalities are elevated much sooner; for example, they rise above 1500 mOsm/l within 2-3 hr after sc injection of 2 ml of 2M NaCl soluiton. The latter findings may imply that vasopressin secretion in rats with vNM lesions is delayed for a few hours after the onset of osmotic dehydration, and we are now examining this possibility. Despite our present uncertainty about the time course of vasopressin secretion after osmotic dehydration, it seems likely that vNM lesions disrupt the normally close association between it and the drinking response.

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58.5 DRINKING ELICITED BY ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMUS AND THE ANGIOTENSIN-RENIN SYSTEM. G. Mittleman, S.J. Block, and E.S. Valenstein (SPON: R. Davis). Psych. Depart. and Neurosci. Lab, University of Michigan, Ann Arbor, MI 48109.

Accumulating evidence indicates that angiotensin II is an important stimulant of thirst. For example, Fitzsimons & Simons (J. Physiol.203:45-57,1969) demonstrated that vigorous drinking could be elicited from water sated rats by intravenous infusions of angiotensin II. The role of angiotensin II in producing thirst has been further supported by the use of its competitive antagonist, saralasin. Rolls & Woods (Pharm.Biochem.Behav.,6:245-250, 1977) have reported that saralasin injected together with angiotensin II blocks drinking normally elicited by the latter. It has been postulated that the neural substrate mediating drinking evoked by electrical stimulation of the lateral hypothalamus (ESLH) may be part of the same "thirst circuit" in which angiotensin II exerts its influence as a thirst cue. The purpose of these experiments was to compare natural drinking (elicited by water deprivation) with drinking elicited by ESLH by using saralasin to attenuate drinking. EXP. 1: Long Evans male rats were implanted with one cannula into the lateral ventricle. They were water deprived for 30 hrs and then allowed access to water for 1 hr. Water consumption was measured by a drinkometer record of the number of licks. Once reliable baseline water consumption was obtained, the rats were again water deprived for 30 hrs and then received an intraventricular infusion of saralasin (5µg/5µl/1 min.). At 60 or 75 min. following the infusion when saralasin has been reported to maximally inhibit drinking these animals were allowed access to water and consumption was recorded as in the baseline condition. Saralasin reduced water consumption by 66% after a 60 min. delay but by only 33% after a 75 min. delay. EXP. 2: A second group of rats was implanted with LH electrodes and a cannula into the lateral ventricle. They were tested for baseline water consumption and for the effectiveness of saralasin on water deprived drinking as in Exp. 1. These rats were then screened for ESLH elicited drinking. After stable and consistent drinking occurred during every 20 sec. trial of hypothalamic stimulation baseline water consumption during ESLH was recorded as the number of licks during 10 trials. This testing was repeated at least 5 times with testing sessions occurring every 48 hrs. These rats were then injected with saralasin as in Exp. 1 and tested for ESLH induced drinking 60 min. later. Saralasin had no effect on ESLH elicited drinking although it again reduced water deprived drinking by an average of 66% when compared to baseline water consumption. Since the angiotensin II blocking agent, saralasin, inhibits drinking caused by water deprivation and does not effect drinking caused by ESLH, it would appear that different physiological mechanisms underlie these two forms of drinking.

58.6 CSF SODIUM CONCENTRATION IS INCREASED IN RESPONSE TO AN EXTRACELLULAR FLUID CHALLENGE. S.P. Frankmann and R.R. Sakai*. Dept. of Psychology, U. Washington, Seattle, WA 98195.

The mechanisms responsible for the initiation of saline ingestion in response to an extracellular fluid challenge(ECF) are not well understood. ECF challenges can be induced by polyethylene glycol (PEG), a colloid which osmotically draws salts and water from the ECF to form an edema at its injection site. In response, the animal renally conserves fluids as well as replenishes the losses by ingestion of water and salts. It has been proposed (Stricker,EM.,JCPP,95:1,1981) that a lowering of cerebrospinal fluid (CSF) sodium (Na+) may represent the critical stimulus for the initiation of sodium ingestion.

Forty male Long Evans rats were given a one week acclimation to a choice of 0.3M NaCl and water. At 8am on test day each rat was injected s.c. with 16.7ml/kg of 20% PEG (PEG) or saline vehicle (CTRL). Each rat was then returned to its home cage from which all food had been removed. One group of rats (sampled at 8 hrs) had access to 0.3M NaCl and water, all others were denied fluid access. At an interval of 2, 4 or 8 hrs a given animal was weighed and anesthetized with Ketamine. At this time 100µl of CSF was collected followed by 1 ml of blood for determination of hematocrit and sodium concentration ([Na+]) by flame photometry.

In those rats tested without access to fluids there was no difference in the P[Na+] at 2, 4 or 8 hrs between PEG or CTRL. At 4 and 8 hours, PEG rats had significantly elevated CSF[Na+], ($t=3.41$, $t=4.5$, $p<.01$). When rats were given access to fluids during the 8 hours, PEG rats had significant elevation of hematocrit and P[Na+], ($t=3.27$, $t=2.82$, $p<.05$) however there was no difference in CSF[Na+] from CTRL. There was no significant difference in percent body weight loss, hematocrit of CSF[Na+] in PEG rats whether or not they were allowed access to fluids. The animals given access to fluids did have decreased Plasma[Na+] compared to those without access to fluids. PEG rats consumed 6.03+ 2.8 ml of water and 1.28 + 1.2 ml of 0.3M NaCl and CTRL rats consumed 0.04 + 0.0 ml water and 0.8 + 1.8 ml of 0.3M NaCl (mean ± SD). There was no relationship between volume or ratio of fluid ingested and CSF [Na+].

At 8 hours post PEG injection CSF[Na+] is increased but P[Na+] unchanged when no fluid access is allowed. In contrast when fluid access is allowed, CSF[Na+] is not increased but P[Na+] is. These data do not support the hypothesis of a lowered CSF [Na+] being a stimulus for sodium ingestion. The observed elevation of CSF[Na+] may reflect altered fluid and electrolyte transport at tissues sensitive to the hormonal changes which occur during an ECF challenge. Possibly there is a synergistic action between elevated CSF[Na+] and circulating levels of hormones which triggers a sodium appetite. Supported by HL 21800.

FEEDING AND DRINKING: CENTRAL MECHANISMS IV

59.1 EFFECTS OF CENTRAL CATECHOLAMINE DEPLETION ON INSULIN LEVELS IN THE GENETICALLY OBESE AND DIABETIC MOUSE (db/db). J. F. Lorden, Dept. of Psychology, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The diabetes mouse (C57BL/Ks-db) is an autosomal recessive mutant that displays elevated plasma insulin levels at weaning. Levels peak at 2-5 mo of age and subsequent islet cell degranulation is accompanied by loss of body weight, hypophagia and premature death. Diabetes mice also have elevated hypothalamic norepinephrine (NE) levels in comparison with lean littermate controls, suggesting the possibility of central nervous system involvement in the diabetes syndrome. Intraventricular injections of 6-hydroxydopamine (6-OHDA) that deplete central NE cause weight loss and decreases in blood glucose and body fat. Furthermore, 6-OHDA treated mice show greater pancreatic islet granulation than vehicle-treated mice of the same age.

The improved islet granulation and lower blood glucose levels of the 6-OHDA treated group suggested that the lesion or its consequences had a protective effect on the pancreas of the diabetes mice. To test this diabetes mice were treated with an 80 nmol intraventricular injection of 6-OHDA or a vehicle solution at 6-7 wk of age. Food intake and body weight were depressed in the 6-OHDA group in comparison with vehicle-treated controls. At sacrifice 7 wk later, blood glucose and plasma insulin levels were also significantly lower in the 6-OHDA treated group than in controls. Thus, the 6-OHDA treatment may have slowed the increase in plasma insulin levels usually seen in diabetes mice. A similar experiment was carried out in 100 day old mice. Diabetes mice of this age would be expected to show a decline in body weight, food intake and plasma insulin if left untreated. In this experiment food intake was significantly depressed in the 6-OHDA group in comparison with controls for only two days after surgery. At sacrifice 3 wk later, there were no reliable differences between the 6-OHDA and vehicle groups in food intake, body weight or blood glucose. However, plasma insulin levels in the 6-OHDA group were significantly higher than those in the control group which had begun to show a decline in comparison with younger mice. These findings suggest that the 6-OHDA lesion does not prevent the increase in plasma insulin seen as the diabetes syndrome progresses. However, the earlier histological data that indicated that the 6-OHDA-treatment prolonged the insulin-secreting capacity of the pancreas in diabetes mice were confirmed. The results obtained in the older mice also indicate that the effect is not due solely to a reduction in food intake. The lesions may reduce circulating levels of diabetogenic hormones and reduce insulin need. (Supported by NINCDS grant NS14755).

59.2 HYPOTHALAMIC NEUROTRANSMITTERS DURING ANOREXIA INDUCED BY ACUTE DIETARY ZINC RESTRICTION OF ADULT RATS. D.L. Sparks , J.T. Slevin, and E.J. Kasarskis. Dept. Neurol. VA Med. Ctrs. and Univ. Kentucky, Sanders-Brown Aging Ctr., Lexington, KY 40536.

Reduced food intake is an early manifestation of zinc (Zn) deficiency. Other investigators have shown increased whole brain norepinephrine (NE) and we have demonstrated elevated levels of NE, dopamine, and 5-HT in hypothalamus of rats fed Zn-deficient diets for 6 wks from weaning. Although hypothalamic NE has been implicated in regulation of food intake, the results of ourselves and others may reflect altered maturation of noradrenergic pathways rather than metabolic changes in severely growth retarded, Zn-deficient rats. In order to separate developmental from metabolic effects of Zn deprivation, we have determined levels of hypothalamic neurotransmitters following acute dietary Zn restriction of adult rats fed Zn-adequate diets from weaning.

Male Sprague-Dawley rats weighing 200 g were fed diets containing <1ppm Zn and offered water supplemented with 30 ppm Zn (as Zn acetate) in order to provide adequate Zn intake. Food consumption and body weights were determined daily for a 1 week equilibration period. Zinc was acutely restricted by offering deionized water ad libitum as the sole fluid source (Day 0). Groups of rats were sacrificed on Day 0 and 1,4, and 7 days following Zn restriction in order to evaluate neurotransmitter levels prior to the period of profound anorexia and weight loss. Plasma was obtained for determination of Zn by flame atomic absorption spectrophotometry; the hypothalamus was isolated and extracted with 0.1M perchloric acid for neurotransmitter assay using HPLC. Plasma Zn fell from 149 ug/dL on Day 0 to 48 and 28 ug/dL on Days 1 and 7 respectively, preceeding a 20% decline in food consumption starting on Day 4 of Zn restriction. Hypothalamic NE did not change during 7 days of acute Zn restriction from pre-treatment levels (2.39 ng/mg wet wgt). However, the levels of dopamine and 5-HT decreased by 18% during the phase of reduced food intake and weight loss beginning on Day 4. The results of this study do not support the hypothesis that acute Zn restriction induces changes in hypothalamic NE which may cue a reduction in food intake in developmentally mature rats.

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59.3 NALOXONE INHIBITS AND THE KAPPA-OPIATE KETOCYCLAZOCINE FACILITATES INTAKE OF PREFERRED SWEETS IN NONDEPRIVED RATS. W. C. Lynch
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Suppression of food and water intake by opiate antagonists appears to depend on oral, palatability, factors, (Apfelbaum and Mandenoff, Pharmac. Biochem. Behav., 15: 89, 1981). On the otherhand, while certain opiate agonists appear to facilitate feeding (Morley and Levine, Life Sci., 29: 1901, 1982), it remains unclear whether oral factors may also influence their effects. In preliminary work we showed that a moderate dose of naloxone (1 mg/kg, sc) selectively suppressed intake of preferred concentrations of sodium saccharin, a non-nutritive sweetener. In the present work we extend our studies to include very low naloxone (NAL) doses and moderate doses of the kappa-opiate agonist, ketocyclazocine (KC).

Two groups of adult male albino rats (n=10 each) were given repeated 2-bottle taste preference tests. Each test consisted of recording 1 hr intake of water or 1 of 3 concentrations of saccharin (.03%, 0.1% or 0.3%, w/v). All 3 concentrations were presented to each rat once per week in counterbalanced order. Drug treatments varied from week to week. In Group 1 saline and NAL treatments were alternated and NAL doses ranged from 0.01 to 1.0 mg/kg, sc. Both saline and NAL injections were given 15 min before intake tests. In Group 2, saline and KC treatments alternated, with KC doses ranging from 1.0 to 5.0 mg/kg, sc, given 30 min before tests. Due to the general inhibition of motor activity produced by KC at 30 min post-injection, a follow-up experiment examined the time-course of KC effect by measuring cumulative intake of 0.1% saccharin hourly for 10 hr following injection of KC (5 mg/kg, sc) or its vehicle (1 ml/kg).

Repeated preference tests yielded a progressive increase in intake of all 3 saccharin concentrations over weeks. NAL at a dose of 0.1 mg/kg or above consistently suppressed intake of all 3 saccharin concentrations by at least 50% but did not affect intake of water. The lowest NAL dose (0.01 mg/kg) suppressed intake of the lowest saccharin concentration but did not affect intake of the ? sweeter solutions, thus suggesting that at very low doses NAL is only effective near the threshold of sweet taste perception. KC (5 mg/kg) produced a generalized inhibition of motor activity for approximately 2 hrs post-injection but following this, saccharin intake was markedly enhanced such that by 6 hrs post-injection, KC had facilitated saccharin intake by approximately 50%. These data suggest that endogenous opioids, possibly acting at κ-receptors, may normally modulate the affective quality of gustatory stimuli. (Supported by MONTS-NSF project ISP-8011449.)

59.4 **REDUCTION OF CLONIDINE-INDUCED FOOD INTAKE BY NALTREXONE AND A STEREOSPECIFIC OPIATE RECEPTOR ANTAGONIST IN RABBITS.** **N.L. Katz, R.F. Schlemmer and D.P. Waller*. Dept. of Pharmacodynamics, Univ. of IL at Chicago, Hlth. Sci. Ctr., Chicago, IL 60680.**

Acute administration of the α-noradrenoreceptor agonist, clonidine, to rabbits stimulates a short-term vigorous increase in food intake, a typical response characterized by 2-3 eating binges over one hour, each binge lasting about 5-10 minutes (Waller et al., Pharmacologist, 24(3): 163, 1982). Studies using selective antagonists have suggested that presynaptic α_2-noradrenoreceptors may be involved in the clonidine response (Katz et al., Fed. Proc., 42(4): 159, 1983).

Since narcotic antagonists have been shown to reduce food intake in various species of rodents (e.g., Holtzman, J. Pharmacol. Exp. Ther., 189: 51, 1974), we determined the influence of the antagonist, naltrexone, on the clonidine feeding response. In a randomized block design, 8 male rabbits were given either 1 mg/kg naltrexone HCl, 2 mg/kg naltrexone HCl, or a saline control injection 10 minutes before receiving an i.m. injection of 0.075 mg/kg clonidine HCl or saline. The amount of food eaten during the first hour following the injections was measured. Rabbits given the saline + clonidine combination exhibited increased feeding behavior. Acute injections of naltrexone in saline blocked the clonidine response, the food intake reverting to control (saline + saline) levels. There was no significant difference between 1 and 2 mg/kg naltrexone.

The stereospecific requirements for the antagonism of clonidine-induced food intake were studied with optical isomers of 5,9α-diethyl-2-(3 furylmethyl)-2'-hydroxy-6,7-benzomorphan. Opiate antagonism resides predominantly in the (-)-isomer (MR2266), whereas the (+)-isomer (MR2267) is not a potent opiate antagonist. In a randomized block design, 3 sets of paired rabbits were given either 1 mg/kg MR2266, 1 mg/kg MR2267, or a vehicle (0.1N HCl) control injection 10 minutes before receiving an i.m. injection of 0.1 mg/kg clonidine. MR2266 significantly decreased the clonidine feeding response, while MR2267 had no effect. Thus, the results suggest that the reduction by opiate antagonists of clonidine-induced food intake is due to an interaction with opiate receptors.

59.5 SHAM FEEDING OF SUCROSE INCREASES HYPOTHALAMIC DOPAC/DA. K. Bourbonais*, C. Jerome*, K.J. Simansky and G.P. Smith (SPON: [illegible] Halmi). Department of Psychiatry, Cornell Univ. Med. Coll. and E.W. Bourne Behav. Res. Lab., New York Hosp., White Plains, NY 10605

Ingestion of food increases central dopaminergic (DA) activity in hypothalamus, accumbens and amygdala (Heffner et al, 1980). It is not clear which aspects(s) of food intake (taste, reward, eating movements, satiation and/or metabolic effects) was correlated with this increased DA activity. On the basis of the effect of DA postsynaptic receptor blockade by systemic administration of pimozide, Wise et al (1978) suggested that central DA activity mediated the rewarding effect of food, and Xenakis and Sclafani (1981, 1982) extended this hypothesis to the reward of sweet taste. We tested the hypothesized relationship between sweet taste and central DA terminal fields after rats sham fed 1, 10 or 40% sucrose. Sham feeding was used because (1) it eliminated the satiating and direct metabolic effects of sucrose, but preserved taste, reward and eating movements; (2) sham intake was a sigmoid function of sucrose concentration (Bernz et al, 1983); and (3) pimozide decreased sham feeding of sucrose in a manner that was similar to decreasing the concentration of sucrose (Geary et al, 1983).

After 17 h food deprivation, rats sham fed one of three concentrations of sucrose--1, 10 or 40%. Control rats were adapted to the deprivation schedule, but they never sham fed. After sham intakes stabilized, rats sham fed for 9 min, then were decapitated and DOPAC and DA content of hypothalamus, olfactory tubercle, accumbens, amygdala, caudate and frontal cortex were measured by HPLC and electrochemical detection. DOPAC/DA increased significantly as a function of sucrose concentration in the hypothalamus, but not in other regions.

Group	n	Hypothalamic DOPAC/DA (±SE)	Sham Intake (ml ± SE)
Control	12	.28 ± .02	0
1% sucrose	9	.30 ± .04	9 ± 2
10% sucrose	26	.35 ± .02	16 ± 1
40% sucrose	16	.50 ± .03*	18 ± 1

*p <.001 compared to 10% sucrose

Since 9 min sham intakes of 40% and 10% sucrose did not differ, the larger hypothalamic DOPAC/DA correlated with sham intake of 40% sucrose is probably not related to ingestive movements, but is more likely to be a function of the sensory and/or rewarding effects of sucrose oropharyngeal stimulation.

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59.6 DIFFERENTIAL EFFECTS OF PIMOZIDE AND SPIPERONE ON HOMEOSTATIC AND NONHOMEOSTATIC DRINKING. J. H. Porter*, J. J. McDonough*, G. F. Heath*, P. A. Goldsmith* and D. N. Johnson. Dept. of Psychology, Va. Commonwealth Univ. and A. H. Robins Pharmaceutical Co., Richmond, VA 23284.

Drinking in response to depletions in the cellular or extracellular fluid compartments may be classified as Homeostatic drinking; whereas, drinking which is insensitive to imbalances in body water and operates heedless of the state of body water may be classified as Nonhomeostatic drinking(see Kissileff, H.R. in The Neuropsychology of Thirst, 1973). Recently, Robbins and Koob (Nature, 285: 409-12,1980) demonstrated that lesions of the mesolimbic dopamine (DA) system attenuated the acquisition of schedule-induced polydipsia (SIP, a form of nonhomeostatic drinking, see Falk, J., Sci., 133: 195-6,1961), but did not attenuate drinking induced by water deprivation (a form of homeostatic drinking). The present study further examined the role of DA in the regulation of homeostatic and nonhomeostatic drinking with the DA blockers, pimozide and spiperone. In Exp. 1, 5 groups of rats were given 15 sessions of Polydipsia Training (FI 1-min food schedule) with vehicle, 0.5 mg/kg pimozide, 1.0 mg/kg pimozide, 0.0625 mg/kg spiperone, or 0.125 mg/kg spiperone injections (ip., 1-hr before test sessions). As seen in Table 1, the acquistion of SIP was significantly suppressed by the DA blockers at all doses. The reduced water intakes were not due to any changes in food motivation as barpressing remained unchanged at all doses. In Exp. 2, injections of pimozide and spiperone did not suppress established SIP. In Exp. 3, pimozide and spiperone did not suppress deprivation-induced drinking(23.5 hr deprivation schedule). The results of Exps. 1 and 3 confirm Robbins and Koob's findings of the differential role of DA in the regulation of homeostatic (deprivation-induced drinking) and nonhomeostatic (SIP) drinking. However, once SIP was established (Exp. 2), pimozide and spiperone were ineffective in attenuating the SIP drinking. These data support the suggestion that an intact DA system is necessary for the acquisition of SIP, but also suggest that other mechanisms may be important for the regulation of established SIP.

TABLE 1. Mean water intakes (ml) for each experiment.

	Vehicle	0.5mg/kg Pimozide	1.0mg/kg Pimozide	0.0625mg/kg Spiperone	0.125mg/kg Spiperone
Exp. 1	10.7	2.5*	3.6*	1.9*	2.3*
Exp. 2	16.3	15.2	16.2	16.3	15.5
Exp. 3	25.2	28.0	26.5	25.9	26.3

*p<0.05

59.7 SEROTONERGIC MODULATION OF NEUROPEPTIDE FEEDING SYSTEMS. B.A. Gosnell, A.S. Levine and J.E. Morley. Neuroendocrine Research Lab, Minneapolis VAMC, Minneapolis, MN, 55417.

Serotonergic systems have been implicated in the regulation of food intake and body weight, though the exact nature of their role is unclear. Depending on the route and locus of administration, serotonin (5-HT) has been reported to enhance or depress food intake. Similarly, conflicting results have been obtained following the administration of the serotonergic neurotoxin 5-6-dihydroxytryptamine (5-6-DHT). As a part of a larger effort in pharmacological modelling of the feeding system, we depleted 5-HT in the rat by injecting 5-6-DHT (75 µg) or vehicle (0.1% ascorbic acid) into the right lateral ventricle. At least ten days were allowed for recovery. During normal feeding, the 5-6-DHT animals ate 26.8 g/24 hrs (80% nocturnal) and the shams ate 27.6 g/24 hrs (82% nocturnal). Spontaneous nocturnal feeding was measured after s.c. injections of naloxone (0-5 mg/kg) and after i.p. injections of cholecystokinin-octapeptide (CCK, 0-20 µg/kg) and calcitonin (0-40 Units/kg). In a separate experiment, daytime feeding was induced by the s.c. injection of ketocyclazocine (KC, 0-10 mg/kg). At one hour after injection, sham and 5-6-DHT rats were approximately equal in their response to naloxone, with the 5 mg/kg dose suppressing intake by 49 and 47%, respectively. At two hours, this dose suppressed intake of 5-6-DHT rats by 44% compared to a 20% suppression in sham rats. Both groups responded approximately equally to the induction of intake by KC. For example, the 1 and 10 mg/kg KC doses increased 4 hour intake by 143 and 137% respectively, in sham rats and by 144 and 171% in 5-6-DHT rats. Both groups were equally responsive to 20 and 40 U/kg doses of calcitonin, though at a lower dose (10 U/kg), one hour intake was reduced by 69% in sham rats and only 24% in 5-6-DHT rats. Similarly, both groups reduced 0.5 hr intake in response to the highest dose of CCK (20 µg/kg), whereas two lower doses (5 and 10 µg/kg) were effective only in sham rats. These results suggest that 5-HT depletion may have caused slightly decreased sensitivity to the feeding effects of CCK and calcitonin, possibly indicating a partial role for serotonin in the mediation of their actions. Responses to an opiate agonist (KC) and antagonist (naloxone) were essentially unchanged, if not improved, by 5-HT depletion. At sacrifice, there were no differences in ir-dynorphin between the 5,6-DHT and sham animals in the anterior cortex, striatum, hypothalamus, brain stem and thalamus. Further use of selective neurotoxins may provide valuable insights into the mechanisms of feeding and satiety.

59.8 SELECTIVE ANTAGONISM OF THE DIPSOGENIC, BUT NOT THE ANOREXIC, ACTION OF SYSTEMIC SEROTONIN BY METHYSERGIDE: COMPARISON WITH DIHYDROERGOTAMINE AND OTHER SEROTONIN ANTAGONISTS. K.J. Simansky and T.A. Connell*. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129

Peripheral administration of serotonin increases drinking and decreases food intake in rats. These behavioral effects of systemic serotonin are mediated by different neural mechanisms because bilateral subdiaphragmatic vagotomy blocks the dipsogenic, but not the anorexic, action of this monoamine (Simansky, Bourbonais, & Smith, Soc. Neurosci. Abs., 1982, 8:605). The present study examined whether the pharmacologic mechanisms of the dipsogenic and anorexic actions of serotonin also differed.

We determined the effects of four doses of methysergide (5-40 umoles/kg, IP), an ergot amide antagonist of serotonin, on drinking produced by a 10 umole/kg (SC) dose of serotonin in male rats. The rats had free access to food pellets and water except during the drinking test--when food was removed. Rats injected with serotonin (n=6) drank 14.0 $\pm$ 1.4 ml/kg during the two-hour test period. Administration of methysergide (n=6/dose) 30 min prior to serotonin produced a dose-related decrease in drinking ($F_{4,25}$= 7.1; $p < .01$). The apparent ID_{50} for the antagonism of the dipsogenic response by methysergide was 10 umole/kg ($p < .01$). In contrast, methysergide (15 umole/kg; n=8) failed to affect the anorexic action of serotonin (n=7) on 30-min intake of a liquid milk diet after 17-h food deprivation. Serotonin reduced eating by 32% ($p < .05$) compared to control intake (17.1 $\pm$ 2.3 ml). Methysergide altered neither the inhibition of feeding produced by serotonin (-35%) nor the baseline intake of rats injected with saline (17.5$\pm$ 1.7 ml). In this same experiment, however, methysergide decreased the 2-h water intake of rats injected with serotonin by 36%.

Dihydroergotamine (.5, 5 and 50 umoles/kg, IP), an ergot peptide antagonist of serotonin and α-adrenoreceptors, also failed to antagonize the effect of serotonin on feeding, but the highest dose reduced drinking by 49%. In contrast, neither ketanserin (1-8 umoles/kg) nor the putative peripheral serotonin antagonist, xylamidine (1.8-20 umoles/kg 4 h prior to serotonin) antagonized the drinking produced by serotonin.

Our results demonstrate that the dipsogenic and anorexic effects of systemic serotonin are mediated by different pharmacologic mechanisms. Moreover, the selective antagonism of the dipsogenic effect of serotonin by the ergot derivatives, but not ketanserin or xylamidine, suggests that this behavioral action of serotonin is mediated by a distinct class of peripheral receptors.

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59.9 Binge-eating Following Chronic, Continuous Administration of Picrotoxin. R. E. Davis and G. D. Ellison. Dept. of Psychology, University of California, Los Angeles, CA 90024

Gamma-aminobutyric acid (GABA) may play a role in the control of appetite and other food-related behaviors in animals. Yet, the effects of chronic GABA deficiencies on appettive behavior have not been extensively studied. We have developed techniques for the chronic continuous administration of picrotoxin, an antagonist of GABA-mediated changes in membrane chloride-ion conductance. Using these techniques we examined the long-term consequences of continuous picrotoxin treatment on variety of consummatory behaviors.

Male, Long-Evans hooded rats (280-320) were housed individually with food and water available ad libitum except as noted. Picrotoxin was administered continuously for 10 days via chronically indwelling silicone reservoirs. Twenty-four hour food and water intake was monitored during the 10 days of picrotoxin administration and for the next 28 days following cessation of treatment. All animals were then food-deprived for 24 hours. After this the food was replaced and hourly food and water intake measures were taken. In addition, the number of food-pellets eaten and the average weight of food pellets was recorded.

Chronic, continuous picrotoxin treatment did not alter 24 hr food or water intake during or after drug treatment nor did it alter the body weights of the treated animals. However, this drug regimen did alter food and water intake following food-deprivation. Picrotoxin-treated animals consumed more food, sampled more food pellets and drank more water during the first 4 hrs after reintroduction of food. During the next 20 hrs food and water intake decreased in compensation for the overconsumption in the preceding period. These data suggest that chronic, GABA deficiencies do not influence the long-term regulation of food and water intake or body weight. However, such neurochemical deficiencies may be important to short-term energy regulation and the control of appetite.

60.1 CHOLECYSTOKININ OCTAPEPTIDE DOES NOT DIFFERENTIALLY INHIBIT SHAM FEEDING OF SUCROSE DURING THE DAY AND NIGHT PHASES OF THE RAT'S DIURNAL CYCLE. J.A. DiPoala*, J.A. Goldstein* and F.S. Kraly (SPON: W.E. Edmonston). Psychology Dept., Colgate Univ., Hamilton, NY 13346

The satiating potency of exogenous cholecystokinin octapeptide (CCK-8) varies diurnally when rats eat milk normally and when they sham feed (pregastric stimulation of oropharynx and esophagus) liquid diet 116EC. The satiating potency of CCK-8 also varies inversely with the concentration of sucrose sham fed during the day phase. To determine whether CCK-8 differentially inhibits sham feeding of sucrose during the day and night, we tested rats sham feeding various concentrations of sucrose at the midpoints of the day and night phases after i.p. CCK-8.

Sprague-Dawley male rats (n=13; 400-600g), equipped with stainless-steel gastric cannulas, were deprived of food for 20 hr prior to testing. Rats sham fed (with open gastric fistula) sucrose (2.5, 5 or 10% w/v) for a 30-min test which began immediately after i.p. .9% NaCl or CCK-8 (1 or 2 µg/kg). Rats sham fed at the midpoints of the day and night phases of a 12:12-hr cycle.

The inhibition of sham feeding by CCK-8 was related to the concentration of sucrose for both the 1 and 2 µg/kg doses: The percentage inhibition of sham feeding was inversely related to the concentration of sucrose with the exception of the inhibition score for the effect of 2 µg/kg CCK-8 upon 5% sucrose sham fed during the day (Table 1).

Table 1 (Median percentage inhibition)

	1 µg/kg CCK-8			2 µg/kg CCK-8		
Sucrose	Day	p	Night	Day	p	Night
2.5%	60	ns	64	41	ns	50
5%	35	ns	34	5	.065	44
10%	29	ns	30	35	ns	24

The CCK-8 did not differentially inhibit sham feeding of sucrose during the day vs. night for each concentration of sucrose and for both doses of CCK-8, with the exception of the effect of 2 µg/kg CCK-8 upon sham feeding of 5% sucrose: This dose produced less inhibition of sham feeding during the day than at night, but this difference did not reach statistical significance (Table 1).

In summary, there was no systematic differential effect of CCK-8 upon sham feeding of sucrose in the day vs. night. Because CCK-8 is reported to have differential effects in the day vs. night when rats eat sweet milk normally and when they sham feed a complex sweet liquid diet (GIBCO 116EC), the findings reported here suggest that the interaction of CCK-8 with pregastric stimulation by sucrose (i.e., sweet taste) is not the primary factor determining the diurnal variation in the satiating potency of exogenous cholecystokinin in the rat.

60.2 CHOLECYSTOKININ, SUBDIAPHRAGMATIC VAGOTOMY AND FOOD INTAKE IN THE GOLDEN HAMSTER. E. S. Corp*, D. A. Fitts* and S. C. Woods. (Spon: G. Clark). Dept. of Psych., University of Washington, Seattle, WA 98195.

Golden hamsters (Mesocricetus auratus) are known to be insensitive to a variety of stimuli which increase (food deprivation, 2-deoxy-D-glucose) or decrease (naltrexone, dexamethasone) food intake (FI) in rats. By contrast, treatments such as insulin and progesterone stimulate feeding in both species. We now report that the octapeptide of cholecystokinin (CCK) suppresses meal size in hamsters in a dose-related fashion, as it does in rats.

Hamsters (n=39, 4-6 wks old) were adapted to a daily, 3-hr deprivation of food and to intraperitoneal injections of .15 M saline. FI was measured for 30 min immediately following the injection. On the test day, hamsters were given an injection of CCK or saline. Percentage change from the preceding day's post-fast meal size showed a dose dependent suppression [$F(4,34)=8.82$, $p<.001$].

	Dose of CCK (µg/kg)				
	0	1.45	2.9	5.8	12.0
% Change	+24.1	-32.7	-38.7	-58.2	-67.3
± SEM	(14.4)	(6.9)	(12.5)	(4.8)	(15.5)

For the lowest dose, but not the higher doses, the suppression of FI in hamsters is comparable to that generally reported in rats. FI returned to baseline levels on the day following the test.

In rats, subdiaphragmatic vagotomy attenuates the suppression of FI by peripheral CCK administration (Smith, G. P. et al., Science 213:488, 1981). In a second experiment, 18 hamsters from experiment 1 received either a vagotomy or a sham surgery (laparotomy without vagal transection). Vagotomy was verified by anatomical inspection. After one week of recovery, the animals were again adapted to the 3-hr food deprivation procedure. Control 30-min FI for the post-fast meal was not significantly different from the levels attained prior to surgery. Injections of CCK (12 µg/kg) into the sham operated group resulted in a mean percentage reduction of -59 ±8.4%SEM, with nine of nine animals reducing meal size (range -85% to -8%). Of the vagotomized hamsters, four increased FI to CCK, while five reduced FI, for a net suppression of -22 ±19.3%SEM (range -90% to +62%). No significant difference from the sham controls was observed, owing to the variability in the response after vagotomy.

These results show a clear effect of CCK on short-term food intake in hamsters, although the role of the vagus nerve in mediating this effect is not conclusively demonstrated.

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60.3 Brief Exposure to a Saline Stimulus Promotes Latent Learning in the Salt Hunger System. Robert E. Breger, Nick Strombakis and Robert W. Allan Dept. of Psychology N.Y.U. and Jay Schulkin Dept. of Anatomy, Univ. of Penn. (Spon. George Wolf)

We all seem to learn things incidentally, and then later exploit the knowledge when it is useful to us. Yet this phenomenon, so evident to us in our sense of our daily life, was difficult to demonstrate in the laboratory (but see Rescorla 1981). Then, in an experimental setting using the salt hunger system, Krieckhaus and Wolf (1968,70) showed that salt hungry rats would remember how or where they found saline in the past even though they had no need for it at the time. In this and other studies the rats were given many repeated exposures to the saline and over a long period of time. But in one study Wirsig and Grill (1982) discovered that a strikingly brief exposure to the saline produced the same effect. We thought this phenomenon important and in this preliminary study, we also found that in an innate modular system, like salt hunger, a brief exposure to the saline is sufficient to trigger such learning.

Rats were trained to bar press for water in a Skinner box when they were thirsty. Then, they were given 5 reinforcements of .05 ml of .15 molar saline instead of water within a five minute period on two successive days. Rats were then made salt hungry for the first time in their lives and then tested in the Skinner box under extinction conditions. They were not deprived of water at this time. We found that resistance to extinction was about four times higher in these rats than in control rats which were treated identically except that they had never received the saline in the Skinner box.

60.4 EFFECT OF PERIPHERAL PROSTAGLANDIN E_2 ON SODIUM INTAKE AND EXCRETION OF ADRENALECTOMIZED RATS. K. M. Skoog, Michelle Muggli*, and Nancy J. Kenney. Department of Psychology, University of Washington, Seattle, WA 98195.

Intraperitoneal (IP) injection of 10-100 ug/kg prostaglandin E_2 (PGE_2) is effective in reducing intake of a 1.5% NaCl solution by intact rats maintained on sodium deficient food (Moe & Kenney, Neurosci. Abs., 1981). The following experiment was conducted to determine if IP PGE_2 treatment would reduce sodium intake of adrenalectomized (ADX) rats in which the need for salt is elevated. Six rats were maintained on sodium-replete laboratory chow and presented with a 1.5% NaCl solution for 2 hr each day. After a 7-day adaptation period, all of the rats were adrenalectomized. PGE_2 was administered IP in doses of 5, 10, 50 and 100 ug/kg immediately prior to sodium-solution access. Sodium intake and urine sodium concentrations were measured over the next 2 hr and at the end of the 24-hr following injections. PGE_2 test days were separated by at least 2 control days on which the carrier solution for the PGE was injected.

ADX rats fed sodium-replete chow reduced their total 2-hr sodium-solution intakes following administration of either 50 ($F(2,12)=5.80$, $p<.05$) or 100 ($F(2,12)=3.49$, $p<.05$) ug/kg PGE_2. Following the administration of 10 ug/kg PGE_2, sodium-solution intakes of ADX rats were reduced for the first 15 min ($F(2,12)=5.02$, $p<.05$) but returned to control levels within 20 min after the injection. Sodium intake from food was unaffected by any dose of the PGE tested.

Corresponding to the decreases of sodium-solution intake, urine sodium concentrations were reduced during the 2-hr immediately following administration of 50 ($F(2,12)=3.18$, $p<.05$) or 100 ug/kg ($F(2,12)=4.86$, $p<.05$) PGE_2. Urine volumes were unaffected by administration of PGE_2 in any of the doses tested. Apparent sodium balance (total sodium intake from the solution and from food - excretion of sodium in urine) was unaffected by any PGE_2 treatment.

Thus, although the dose required to effectively reduce sodium intake of ADX rats may be higher than that required to reduce intake of intact animals fed sodium-deficient chow, IP PGE_2 does suppress sodium intake to ADX rats.

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60.5 ALDOSTERONE AND BODY FAT. L. D. Devenport, K. G. Goodwin*, P. M. Hopkins*, G. L. Manes*, and K. L. Oltmanns*. Depts. Psychology and Zoology, University of Oklahoma, Norman, OK 73019.

Long-term aldosterone (ALD) injections elevate food intake and body weight in rats in the absence of measurable fluid retention (Devenport et al, Behav. Neurosci., in press), suggesting an accelerated body fat accumulation. And because body weight gains in ALD rats occur even when pair-fed we have suggested that aldosterone may inhibit lipolysis and/or enhance lipogenesis (Devenport et al., Neurosci. Abstr., 1982). The present studies sought to determine if the weight gains of ALD-treated animals could be attributed to body fat and if this hormone impedes the mobilization of fat stores during starvation.

For the first question, 44 d male rats were adrenalectomized (ADX) and implanted with continuous infusion devices (Alza Corp.) that provided sustained release of one of 6 doses of ALD (1.3-10.4µg/hr) against a constant background of corticosterone (CRT). Other animals received only CRT. Following 13 d of free-feeding, the rats were exsanguinated by decapitation for RIA determination of ALD levels. These values ranged from 0-.5-750ng/ml. Most importantly, a significant increase in epididymal fat pad mass was found to accompany ALD administration, peaking at moderate doses and falling off at high doses. These changes paralleled differences in total body weight.

In order to address the question of lipolysis, 350 g male ADX rats were implanted with infusion pumps containing maximally effective doses of ALD (2.6µg/hr from previous study), CRT, a combination of these hormones, or no replacement at all. Two experiments were conducted. In one, rats were placed on a severely limited feeding regimen; in the other they were totally deprived. Although these were terminal deprivation experiments, animal discomfort was minimized by basing imminent death on the appearance of hypothermia. Rectal temperature was monitored every 8 hr and when it dropped by >3°C the rats were sacrificed by anesthetic overdose and autopsied. All measurements including body temperature were taken without knowledge of hormone assignment. Regardless of deprivation condition, ALD alone and in combination with CRT significantly hastened the onset of hypothermia as compared to the other groups. Some ALD-infused rats became hypothermic in less than 24 hr. Besides bearing heavier fat pads at autopsy, the ALD rats lost weight at a rate significantly slower than that of other groups.

Since this same dose of ALD increased body weight and fat pad mass in free-feeding rats, we suggest that ALD shifts the balance of fat metabolism toward lipogenesis and away from lipolysis. This chronic diversion of calories away from lean body mass may account for ALD's stimulation of hunger.

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60.6 DOSE DEPENDENT REGULATION OF VOLUNTARY INTRAVENOUS INSULIN INTAKE IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT. E.K. Walls* and T.B. Wishart. Dept. of Psychology, Univ. of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0.

Although intravenous self-administration if insulin has been observed to be stable in normal rats, insulin intake increased initially and then paradoxically decreased to levels well below the prediabetic intake when these rats were made diabetic (Jouhaneau, J., & LeMagnen, J., Physiol. Behav., 20, 739, 1978). Recently it has been shown that diabetic rats rapidly learn to regulate their insulin intake when they have previous experience with a food delivery operant lever and are in a relatively stable metabolic state as a result of daily subcutaneous Lente Insulin treatment (Walls, E.K., & Singer, G., Proc. Int. Conf. Physiol. Food & Fluid Intake, Melbourne, 1983).

To determine the capability of the diabetic rat to regulate its insulin intake six streptozotocin-induced diabetic rats with chronic vena caval catheters were maintained throughout the experiment with 1.2 U of regular insulin/day infused continuously at a rate of .05U/hour. In addition to the chronic infusion, rats were given continuous access to two operant levers; one delivering 45 mg food pellets and the other delivering a fixed bolus infusion containing .0125U, .025U, or .050U of regular insulin. All rats initially learned to self-administer insulin with the .025U dose for a period of at least 5 days and were then tested for periods of 5 days with each of the .0125U and .050U infusions. Compared to the .025U condition, daily infusions were significantly increased 53% in the .0125U condition and significantly decreased in the .050U condition. However, daily voluntary insulin intake did not differ significantly across infusion conditions suggesting that diabetic rats compensate for insulin dosage. Because daily water intake accurately reflects urinary glucose excretion as a result of urine volume, it was used as a measure of metabolic stability. Daily water intake initially decreased by 22% of preself-administration levels within the .025U condition and was decreased by 41% and 39% in the .0125U and .05U conditions respectively.

In addition to the finding that insulin self-administering diabetic rats learn to regulate their insulin intake in a dose dependent manner, reduced water intake reveals that substantial therapeutic benefit may accrue from voluntary insulin intake in the diabetic rat.

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60.7 LIMITATIONS OF MEAL SIZE DO NOT PREVENT OVEREATING AND WEIGHT GAIN FOLLOWING OVARIECTOMY. Naomi J. Tomoyasu*, David B. West, and Nancy J. Kenney. Department of Psychology, University of Washington, Seattle, WA 98195.

Ovariectomy results in an increase of food intake primarily due to an increase of meal size. Since ingestion of a fixed caloric load in a few, large meals results in greater fat deposition than eating the same number of calories over many smaller meals, the obesity following ovariectomy may be due in part to the shift of meal pattern toward larger meals. To examine this possibility, the effect of restriction of meal size on weight gain and total food intake following ovariectomy was examined. Spontaneous meal patterns of 11 adult, female Long-Evans rats were monitored over a 5-day baseline period. Noyes pellets were available ad libitum by means of a pellet-detecting eatometer and meal size and intermeal interval were recorded by means of a microcomputer. At the end of this baseline period, all rats were ovariectomized. Six ovariectomized (OVX) rats were then returned to the ad-libitum feeding situation. For the remaining 5 OVX rats, meal size after ovariectomy was limited to a maximum of 80% of their average presurgery meal size. Separate meal-size limitations were imposed for the light and the dark portions of the light:dark sequence. Meal frequency and total food intake were not restricted. Body weight was monitored for 10 days after ovariectomy and meal pattern recorded for days 6-10 post-surgery.

OVX rats with ad libitum pellet access showed significant increases of rate of weight gain, total food intake, and meal size compared to presurgery measures. Restriction of maximum meal size of OVX rats to 80% of presurgery average meal size did not prevent either the increase of body weight or the increase of total food intake. The increase of rate of weight gain and the increase of total food intake of restricted OVX rats did not differ from those of free-feeding OVX animals. Following gonadectomy, the rate of weight gain of the restricted OVX rats averaged 1.9 + 1.0 g/day while that of the free-feeding OVX rats averaged 2.0 + 0.5 g/day. Meal-size restricted OVX animals increased their daily food intake by an average of 28.1 + 7.1% compared to an average increase of 27.7 + 6.8% for the free-feeding OVX rats. Thus, the increase of body weight following ovariectomy is apparently not dependent upon an increase of meal size. Increases of both food intake and body weight are observed following ovariectomy even if artificial meal-size limitations are imposed.

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60.8 OVARIECTOMY INFLUENCES DIETARY SELF SELECTION DIFFERENTLY AS A FUNCTION OF AGE OF THE RAT AT SURGERY. D. A. VanderWeele and V. I. Pulido* Dept. of Psychol., Occidental College, Los Angeles, CA 90041.

Several studies have assessed the effects of ovariectomy (OVX) upon self selection of dietary macronutrients in rats. Geiselman, et al, found that OVX reliably increased animal's intake of fat while decreasing carbohydrate consumption but Kanarek, et al, found no effects of OVX on macronutrient selection. Recently, Bartness, et al, have reported that carbohydrate intake was increased following OVX while fat ingestion was decreased and they indicated that OVX might produce preferences for more calorically dense diets. Other variables differ among these studies but age of the animal at OVX might be important. In Geiselman's study, rats were neonatally OVX while, in the others, adults were surgically altered.

We OVX rats at 3 days of age (n=4), or as adults (n=10) or gave them sham surgery (n=6). As adults, all animals were exposed to dietary regimes of solutions of 30% sucrose, 15% casein, pure vegetable oil and a mixture of alphacel, vitamins and essential inorganic salts, each in separate containers. Animals were then allowed to self select diets while separate nocturnal and diurnal ingestions were recorded and body weight gain noted. Intake data and body weight changes were analyzed by ANOVA.

Analyses showed no significant effects on macronutrient selection when rats were OVX as adults, however, there was a tendency to increase fat intake in spayed animals. OVX animals did display macronutrient preferences; see Table below. Rats OVX as neonates showed a significant elevation in fat and a decrease in carbohydrate intakes when adults. Comparisons appear in the Table. Both groups of OVX animals showed mild hyperphagia and increased body weight; body weight: neonate OVX=361±15.2, adult OVX=348.5±9.8, control=326±13.5 g. Rather than conclude that OVX induces rats to eat more calorically dense diets (an explanation of their increased weight), we conclude that removing estrogen during its organizational as opposed to its activational period, induces the rat to select diets high in fat (most often more calorically dense).

TABLE: % of nutrient intake as this macronutrient:

	FAT	CARBOHYDRATE	PROTEIN
NEONATE OVX	59.6%	30.2%	10.2%
ADULT OVX	28.5%	60.6%	11.3%
CONTROL	22.9%	63.2%	13.9%

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61.1 PERIPHERALLY-ADMINISTERED CAPTOPRIL: EFFECTS ON SODIUM APPETITE.
K. E. Moe, M. A. Stevenson* and A. N. Epstein. Dept. of Biology, University of Pennsylvania, Philadelphia, PA 19104.

In experiments designed to assess the role of the hormone angiotensin II (Ang II) in the arousal of sodium appetite (natriorexia), we have previously shown that peripheral administration of captopril (a blocker of Ang I→Ang II) at doses which block Ang II formation only in the periphery enhances depletion-induced sodium appetite but that higher doses which block conversion universally suppress it. This suppression is not the result of malaise or behavioral incompetence.

To examine the specificity of these effects, animals exhibiting a mineralocorticoid-elicited sodium appetite were infused with captopril (Squibb). This form of sodium appetite is independent of the renin-angiotensin system.

Rats received daily subcutaneous injections of 2 mg deoxycorticosterone acetate (DOCA), a precursor of aldosterone, which produced a reliable sodium appetite (intake > 4 ml of 3% NaCl in a daily 1-hr test). Some were subsequently infused continuously through jugular vein catheters with saline for 2-3 days, followed by 22-hr IV infusion of 15 mg/hr captopril, a dose shown previously to reliably suppress depletion-induced sodium appetite. It had no effect on DOCA-induced sodium appetite.

Other rats showing the DOCA-elicited appetite received a much lower dose of captopril administered in the drinking water (0.1 mg/ml) rather than IV, a procedure which enhances depletion-induced appetite and which can generate a sodium appetite in otherwise untreated rats. As reported here, this dose of captopril also enhanced DOCA-induced sodium appetite (average increase = 68%).

These results support the synergy hypothesis (Fluharty & Epstein, 1980) which proposes that during sodium deprivation or loss the two hormones aroused by sodium depletion, aldosterone and Ang II, act synergistically on the brain to produce sodium appetite, and which acknowledges that a very high level of either hormone is sufficient to arouse the appetite. Thus, universal blockade of endogenous angiotensin formation (by 22-hr of 15 mg/hr captopril IV) does not suppress the sodium appetite elicited by excess amounts of mineralocorticoid. The enhancement of the appetite by a treatment with captopril which blocks angiotensin formation only in the periphery (0.1 mg/ml in the drinking water for 1 night) is best explained by a "spillover" hypothesis; that is, the increase in peripheral Ang I known to result from this treatment can "spill over" into the brain where, in the absence of captopril, it can be converted to Ang II and can add its natriorexigenic effect to a pre-existing DOCA-induced sodium appetite.

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61.2 SODIUM APPETITE INDUCED BY SODIUM DEPLETION IS SUPPRESSED BY INTRACEREBROVENTRICULAR CAPTOPRIL. M. Weiss* and A.N. Epstein (SPON: G.B. Koelle). Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Angiotensin and aldosterone are the hormones of sodium conservation. Their levels in plasma rise during sodium depletion and they play important roles in the renal retention of sodium. We have suggested elsewhere (Peptides, 3: 1983) that the sodium appetite of sodium depletion may be aroused by their synergistic actions, and have shown in recent work (Fluharty & Epstein, Behav. Neurosci.) that an appetite can be rapidly and reliably aroused in the sodium replete rat by treatment of it with both angiotensin and aldosterone (given as its precursor DOCA). In work described here the role of angiotensin in the arousal of sodium appetite was studied with captopril, a competitive inhibitor of angiotensin converting enzyme, administered directly into the cerebral ventricles of sodium depleted rats at doses sufficient to block angiotensin formation in the brain. Sodium depletion was produced by furosemide (10 mg, sc) followed by sodium deficient diet in rats fitted with intracerebroventricular (ICV) cannulae, through which they were infused overnight with captopril (1.2 ng, 12 ng, 120 ng, 1.2 µg, 12 µg/hr) or its vehicle during the entire 18-hr depletion period (when sodium is not available) and during a subsequent 2 hr sodium appetite test when 3% NaCl was offered. This was followed by confirmation of the Ang I to Ang II blockade by failure of dipsogenic response to pulse ICV Ang I, followed by drinking to pulse ICV Ang II. The experiment was repeated (1.2 µg/hr captopril, cICV) in sodium replete rats in which a sodium appetite had been raised with excess DOCA (2 mg/day, 1 week).

Depletion-induced sodium appetite, but not overnight food and water intake or the sodium appetite induced by DOCA, was reduced in a dose related manner by the 12 ng, 120 ng, 1.2 µg/hr doses. The highest dose (12 µg/hr) had nonspecific effects on overnight water intake. The lowest dose (1.2 ng/hr) failed to block Ang I to Ang II conversion and did not block the salt appetite. The 1.2 µg/hr dose did not alter sodium excretion.

Thus, the infusion of captopril into the ventricles of the rat at doses that block conversion of Ang I to Ang II suppresses the sodium appetite induced by sodium depletion without reducing the animals behavioral competence to eat or drink overnight, or to drink 3% NaCl when a sodium appetite is induced by high doses of DOCA.

The cerebral action of angiotensin II may be essential for the arousal of sodium appetite.

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61.3 SUPPRESSION OF SODIUM APPETITE BY INTERFERENCE WITH THE CEREBRAL ACTION OF ANGIOTENSIN. Jacob F. Schultz* and Alan N. Epstein. Dept. of Biology, Univ. of Pennsylvania, Philadelphia, PA 19104.

If a synergy of angiotensin (Ang) and aldosterone is the cause of sodium appetite (Epstein, A.N., Peptides 3: 1983) then prevention of the action of endogenous Ang should suppress it and the suppression should be especially effective in animals in which aldosterone can not participate. This prediction was successfully tested in 5 adrenalectomized rats drinking excess volumes of 3% NaCl. The animals had been treated with dexamethasone (20 µg/day, sc) for at least 3 days before the experiment began. Their appetites for salt were reduced by 83% by continuous (overnight) intracerebroventricular (cICV) infusion with a dose of captopril (120 ng/hr) that was sufficient to block conversion of Ang I to Ang II in the cerebral ventricles. Concurrent water intakes were reduced by 55%.

To address the question of specificity we induced another type of salt appetite in 4 adrenalectomized rats. They were treated with deoxycorticosterone acetate (2-6 mg/day, sc, 4-6 days) until their preexisting salt appetite was abolished and then replaced by a DOCA-induced appetite. Indentical (cICV) infusion of captopril did not reduce that appetite.

The recent suggestion (Weisinger, R.S. et al., Amer. J. Physiol. 242: 1982) that CSF Na^+ is crucial for salt appetite was tested with overnight (cICV) infusion of hyperosmotic NaCl (0.2 M to 1.0 M at 5 µl/hr). It did not reduce the established appetite for 3% NaCl in 4 adrenalectomized rats.

These results encourage the proposal that in the adrenalectomized rat, hungry for salt, sodium appetite is dependent on the cerebral action of Ang II.

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61.4 COMPARISON OF INTRACEREBROVENTRICULAR AND INTRAVENOUS ANGIOTENSIN II ON THE Na APPETITE OF Na-REPLETE SHEEP. R.S. Weisinger*, D.A. Denton*, M.J. McKinley*, J.B. Simpson and E. Tarjan*. Howard Florey Institute, Univ. of Melbourne, Australia.

Previous research from this laboratory has shown that intracerebroventricular (IVT) infusion of angiotensin II (AII) causes increased Na intake of Na-replete but neither IVT nor intravenous (IV) infusion of AII cause increased Na intake in Na-deplete sheep. The present experiments evaluated the effect of IVT or IV infusion of AII on Na intake of Na replete sheep with limited Na access. In the present experiments, sheep were trained to bar press for water (50 ml/delivery) and 0.5M NaCl (12.5 mmol/delivery). Water was available continuously while Na was available for 2h/day. At the end of a Na access period a 48h IVT infusion of AII (3.8 µg/h) or IV infusion of AII (24 µg/h) was begun. Thus, the effect of AII on urinary Na loss as well as subsequent Na intake (i.e., after 22 and 46h of infusion) could be determined.

IVT infusion of AII caused a marked increase both in Na excretion and in Na intake. Without infusion of AII, the sheep bar pressed for 3.6 ± 0.7 deliveries or 45 mmol of Na. During IVT infusion of AII, Na intake was increased (p's <0.001) to 220 mmol of Na on day 1 and 335 mmol of Na on day 2. Daily Na deficit caused by urinary loss prior to the two Na access periods were 250 ± 96 and 295 ± 73 mmol. For the two days of infusion combined, there was a significant correlation ($r(8) = 0.73$, $p<0.02$) between Na deficit (273 ± 57 mmol) and Na intake (278 ± 44 mmol). IV infusion of AII caused an increase both in Na excretion and Na intake. Without infusion of AII, the sheep bar pressed for 3.4 ± 1.1 deliveries or 42 mmol of Na. During IV infusion of AII, Na intake was increased (p's <0.001) to 205 mmol of Na on day 1 and 208 mmol of Na on day 2. Na deficit prior to the first Na access period was 75 ± 37 mmol. No Na deficit was evident prior to the second Na access period.

These results demonstrated that IV infusion of AII can cause increased Na intake in Na-replete sheep in excess of previous loss. In addition, the increase in Na intake caused by IV infusion of AII was similar to that produced by IVT infusion of AII even though only the latter infusion was clearly associated with a marked Na deficit. One possible interpretation of these data is that the IV as well as the IVT hormone acted at neural structures involved in salt appetite located outside the blood-brain barrier (e.g., circumventricular organs). AII infused IVT could have acted at additional loci within the blood-brain barrier, thus inaccessible to the circulating hormone, to provoke the Na deficit.

61.5 TOGETHER INTRACRANIAL ANGIOTENSIN AND SYSTEMIC MINERALOCORTICOID PRODUCE AVIDITY FOR SALT. D.-M. Zhang*, E. Stellar and A. N. Epstein (SPON: J. M. Sprague). Depts. of Anat. and Biol., Univ. of Pennsylvania, Philadelphia, Pa. 19104.

These experiments use runway performance rewarded by minute volumes of 3% NaCl as a measure of increased avidity for salt. They were undertaken to assess the power of the synergy hypothesis to predict the appetitive as well as the consumatory aspects of the motivation that yields a sodium appetite.

Adult, male Sprague-Dawley rats were first trained under 23 hrs water deprivation to run to a small water reward (2 drops) at the end of a one-meter runway. 18 rats were then implanted with intracerebroventricular (ICV) cannulas, were injected with different doses of angiotensin, for each of 3 days, and were allowed to run the runway to either water rewards or 3% sodium chloride rewards. These tests were repeated when the animal received no angiotensin but was primed for 3 days with subcutaneous DOCA (500 µg) injections, and when it received DOCA plus ICV angiotensin (60 ng).

The major finding is that when Ang II and DOCA are used together, the animal runs for 3% NaCl and the effect appears to last for 60 minutes whereas DOCA or Ang II alone do not compel the animal to run for salt. Adding DOCA to Ang II produces only a slight potentiation of running for water. While running speed to water is twice the running speed to 3% NaCl under all conditions, it is clear that only the combined treatment with Ang II and DOCA produces a robust appetitive response to 3% NaCl.

The combination of treatments with doses of 1) systemic DOCA that are too low to elicit the search for strong salt solutions, and 2) intracranial angiotensin that does not lead to an approach to salt, produces a remarkable change in the animal's behavior. It now leaves the start-box and runs the runway for the taste of salt. The treatment with angiotensin and a mineralocorticoid has aroused appetitive behavior for salt.

Our animals were replete with sodium throughout the experiment. They were eating standard lab diet which is rich in NaCl. They had been treated for several days with a hormone that promotes renal conservation of sodium. And the behavior of salt seeking occurred within minutes after intracranial injection of angiotensin, long before it could have had natriuretic effects. The animals were therefore seeking salt that they did not need. These results are compatible with the idea that the brain is apprised of the need for salt not by salt depletion but by a synergy of the hormones that are mobilized to reverse the depletion. By showing that the two hormones of sodium conservation generate salt-seeking behavior when given together at doses that are each too low when given alone to alter behavior toward salt, these results suggest that the same hormones that act in the kidney to promote the saving of sodium also act in the brain to promote the search for salt.

61.6 ANGIOTENSIN II AND OSMOSENSITIVE NEURONS IN THE OVLT REGION. D.O. Nelson and Carol A. Graham*. Depts. Physiology, Surgery and Medicine, Northwestern Medical Sch., Chicago, IL 60611.

Intraventricular injections of angiotensin II (AII) and hyperosmolar solutions produce increased vasopressin release, water intake, intravascular volume and blood pressure. Binding studies have localized AII receptors in tissue near the organum vasculosum lamina terminalis region (OVLT) and extracellular recordings show neurons in this region to be sensitive to AII. Similarily, lesioning and microinjection studies in intact animals and hypothalamic explants indicate that the integrity of the OVLT is important for transduction of osmotic stimuli to appropriate behavioral and hormonal responses. In the present study we have examined the sensitivity of spontaneously active single OVLT neurons to iontophoretically applied AII and to changes in extracellular osmolarity using the in vitro brain slice technique and extra cellular recording. Hypothalamic slices containing the OVLT region were prepared from 10-12 wk. old Sprague-Dawley rats. Multibarrelled electrodes were used to simultaneously record and apply AII, Saralasin and other agents. Of 65 spontaneously active neurons examined for sensitivity to AII, 48 showed increases in activity to low current (50uA) iontophoretic applications of AII. Application of Saralasin reduced or eliminated spontaneous ongoing activity or reduced the response to simultaneously applied AII. Approximately 65% of the cells were also responsive to application of either glutamate or acetylcholine. Addition of the calcium channel blocking agent Verapamil, to the perfusing medium or reduction of extracellular calcium levels reduced or eliminated responses to AII application. In the majority of the neurons examined, increased activity outlasted the period of AII application by several minutes. Application of the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) prolonged response periods to AII.

Twenty-two of 57 cells showed increases in spontaneous activity during perfusion with medium made hyperosmolar with mannitol or sodium chloride. Increases in activity were dose dependent and were detected with changes in osmolarity of approximately 5mOsm. The results of this study support the work of other investigators which implicate the OVLT as an AII and osmotic sensitive brain region. Preliminary results suggest the possibility that calcium influx and cyclic nucleotides may partially mediate the excitation to AII application. Current studies are concentrating on further understanding the cellular mechanisms responsible for AII and osmosensitivity in OVLT neurons and possible interactions. (Supported by USPHS HLB 29033).

61.7 THE PRESSOR RESPONSE TO INTRAVENOUS INFUSION OF ANGIOTENSIN II INHIBITS THE DRINKING RESPONSE. M. M. Robinson and M. D. Evered*. University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Intravenous infusions of Angiotensin II (Ang II) at doses commonly used to study thirst mechanisms cause large increases in arterial pressure yet relatively small drinks. We have investigated the possibility that the pressor response suppresses the water intake. Ang II was infused intravenously into rats (200 - 400 g) at a rate of either 16.7 or 100 ng/min. Captopril (0.33 mg/min), an angiotensin-converting enzyme inhibitor, was infused during all experiments to block any endogenous synthesis of Ang II. Fifteen minutes after the start of the Ang II infusion, isoproterenol or diazoxide was injected subcutaneously in doses which returned blood pressure to normal. For example, Ang II infused at 100 ng/min increased mean arterial pressure 40 - 60 mm Hg (n = 6) and caused rats to drink 3.7 ± 0.5 ml/90 min (Mean ± S.E., n = 28). Injections of diazoxide (75 mg/kg) or isoproterenol (0.1 mg/kg), which returned blood pressure to normal, increased Ang II-induced drinking to 7.8 ± 0.6 (n = 7) and 7.3 ± 1.1 (n = 9) ml/90 min respectively. Similar results were obtained at the lower rate of Ang II infusion (16.7 ng/min) which alone increased arterial pressure 40 - 50 mm Hg and caused rats to drink 1.6 ± 0.5 ml/90 min; diazoxide (20 mg/kg) returned blood pressure to normal and again increased intake, to 4.2 ± 0.7 ml/90 min (n = 6). Increasing the dose of diazoxide to cause blood pressure to fall below normal did not increase the drinking response to Ang II further. The increased water intake cannot be attributed to a drinking response to the hypotensive agents themselves since the captopril infusion was adequate to completely block drinking to either isoproterenol or diazoxide alone. These results suggest that: (1) the rise in arterial pressure reduces the dipsogenicity of Ang II, and (2) with Ang II levels clamped, hypotension is not a stimulus to drink. Supported by Canadian MRC.

61.8 A LIMITED ROLE FOR ANGIOTENSIN II IN DRINKING ELICITED BY HISTAMINE IN THE RAT. F.S. Kraly, A.F. Moore*, L.A. Miller* and A.P. Drexler*. Psychology Dept., Colgate Univ., Hamilton, NY 13346 and Research & Development Div., Norwich-Eaton Pharm., Norwich, NY 13815.

Sprague-Dawley male rats (n=4; 310-335g), equipped with catheters in the abdominal aorta for measurement of blood pressure, were tested for drinking after s.c. histamine diphosphate (1.25, 2.5, 5 and 10 mg/kg). Histamine decreased mean blood pressure ($p < .005$) and increased water intake ($p < .005$) from baseline. The 2.5 mg/kg dose was the smallest dose to significantly ($p < .05$) decrease mean blood pressure (mean decrease ± SE: 15.5 ± 5.7 mmHg) and it was the smallest dose to significantly increase ($p < .05$) water intake (mean increase: 1.8 ± .6 ml) in 45 min after the injection. Both hypotensive ($r = .96$) and dipsogenic ($r = .89$) effects were correlated with the dose of histamine.

Because hypotension accompanied drinking in response to doses of s.c. histamine above and including 2.5 mg/kg, and because doses of s.c. histamine above and including 6.6 mg/kg are reported to increase plasma renin activity in the rat (Leenen et al., 1975), it is possible that a renal-mediated angiotensin II (AII) response to restore circulatory homeostasis could contribute to drinking elicited by s.c. histamine. This hypothesis was tested by blocking the conversion of angiotensin I to AII using SQ14,225 (Captopril) p.o. prior to s.c. histamine (1.25, 2.5, 5, 10 and 20 mg/kg) in 12 rats (500-650g). Each dose of histamine was preceded by 60 min with either 50 mg/kg SQ14,225 or .9% NaCl. Tests (60 min) occurred every fourth day. Histamine preceded by .9% NaCl p.o. elicited drinking in a dose-related manner ($p < .001$). SQ14,225 p.o. inhibited drinking ($p < .05$) in response to only the largest dose (20 mg/kg) of histamine (mean water intake, after .9% NaCl: 8.6 ± .8 ml; after SQ14,225: 6.4 ± .8 ml). SQ14,225 failed to inhibit drinking elicited by doses of histamine ranging from 1.25-10 mg/kg.

That SQ14,225 attenuated drinking in response to only the highest dose of histamine is indirect evidence that peripheral AII, mobilized in response to the hypotensive effects of s.c. histamine, contributes to a normal drinking response to histamine only when hypotension is pronounced. This, together with the finding that peripheral AII is not necessary for drinking in response to 1.25-10 mg/kg s.c. histamine, is consistent with the report (Kraly & Miller, 1982) that selective transection of gastric vagi abolishes drinking in response to these doses of s.c. histamine and that vagotomy together with SQ14,225 is necessary to abolish drinking in response to 20 mg/kg s.c. histamine in the rat.

61.9 IMMUNOHISTOCHEMICAL EVIDENCE FOR CENTRAL ANGIOTENSIN II-CONTAINING PROJECTIONS TO THE SUBFORNICAL ORGAN. R.W. Lind, L.W. Swanson and D. Ganten*, The Salk Institute, La Jolla, CA 92037 and The University of Heidelberg, Germany.

Angiotensin II (AII) is a peptide hormone that acts at peripheral and central sites to promote fluid balance. Considerable evidence now indicates that there is a central source of AII, in addition to the well known peripheral source, and immunohistochemical studies have identified an AII-like substance in neuronal cell bodies and axons in the brain.

We recently mapped the distribution of AII immunoreactivity in the rat central nervous system and identified several cell groups and fiber systems. Of particular interest was the consistent appearance of a dense fiber plexus in one of the circumventricular organs, the subfornical organ (SFO). Since the SFO is a likely central receptor site for circulating AII, it was first necessary to determine whether the immunoreactivity in the SFO was due to receptor-bound peptide. Nephrectomy, which greatly diminishes plasma titers of AII, was performed 48 h before perfusion. No apparent diminution was seen in subsequent immunohistochemical staining for AII in the SFO. Conversely, 3-day water deprivation greatly increases circulating levels of AII, and this treatment produced no visible enhancement of AII immunoreactivity in the SFO. These results indicate that circulating AII is not the source of immunohistochemical staining in the SFO.

The next experiment employed knife-cut lesions to interrupt possible AII-containing pathways to the SFO. It was found that horizontal cuts of the ventral stalk of the SFO consistently diminished AII immunoreactivity in the SFO. Furthermore, bright, varicose fibers containing AII immunoreactivity were observed ventral to the cut. These findings suggest that an AII-containing neural input enters the SFO through its ventral stalk.

Finally, several double labeling experiments involving injections into the SFO of the retrograde tracer True blue, combined with immunohistochemical staining for AII, have been conducted. Neurons containing both the retrograde tracer and AII immunoreactivity were found in perifornical parts of the lateral hypothalamic area, in the zona incerta, and in the nucleus reuniens. Other known inputs to the SFO were also retrogradely labeled, but none were AII-positive.

These findings suggest that certain diencephalic projections to the SFO contain AII. It is possible, therefore, that humoral as well as neural signals involving AII converge in the SFO to produce both visceral and behavioral responses related to fluid balance.

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61.10 METABOLIC STIMULATION OF THE SUBFORNICAL ORGAN BY PERIPHERAL ANGIOTENSIN II. D.W. Andrews*, P.M. Gross, M. Kadekaro, and L. Sokoloff. Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, Maryland 20205.

The subfornical organ has an important role in the dipsogenesis produced by peripherally administered angiotensin II (AII). We examined the effect of AII on glucose metabolism in the subfornical organ of conscious rats in two sets of experiments: 1) intravenous infusion of AII (2.5 µg/min for 45 min) which increased arterial pressure and stimulated drinking in water-sated Sprague-Dawley rats; and 2) intraperitoneal injection of captopril, an angiotensin converting-enzyme inhibitor (50 mg/kg 60 min before experiment + 1/3 total dose given i.v. 15 min before experiment) to homozygous Brattleboro rats, which normally have high circulating levels of AII and high rates of glucose metabolism in the subfornical organ. Captopril caused a sustained reduction in arterial pressure. We determined rates of glucose utilization by the quantitative autoradiographic [^{14}C]deoxyglucose method with the assistance of computerized image processing. Results are presented as the mean µmol/100 g/min ± SEM for the subfornical organ. The number of animals is indicated in parentheses.

	Saline	Angiotensin II	% Diff.
Experiment 1	44±4 (4)	66±4* (4)	+50
	Untreated/Saline	Captopril	
Experiment 2	69±3 (11)	53±1* (4)	-23

* $p < 0.05$ compared to control animals by independent t-test.

Intravenous AII also increased glucose utilization in the pituitary neural lobe (+76%), but other structures having connections with the subfornical organ were not affected. Captopril did not lower the high rate of metabolism found in the neural lobes of Brattleboro rats. We conclude that circulating AII stimulates metabolic activity in the subfornical organ of normal and homozygous Brattleboro rats.

61.11 NEUROCHEMICAL LESIONS IN SUBFORNICAL ORGAN OR MEDIAN PREOPTIC NUCLEUS AFFECT DRINKING, BUT NOT PRESSOR, RESPONSES TO ANGIOTENSIN S.I. Bellin, R.K. Bhatnagar and A.K. Johnson. Depts. of Psychology and Pharmacology, and the Cardiovascular Center, University of Iowa, Iowa City, Iowa 52242.

Recent work in our laboratory has demonstrated anatomical connectivity between several anterior forebrain structures that comprises a portion of the functional pathway involved in angiotensin II (AII)-elicited thirst and pressor responses (Johnson, A.K., Circulation, Neurobiology and Behavior (Smith, Galosy and Weiss, eds.), p.277, 1982; Lind, R.W. and A.K. Johnson, J. Neurosci. 2(8):1043, 1982). Using electrolytic lesioning and experimental knife cut techniques, several investigators have defined a neural substrate for the mobilization of thirst which involves the Subfornical Organ (SFO) and the Median Preoptic Nucleus (MnPO).

In the present study, 6-hydroxydopamine (6-OHDA, 4ug/2uL) was injected into either the SFO (SFOX) or MnPO (MnPOX) nucleus of ether-anesthetized rats. Following the recovery of normal overnight drinking behavior and rates of body weight gain, thirst and pressor responses to systemic and central challenges were tested. MnPOX animals exhibited a significant attenuation of water intake following peripheral AII injections (3 mg/kg; 3 mg/ml, S.C.) when compared to values observed in vehicle injected controls (1.2±1.0 vs 4.4±1.4 ml, respectively, $p \leq .001$). Although there was a tendency for SFOX rats (6.9±2.6 ml) to drink more than control animals in response to systemic AII, this difference was not statistically significant. Peripheral osmotic challenges (12% NaCl, 1 ml/100gm, S.C.) caused SFOX animals to drink more water than that consumed by animals in either MnPOX or control groups (13.5±2.6 vs 10.5±2.4 or 9.7±2.1 ml, respectively, $p \leq .05$). The drinking response to central AII (100ng/2uL, ICV) by rats from either of the lesioned treatment groups was indistinguishable from values observed in control animals. Pressor responses to central AII, NaCl (2uL of a 600 mOsm solution) or carbachol (250ng/2uL) were comparable across groups. Similarly, the immediate and robust rise in blood pressure that accompanied a systemic AII injection was virtually identical in all rats. These data suggest that there is an interaction between catecholamines and the thirst elicited by systemically administered AII at the level of the MnPO nucleus.

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61.12 EVIDENCE FOR THE INVOLVEMENT OF THE LATERAL LATERAL PARABRACHIAL NUCLEUS IN THE MAINTENANCE OF BODY FLUID BALANCE. L.E. OHMAN AND A.K. JOHNSON. Department of Psychology, University of Iowa, Iowa City, Iowa 52242.

The lateral parabrachial nucleus (LPBN) has been reported to receive ascending projections from the nucleus of the tractus solitarius (NTS) (Loewy and Burton, J. Neurol., 181, 421-450, 1978), and area postrema (AP) (Shapiro and Miselis, Neuroscience Abstracts, 74.4, 8, 269, 1982), a circumventricular organ that has been shown to enhance drinking to systemic administration of AII (Edwards and Ritter, Physiology and Behavior, 29, 943-947, 1982). The LPBN innervates a number of diencephalon nuclei, including the median preoptic nucleus (MNPO), a prominent component of the periventricular anteroventral third ventricle, or AV3V region. The existence of these projections suggests that LPBN may play a role in the maintenance of body fluid homeostasis.

To test this hypothesis, rats with bilateral lesions of the lateral portion of the lateral parabrachial nucleus (LLPBN) or bilateral sham tract lesions were tested for drinking in response to systemic administration of AII (3.0 mg/kg, 1.5 mg/kg), isoproterenol (100 ug/kg, 30 ug/kg), hypertonic saline (12%, 4%), and isotonic saline.

Animals in the lesion group were found to drink significantly more to both doses of AII than animals in the sham tract group. ($p<.05$). No differences were found between groups in response to the other dipsogens. A third group of animals became apparent after histological examination. In this group of rats, the LLPBN sustained partial but not complete bilateral damage. An analysis of the drinking response for this partial miss lesion group revealed no significant difference between their water intake and that of the control group in response to the low dose of AII. However, in response to the high dose of AII the partial miss group did not drink significantly less than LLPBN lesioned animals, showing a graded effect of the lesion. The majority of complete and partial miss lesions appeared to bilaterally destroy an area through which AP afferents projecting to LLPBN have been reported to travel (Shapiro and Miselis).

These data lead us to conclude that LLPBN is involved in an inhibitory thirst pathway and further, we speculate that this nucleus may be a point of convergence for cardiovascular (NTS) and endocrine (AP) information.

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62.1 CENTRAL VS. PERIPHERAL ORIGINS OF FEEDING: BEHAVIORAL AND METABOLIC EFFECTS OF GLUCOSE AND FRUCTOSE RE-EXAMINED FOLLOWING INSULIN. J. Carlton and N. Rowland. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

The finding that intravenous (iv) infusions of glucose and fructose effectively suppressed the feeding which occurs in the 2hr after insulin administration to rats was taken as evidence for a peripheral origin of feeding, since fructose does not enter the brain (Stricker, et al.1977). However, it was reported that fructose, but not glucose, suppressed the feeding observed 6hr after insulin (postglucoprivic feeding; Bellin & Ritter, 1981). This ineffectiveness of glucose is paradoxical, since it is used in both brain and periphery.

In the present study we first attempted to replicate the findings of Bellin & Ritter. Male Sprague-Dawley rats were injected s.c. with regular insulin (2U/kg) or saline, and chow was removed for 6hr. They were then given a 2hr feeding test, and those eating more after insulin than saline were included in the study. Jugular vein catheters were implanted and, after recovery, insulin was administered followed 90 min later by a 30 min iv infusion of 0.9% saline, D-glucose, or β-D-fructose (2.4 M; 4.4 ml). A feeding test was then performed 6 hr postinsulin as before. In three separate experiments (data combined in Table) both glucose and fructose abolished the

Mean Chow Intake (g)	Injection/Infusion SAL/SAL	INS/SAL	INS/GLU	INS-FRUCT
30 min	0.9	4.4*	1.6	2.0
120 min	1.7	5.2*	2.4	2.6

postglucoprivic feeding. The reductions in food intake were calorically equivalent to the calories infused. The glucose suppression does not confirm the data of Bellin & Ritter, but is consistent with the expected metabolic effects.

In parallel experiments we examined the plasma concentrations of these sugars following iv infusions. Two hr following insulin rats had plasma glucose (PG) levels of 60mg/dl (PG of non-treated rats = 130-160 mg/dl). Rats infused with glucose between 90 and 120 min postinsulin as above had PG 450 mg/dl at the end of the infusion, and this fell exponentially to 90 mg/dl after 1 hr (Conard K coeff. =-.031). Similar infusions of fructose produced plasma fructose (PF) levels of 250 mg/dl, falling to 61 mg/dl 1 hr later (K for fructose = -.023). In these cases, small amounts of glucose and fructose (ca. 0.1 g) were lost in the urine.

Thus, both glucose and fructose suppress postglucoprivic feeding, but the effective infusions produce abnormally high PG and PF values. We are currently extending these data with studies of fructose utilization, and effects on hypothalamic NE turnover.

62.2 DIABETES IN RATS: EFFECTS OF DIETARY FAT TYPE AND CONCENTRATION ON FOOD INTAKE AND PLASMA METABOLITES. L.L.Bellush & N.Rowland. Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Mildly diabetic (D) rats showed a preference for dietary fat compared with nondiabetics (ND) but severely D rats self-selected a high protein regimen (Bartness & Rowland, Soc.Neurosci.'82). We also confirmed Friedman's ('78) observation that D rats are no longer hyperphagic when fed the "Corbit & Stellar" high fat diet (1/3 Crisco shortening, 2/3 chow). However, many D rats deteriorated when fed this diet and, given the choice, preferred chow. We now report the metabolic effects of these diets.

In the first experiment D (streptozotocin 70 mg/kg) and ND rats received either high protein (50%P, 40%C, 10%F), high fat-protein (50%P, 15%C, 35%F) or normal (25%P, 65%C, 10%F) purified equicaloric diets for 4-6 weeks. Food intakes (FI), weight gain (W) and plasma glucose (PG) are shown in Table 1. The D rats fed the high fat-protein diet showed the greatest W gain for the lowest FI, suggesting that the fat calories were effectively utilized. These rats were clearly in better health that D rats on the Corbit-Stellar diet. One difference between the two diets is that the latter is made with saturated fat while the purified and self-selection diets that we have used are made with oil (relatively unsaturated).

TABLE 1		HiPro	HiFat	Normal
FI(g/d)	D	45	39	53
	ND	31	33	34
W(g/d)	D	-1.0	+1.2	-2.3
	ND	+3.7	+2.8	+4.1
PG(mg/dl)	D	551	473	664
	ND	131	138	139

In the second experiment we investigated whether the saturation of the dietary fat was an important factor. D and ND rats received high fat diets of either saturated (1/3 w/w coconut oil + 2/3chow) or unsaturated (1/3 w/w safflower oil + 2/3 chow) components for 10 days. The data are shown in Table 2. D rats on the unsaturated fat diet showed less weight loss per day and less elevation of triglyceride levels compared with D rats on saturated fat diets. Dietary fat saturation (Table 2) as well as protein concentration (Table 1) are important determinants of food utilization in D rats.

TABLE 2 Safflower (UNSAT)	FI g/d	W g/d	PG mg/dl	Triglyc mM	Ketone mM	BUN mg/ml
D chow	59	-0.4	553	6.6	1.8	21
high fat	22	-0.6	415	12.8	11.1	19
ND chow	41	+5.1	174	2.9	0.2	18
high fat	26	+5.0	160	3.3	0.2	18
Coconut (SAT)						
D chow	65	-1.0	561	7.9	2.0	30
high fat	20	-3.1	380	31.4	12.1	26
ND chow	42	+3.8	168	2.8	0.3	27
high fat	28	+3.9	170	3.8	0.4	14

62.3 CENTRAL EFFECTS OF A NOVEL FATTY ACID-OXIDATION INHIBITOR: BIOCHEMICAL AND BEHAVIORAL ALTERATIONS. A. Y. Deutch, T. R. Kasser§ and R. J. Martin. Department of Foods & Nutrition, University of Georgia, Athens, GA 30602.

Previous data from our laboratory indicate that there is regional heterogeneity in the ability of the CNS to oxidize fatty acids, that such fatty acid oxidation is linked to the uptake of peripherally-derived fatty acids, and that both central uptake and oxidation of fatty acids is altered by food deprivation in certain, but not all, brain areas. We have therefore assessed the central activity of a novel fatty acid oxidation (FAO) inhibitor, MDL 14,514ZA, as a prelude to examination of possible alterations in food intake and body weight regulation induced by suppression of central fatty acid oxidation.

Adult male rats, trained to a 22 hour food deprivation schedule, were sacrificed two hours after the presentation of food, or after 24 hours of food deprivation. One mm thick coronal slices were prepared from the removed brain, and the following areas microdissected from the slices: nuc. accumbens/olfactory tubercle, septum, striatum, amygdala, pyriform/entorhinal cortices, and ventrolateral and ventromedial hypothalamus (VLH and VMH). Tissue was incubated in the presence of ^{14}C-palmitic acid in Krebs buffer, and generated $^{14}C\text{-}CO_2$ trapped and counted.

MDL 14,514ZA addition to the incubation medium (10^{-9}-10^{-3}M) induced a dose-dependent suppression of FAO in the liver and the two hypothalamic sites. Subsequent experiments were performed in the presence of 10^{-5}M of the inhibitor. All brain areas examined exhibited similar sensitivity to the inhibitor; FAO rates were decreased to 46-63 percent of basal values. The suppression of FAO occured to an equal degree in both fed and fasted animals, although fasting markedly enhanced oxidation rates in the VLH. These data therefore suggest that the process of central fatty acid oxidation is a) similar to FAO occuring in the periphery, and b) similar across different brain regions, although fatty acid oxidation response to food deprivation is regionally distinct.

Preliminary data indicate that continuous osmotic minipump infusion (1 ul/hr for seven days) of the FAO inhibitor MDL 14, 514ZA through cannulae aimed at the lateral ventricle or VLH results in a loss of body weight. The recovery process appears to proceed at a faster rate in those animals sustaining lateral ventricular infusions of the inhibitor. These data therefore suggest that fatty acid oxidation at certain central sites may be an important peripherally-derived signal involved in the regulation of food intake and body weight.

62.4 Oral and Duodenal Hexose Loads Can Affect Food Intake. Paula J. Geiselman and Donald Novin. Department of Psychology and Brain Research Institute, University of California, Los Angeles, CA 90024.

We have found that, following normal ingestion of 10 ml of 0.3M glucose, rabbits showed a significant increase in chow intake. This food-enhancement effect was potentiated by bilateral subdiaphragmatic vagotomy. We have further found that, when infused intraduodenally at a slow rate, glucose suppressed subsequent chow intake but, when glucose was infused into the duodenum at a rapid rate, the rabbits nearly doubled their food intake during the first half hour postinfusion. We have extended these results, investigating the roles of glucose, fructose, and galactose on subsequent food intake in the rabbit.

Female New Zealand rabbits were implanted with intraduodenal cannulae and infused (10 ml/3 kg BW) with 0.3M glucose, 0.3M fructose, 0.3M galactose, and 0.15M NaCl delivered at 3 ml/min.

The results obtained with intraduodenal fructose were the most robust. Following intraduodenal infusion of fructose, the latency to the first meal was significantly reduced, and the size and the duration of the first meal were significantly greater than in the control condition. Further, after duodenal fructose infusion, measures of meal duration, meal size, and total food intake remained significantly greater throughout the four-hour measurement period. The effects of the hexoses have implications for metabolic and gastrointestinal mechanisms that may be controlling food intake.

We also allowed intact rabbits to ingest 10 ml of the following solutions: 0.3M glucose, 0.3M fructose, 0.3M galactose, 0.0049 NaSac, and 0.15M NaCl.

After the ingestion of either fructose or glucose, the rabbits showed an increase in chow intake in comparison with measures in the control conditions; but the food-enhancement effect was not obtained following ingestion of either galactose or NaSac. Preference tests were therefore conducted. The results suggest that the rabbits tended to show the food-enhancement effect in response to the more palatable solutions but not in response to the less palatable solutions.

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62.5 INCREASED GASTRIC EMPTYING PARALLELS INCREASED FOOD INTAKE IN RATS. Monica J. McCann* and Edward M. Stricker (SPON: A. Caggiula). Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

In recent years there has been renewed interest in the contribution of peripheral mechanisms to the control of food intake. We now report our studies of gastric emptying in rats, using treatments known to reliably increase food intake: dietary dilution, and glucoprivation induced by 2-deoxyglucose (2-DG) or pharmacological doses of insulin.

Stainless steel fistulae were implanted in the fundic portion of the stomach of adult male rats. Experimentation began after a 1 wk recovery period. Rats were deprived of food for 6 hr and then given 3.0 ml of 10%, 20%, or 25% glucose solution through the fistulae. After a small bolus was evacuated in the first 2 min, emptying proceeded at a linear rate of approx. 0.35 kcal/min for 20-40 min regardless of the load. These findings replicate the important observations that McHugh and Moran (Am. J. Physiol. 236: R254, 1979) reported in similar experiments involving glucose and other nutrient loads in rhesus monkeys. Collectively, they are consistent with the hypothesis that gastric distention limits food intake, and that dilute diets permit more frequent meals because they leave the stomach more rapidly.

Glucoprivation induced by 2-DG treatment also increases meal frequency and food intake in rats. To determine whether gastric emptying is increased, rats were given a 3.0 ml load of 5% glucose solution 30 min after receiving 200 mg/kg 2-DG, ip. Gastric emptying was increased 5-6 fold. Similar results were obtained when glucoprivation was induced by sc injection of 3 U/kg regular insulin; gastric emptying was increased 7-fold. These results indicate that gastric distention is not sustained under conditions in which the postprandial satiety period is relatively brief, suggesting a causal relation between the two variables.

We have begun to examine gastric emptying under conditions in which food intake is attenuated. Specifically, the food intake elicited by insulin treatment can be eliminated, despite continued hypoglycemia, by infusion of fructose, a sugar that does not cross the blood-brain barrier (Stricker et al., Science 196: 79, 1977). In preliminary experiments we have found that administration of fructose markedly attenuates the increase in gastric emptying that was induced by insulin. Together with other findings (Granneman & Friedman, Am. J. Physol. 238: R346, 1980), the present results indicate that alterations in caloric utilization in some peripheral tissue, perhaps the liver, can influence gastric emptying and may thereby affect food intake, at least in part, by this indirect route. (Supported by research grant MH-25140.)

62.6 BODY WEIGHT REGULATION: IN VITRO NEURONAL TISSUE METABOLISM OF GLUCOSE AND FATTY ACID. R. J. Martin, T. R. Kasser*and R. B. S. Harris.* Dept. of Foods and Nutrition. University of Georgia, Athens, GA 30602.

The purpose of this study was to identify specific neuronal metabolic patterns which may be important in body weight regulation. In vitro glucose and fatty acid metabolism was measured in selected brain sites in rats during hypophagic and hyperphagic recovery of a normal body weight (see R.B.S. Harris et al. this Mtg.) Rats were fed 40, 100 or 160% of normal intake (gastric intubation: thrice daily) for three weeks. As a result, 40%-fed rats weighed 29% less and 160%-fed rats weighed 25% more than 100%-fed rats. Groups of rats were killed at the end of tube feeding and at specific periods during the recovery of a normal body weight. Rats were killed by decapitation and brains quickly removed and dissected into ventromedial hypothalamus ventrolateral hypothalamus, area postrema-nucleus solitary tract and cortex. Tissues were incubated in Krebs Ringer bicarbonate buffer with labelled substrate for 2 hours. Labelled substrate was either 1-C^{14} palmitate or U-^{14}C glucose (1 mM and 10 mM respectively).

During recovery, rats previously fed 40% of normal intake (hungry) were hyperphagic and maintained high rates of VLH fatty acid oxidation until deficient fat stores were repleted. Conversely, rats previously fed 160% of normal intake (anorectic) were hypophagic and maintained low rates of VLH fatty acid oxidation until excessive fat stores were depleted. Other alterations were observed but did not follow the consistant pattern seen between VLH fatty acid oxidation and peripheral energy storage. This experiment was important because our previous studies (Kasser and Martin-Fed. Proc. 42:393,1983) examined central metabolism at the conclusion of tube feeding, not permitting isolation of changes associated with excess energy intake from those associated with excess energy storage. The results demonstrated that rates of VLH fatty acid oxidation respond to peripheral energy storage, while changes in central glucose utilization may be more strongly associated with energy intake.

62.7 BODY WEIGHT REGULATION: DYNAMICS OF RECOVERY FROM OVERFEEDING AND UNDERFEEDING. R. B. S. Harris* T. R. Kasser*and R. J. Martin. (Sponsor: L. Proenza), Univ. of Georgia, Athens,GA 30602.

Evidence for the control of body weight by regulation of body energy stores is provided by studies in which previously overfed rats are hypophagic or underfed rats are hyperphagic until they return to control body weight. In most of these studies body composition was only measured at the end of recovery. We have investigated the pattern of change in body composition with recovery of weight to "set-point" in overfed, restricted and starved rats.

Female Sprague-Dawley rats, weighing 230g, were divided into 27 groups of 6 or 8 rats. Four groups were fed 160%, seven groups were fed 100% and eight groups were fed 40% of control intake. Liquid diet was fed as three tube-fed meals a day. Tube-feeding continued until the 40% fed rats had lost 50g. Six groups were then starved to lose 50g. Initial carcass composition was determined on one group from each treatment. The remaining rats were returned to ad libitum feeding. The 160% rats were initially hypophagic and lost weight. The restricted and starved rats were hyperphagic and gained weight rapidly. Body composition was determined on groups of 40% fed rats after 2,4,6,8,10 and 17 days of refeeding and on starved rats after 1,2,3,4,5 and 11 days. Composition of 100% fed controls was determined after 2,6 and 17 days and of ad libitum controls with the last group of 40% and starved rats. 160% fed rats gained 90 g during tube-feeding. Carcass composition was determined on 100% and 160% fed rats when the overfed rats had lost the same proportions of their 90g gain as the 40% rats had gained of their 50g loss after 2,6 and 17 days of refeeding.

In starved and restricted rats approximately 28g of the weight loss was fat and 10g were protein. Although the composition of loss was similar the pattern of regain was different. Starved rats initially gained protein and then replaced fat so that it was at control levels at the same time as body weight. Restricted rats regained most of their fat before replacing protein. When control weight was reached fat content was still slightly less than in controls. In overfed rats 43g of the weight gain was fat and only 7g were protein. The protein was lost soon after the end of overfeeding. Fat decreased steadily but was still significantly greater than in controls 44 days after the end of tube-feeding. It seems that although all of the rats adjusted food intake to correct body weight to "set-point" different mechanisms were involved for each treatment. The cause of the difference is not clear, but change in body fat seemed closely related to change in weight only in the overfed rats.

62.8 COMPENSATORY INTRA-MEAL RESPONSES OF OBESE WOMEN TO REDUCTION IN THE SIZE OF FOOD UNITS, E. E. Shrager*, T. A. Wadden*, D. Miller*, A. J. Stunkard* and E. Stellar. Obesity Research Group, University of Pennsylvania Sch. of Med., Philadelphia, PA 19104.

It has been suggested that obese humans, like obese rats, display different behaviors as a function of the discrepancy between their current weights and their set points (Nisbett,1972). "Restraint"(Herman & Polivy, 1980) has been proposed as a characteristic of individuals who exert cognitive control over their feeding behaviors to maintain weights below set point. The present study examined the rates and amounts of food intake by 12 obese women after the number of bites needed to ingest a meal was increased by reducing the size of the available units of food. In addition, the intakes of the subjects on the smaller food units relative to the larger were examined with respect to subjects' scores on the Stunkard-Messick Eating Inventory, a 3-factor measure of Restraint.

Test meals consisted of either sandwich quarters (SQ's) or Solid Food Units (SFU's) which were prepared by spreading filling on a slice of bread which was rolled and cut into 8 equal portions. Four SFU's were equal in constituents and calories to 1 SQ; however, the shape of the SFU's effectively obscured this ratio from the subjects. Subjects refrained from eating for 12-14 hr prior to each of three, 30 min test lunches which were separated by intervals of at least one week. Subjects obtained food portions by reaching into the side opening of an insulated box. Removal of each food unit was recorded on a counter in a separate room from which subjects were also observed via a one-way mirror. At 0, 10, 20 & 30 min, subjects marked 100 mm analogue scales to indicate their levels of hunger and fullness. Water intakes were measured at 10 min intervals and food intakes at 5 min intervals. Trials 1 & 3 employed SQ's as the meal; Trial 2 used SFU's. Results indicated that food intake (in SFU or SFU equivalents) per 5 min interval for the two SQ trials was not significantly different over time; however, intakes of SFU's at 5, 10 & 15 min during Trial 2 were significantly reduced ($p < .01$). While subjects did double the rate at which they took food (SFU's) from the box, they failed to adequately compensate for the four-fold difference in size between the SQ's and SFU's. A number of subjects did continue to eat at a relatively constant rate throughout the later time intervals of Trial 2 such that their total intakes approximated or surpassed their intakes on Trials 1 & 3. When SFU intake for Trial 2 was expressed as a percent of the average intake, in SFU equivalents, on Trials 1 & 3, these percent intakes were significantly correlated ($r=0.7742$, $p < .01$) with the scores of the subjects on factor 2, "Disinhibition" of the Stunkard-Messick Eating Inventory.

These data are discussed in light of previous findings from the laboratory regarding the patterns of food intake by lean and obese subjects during SFU test meals.

62.9 DECREASE OF THREONINE IN CSF, PLASMA AND BRAIN OF RATS FED A THREONINE DEFICIENT DIET. W. J. Hartman*, C. C. Calvert* and Q. R. Rogers* (SPON: V. E. Mendel). Dept. of Physiological Sciences, Dept. of Animal Science and the Food Intake Laboratory, Univ. of California, Davis, CA 95616.

It is well recognized that consumption of amino acid (aa) imbalanced or devoid diets by rats results in a rapid decrease in plasma and brain concentrations of the most limiting aa. This decrease is correlated to a decreased food intake.

Rats (185g) on a 12:12 hr light-dark cycle, individually housed in stainless steel suspended wire cages, were allowed to consume a thr limiting aa diet (21% aa) during the first 6 hr of each dark cycle. After a 13 day adaptive period, each rat was assigned to one of three dietary treatments; 1) food deprived (FD), 2) thr devoid (DEV) or 3) an isonitrogenous thr corrected diet (COR) containing 0.6% thr. Cerebrospinal fluid (CSF), blood plasma and brain were sampled from the FD rats at the beginning of the next dark cycle while the rats assigned to treatment 2 and 3 consumed 2.0g of diet. All rats ate the diet in 10-30 minutes and CSF, blood and brain were obtained 2 hr after the initiation of feeding. Amino acids were determined using an LKB 4400 amino acid analyzer. Plasma thr was depressed ($P < .05$) in DEV and FD rats as compared to COR rats; 31.2 µM, 79.9 µM and 163.0 µM, respectively. A similar effect was noted for brain (DEV = 145.7 µM, FD = 222.6 µM, COR = 282.5 µM) although in this case only the DEV rats had a significant reduction ($P < .05$) in thr levels as compared to COR rats. These differences were greater in CSF where thr was depressed ($P < .05$) in both DEV and FD rats as compared to COR rats; 6.6 µM, 13.8 µM and 22.9 µM, respectively. Plasma thr was most affected by dietary treatment, with DEV and COR rats having 39.1 and 204.0%, respectively, of the plasma thr levels found in FD rats. Percent changes in CSF thr concentrations in DEV and COR rats were 47.8 and 165.9%, respectively, of CSF thr in FD rats, while brain from DEV and COR rats had 65.5 and 126.7% of the total brain thr of FD rats.

These results demonstrate that CSF amino acid levels can be altered by short term dietary feeding. This is consistent with the rapidity with which animals can detect an amino acid devoid diet. Changes in CSF amino acid concentrations induced by diet may play a role in altering food intake in the rat.

(Supported in part by NIH grants AM 07355 and AM 13252)

NEUROPEPTIDES AND BEHAVIOR: VASOPRESSIN AND OXYTOCIN

63.1 VASOPRESSIN ANALGESIA:SPECIFICITY OF ACTION & NON-OPIOID EFFECTS J.H.Kordower, R.J.Bodnar, M.M.Manning, and W.H.Sawyer*. Dept. of Psychology, Queens College, Flushing N.Y. 11367;Dept. of Biochemistry, Medical College of Toledo, Toledo Ohio,43699; Dept. of Pharmacology, College of Physicians and Surgeons, Columbia University, N.Y. 10032.

Vasopressin (VP) increases pain thresholds following intracerebroventricular (icv) administration. To characterize VP analgesia, the first experiment examined dose-response relationships of AVP (0,75,150,500ng/5µl,icv), DDAVP (0,150,500ng/5µl,icv) and oxytocin (0,150,500,1000ng/5µl,icv) upon tail-flick latencies in 3 groups of 8 rats. All AVP doses elevated tail-flick latencies at 5 and 15 min post-injection with the analgesic magnitude increasing as a function of dose. DDAVP increased tail-flick latencies up to 5 min (150ng) and up to 45 min (500ng) after injection. Oxytocin increased tail-flick latencies for up to 15 (150,500ng) and 30 (1000ng) min respectively, but these effects were paralleled by barrel roll seizures. The second experiment compared icv pretreatment (10 min) of either a VP antagonist (dPTyr(Me)AVP-500ng), naloxone (1µg), or vehicle to antagonize AVP and DDAVP analgesia. While vehicle pretreated rats display both AVP (500ng) and DDAVP (500ng) analgesia for up to 30 min after injection, pretreatment with dPTyr(Me)AVP eliminated these effects. In contrast naloxone pretreatment failed to alter either AVP or DDAVP analgesia. Moreover, the VP antagonist failed to alter tail-flick latencies at this dose. In a third experiment, pretreatment with naloxone (1µg-icv) significantly reduced morphine analgesia (10mg/kg-sc). In contrast, icv pretreatment with vehicle or dPTyr(Me)AVP (1µg) failed to differ from each other. The fourth experiment examined whether pretreatment with dPTyr(Me)AVP (1µg) would alter prolonged intermittant foot-shock (PIFS) or brief continuous foot-shock (BCFS). These parameters of FS are mediated through different mechanisms (Science,208,623,1980). The VP antagonist significantly reduced PIFS analgesia, yet significantly potentiated BCFS analgesia.

These data indicate that VP but not oxytocin elevates tail-flick thresholds without apparently affecting other neurobehavioral systems. Also, VP appears to be acting independent of the endogenous opioids since the specific VP antagonist, but not naloxone blocks VP analgesia, while naloxone but not the VP antagonist reduces morphine analgesia. Lastly, VP appears to modulate foot-shock analgesia in a reciprocal fashion, indicating that PIFS parameters may be dependent upon VP for its full expression, while BCFS parameters may be inhibited by that same connection. (SUPPORTED BY NIH GRSG 5 SO5 RR07064).

63.2 DIFFERENT MECHANISMS MEDIATE THE CENTRAL AND PERIPHERAL ACTIONS OF AVP ON BEHAVIOR. G.F. Koob, R. Dantzer*, P. Mormède*, R.M. Bluthe*, F.E. Bloom, and M. Le Moal.Lab.Neurobiologie des Comportements, Université de Bordeaux II, Bordeaux, France, and A.V.Davis Ctr. for Behavioral Neurobiology, Salk Institute, San Diego, California, 92138.

Previous work from our laboratories has suggested that the aversive properties of peripherally-injected arginine vasopressin (AVP) may mediate its putative "memory-enhancing" action (Ettenberg et al. Behav.Brain.Res. 7:331,1983). However, many previous studies have shown that AVP directly injected into the brain in nanogram quantities can also have behavioral actions. The purpose of the present study was to examine the possible mediating factors for the aversive effects of peripherally injected AVP by examining the effects of the pressor antagonist of AVP, dPTyr(Me)AVP, on the development of AVP induced taste aversion, and to examine the possibility that centrally- injected AVP could also produce aversive effects. Male Wistar rats were food deprived to insure consumption of the test solutions and then were habituated to drink a sucrose solution for 15 min. per day. On the 6th day the sucrose was replaced by a milk solution and after presentation of the milk each animal was injected with either saline, 6 ug/kg of AVP, or 6 ug/kg AVP plus 30 ug/kg of the AVP antagonist.These pairings continued for 4 days. Results showed a clear and significant taste aversion following the AVP injection that was completely blocked by the concurrent injection of the AVP antagonist. In a second identical experiment, after each milk presentation the rats were injected with AVP intracerebroventricularly in doses of 0.1, 1.0, and 10.0 nanograms/rat (doses shown to prolong extinction of active avoidance behavior). No conditioned taste aversion was observed at the 0.1 or 1.0 doses. At 10 nanograms there was some aversion, but 5 out of 6 rats showed barrel-rolling immediately following the injection. Results suggest that the aversive properties of peripherally-injected AVP are peripherally- mediated, possibly by the vasopressor action of AVP. Results also suggest that some action other than an aversive property of centrally-injected AVP must mediate its behavioral effects, and that the central and peripheral actions of AVP are at some level independent of each other.

63.3 BEHAVIORAL VARIATION BETWEEN DIFFERENT COLONIES OF BRATTLEBORO RATS. J.P. Herman*, D.M. Gash, G.J. Thomas, J.F. Laycock* and I.V. Gartside*. Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642, and Charing Cross Hospital Medical School, London, England (Spon: C.K. Kellogg).

Vasopressin (VP) deficiency observed in Brattleboro rats homozygous for the diabetes insipidus trait (DI rats) has been used extensively as an independent variable in experiments investigating the role of VP in behavioral processes. Unfortunately, behavioral data from some laboratories have proven difficult to replicate. This study addressed the hypothesis that substantial behavioral differences might exist between different colonies of DI rats, accounting for some of the problems in replication.

DI samples were drawn from the population maintained at Rochester (RO/DI animals) and from the colony maintained by Dr. Laycock in Great Britain (GB/DI animals). Samples from both colonies manifested the same degree of polydipsia and polyuria. Control rats were normal Long Evans animals from the respective colonies (RO/N, GB/N). Ten animals from each experimental group were tested in a straight runway approach-avoidance paradigm, consisting of a) 3 approach trials per day for 10 days, where entry into a goal box is rewarded with presentation of food; b) a single shock trial (trial 30), where a 2.5 mA shock is delivered upon contact with the food dish; and c) 3 post-shock approach trials per day for 10 days (goal box entry again rewarded). Pre-shock approach speed is taken to be a measure of adaptation to the novel environment presented by the runway, while post-shock speed is taken to measure retention of shock avoidance. RO/DI animals adapt more slowly to the runway than do RO/N rats ($p<0.05$) while retaining avoidance to a similar degree as RO/N animals. Conversely, GB/DI animals adapt more quickly ($p< 0.05$) to the runway than do GB/N rats, while again retaining avoidance to a similar degree as normals.

These data support the hypothesis that behavioral differences, specifically in adaptation to novel situations, exist between different colonies of DI rats. The presence of such a colony effect introduces a confounding variable into experiments using the DI animal to study the relationship of VP to behavior, since hereditary behavioral traits may be potentially independent of the DI gene. More stringent control of polygenic variability must be employed before conclusions may be drawn linking VP deficiency to behavioral processes. In addition, these data do not support the hypothesis that VP deficiency is deleterious to retention of shock avoidance. Neither RO/DI nor GB/DI animals differ from normals in post-shock approach speed.

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63.4 FACILITATION OF DEVELOPMENT OF RESISTANCE TO MORPHINE ANALGESIA BY ARGININE VASOPRESSIN AND THE ROLE OF BRAIN SEROTONIN IN THE RAT. M.S. Shahid Salles, and K. Sharifi Hossaini. Departments of Physiology & Pharmacology, School of Medicine, Shiraz University, Shiraz, Iran.

Rats were prepared with permanent electrodes for recording EEG and EMG. Morphine injections of 10 mg/Kg given S.C. were followed by the appearance of high voltage EEG slow bursts associated with stuporus behavior. This phase was suppressed by the appearance of behavioral arousal shown by EMG and behavior of rats and afterwards behavioral sleep became apparent on the EEG. Administration of 10 mg/Kg dose of morphine to rats pretreated with arginine-vaso pressin (AVP) potentiated the observed behaviors of morphine. The result of tailflick-tests in another series of animals treated with AVP 5 µg/animal show facilitation of the development of resistance to morphine analgesia. Administration of a 10 mg/Kg dose of morphine to rats pretreated with P-chlorophenylalanine (p-CPA, 100 mg/Kg/day for 3 days) was followed by an almost immediate EEG and behavioral arousal. Combined treatment with AVP and PCPA did not change the effect of AVP on morphine analgesia. This may suggest that there is no interaction between AVP and brain serotonin in mediation of morphine's analgesics effect.

63.5 INTRAVENTRICULAR APPLICATION OF A PRESSOR ANTAGONIST ANALOG OF VASOPRESSIN PREVENTS BOTH THE "MEMORY" AND "AVERSIVE" ACTIONS OF VASOPRESSIN. A. Ettenberg, Department of Psychology, University of California, Santa Barbara, CA 93106

Many studies have reported an improvement in memory with post-training administration of the neuropeptide, arginine vasopressin (AVP). However, it remains to be determined whether the putative memory-enhancing properties of AVP result from some direct action on the neural substrates of memory or from some other modulatory or indirect mode of action. For example, we (Ettenberg et al. Behav. Brain Res.,1983,7,331) recently reported a correlation between the aversive actions of AVP and its performance-improving effects in an appetitive learning task. These data suggested that one might account for the "memory" properties of AVP by the arousing or alerting effects of administering an aversive agent. If this hypothesis is correct, then one would predict that treatments that prevent the aversive effects of AVP should also prevent its memory effects. The present study was designed to examine this possibility.

In previous work (Ettenberg et al.,Pharmacol. Biochem. Behav., 1983,18,in press) we observed that the concurrent administration of AVP and a pressor antagonist analog of AVP prevented the memory facilitation observed with AVP alone. In the present study two additional factors were examined: 1) would intraventricular infusion of the AVP antagonist similarly prevent the memory effects of AVP, and 2) if so, would this same treatment also prevent the aversive properties of AVP.

The aversive actions of 6.0 ug/kg s.c. injections of AVP were demonstrated in two behavioral assays: 1) a Conditioned Taste Aversion Test in which rats learned to avoid a preferred tasting substance (i.e. saccharin) as a consequence of pairing the presentation of that substance with injections of AVP, and 2) a Conditioned Place Test in which rats learned to avoid a distinctive environment associated with AVP administration. The memory-enhancing properties of AVP were observed in a one-trial food-finding task where non-deprived rats were briefly exposed to a large open-field that contained an alcove in which a high-incentive familiar food reward (sweetened milk) was freely available. AVP injections immediately upon removal from the open-field produced faster latencies to relocate the alcove (compared to vehicle controls) when tested 48 h later.Both the memory and the aversive actions of AVP were prevented by immediate pretreatment with intraventricular infusions (10ug /rat) of the AVP pressor antagonist analog 1-deaminopenicillamine-(O-methyl)-tyrosine AVP. Antagonist doses of lug & 5 ug/rat had weak and moderate effects respectively. The large doses required to block AVP's behavioral effects suggest that the critical site of action may be far removed from the lateral ventricles.AVP memory effects may,therefore, occur via peripheral aversive actions.

63.6 NEUROPEPTIDE INFLUENCES ON MEMORY IN YOUNG COCKERELS. J. L. Davis, R. M. Pico* and A. Cherkin. Aging and Behavioral Biology Research Lab. and GRECC. VAMC. Sepulveda, CA 91343.

We have previously described certain proline oligopeptides to be enhancing in a conditioned peck aversion paradigm (Davis, J. L. and Cherkin, A., Neurosci. Abstr., 6:169, 1980). In addition we have described memory enhancement induced in chicks by oxytocin and its C-terminal tripeptide L-prolyl-L-leucyl-glycine-amide (Davis, J. L., Pico, R. M., and Cherkin, A., Neurosci. Abstr., 7:365, 1982). We report here an expansion of these results to include a demonstration of memory enhancement in a similar paradigm produced by the C-terminal tripeptide of arginine vasopressin, L-prolyl-L-arginyl-glycineamide (PAG).

In these experiments two-day-old cockerels were injected intracerebrally with 5 µL/hemisphere of PAG (3.0 µmol/chick) or saline 1, 9, or 59 min after one-trial training to suppress the peck response to a bead coated with EtOH. The aversant effects of EtOH are not noticeable 24 hr later without a memory enhancement manipulation. Enhanced memory was noted, 24 hr later, when chicks showed a lower mean peck rate (number of pecks in 10 sec) than the control chicks when presented with an uncoated bead. Further data (not shown here) indicate that decreased responding is not the result of a general depressive effect of the oligopeptide.

Post-Training Injection (min)	Compound	N	$\bar{P}$	Retention Test ±SD	P values
1	PAG	50	4.56	4.45	<0.001*
9	PAG	50	6.76	5.05	<0.001*
59	PAG	50	7.90	5.93	<0.05*'
1	Saline	50	10.38	5.31	

*significance levels obtained by Dunnett comparisons with saline group.
'significance levels obtained by Tukey-HSD comparisons of the 1 min PAG group to the 59 min PAG group.

The memory enhancing qualities of PAG were not observed when the cockerels were injected intraperitoneally as the following data suggest.

Compound	Dose/Chick (µmols)	N	$\bar{P}$	Retention Test ±SD	P values
PAG	3.0	25	9.60	4.03	NS*
PAG	6.0	25	10.52	6.42	NS*
PAG	12.0	25	9.12	5.61	NS*
Saline	----	25	9.44	3.93	

*significance levels obtained by comparison with saline group.

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63.7 REVERSAL OF INDUCED ISCHEMIC NEUROLOGIC DEFICIT IN GERBILS BY VASOPRESSIN, G. Delbarre, B. Delbarre and A. Ferger*. Lab. Ch. Exp., Faculté de Médecine 37032 TOURS, FRANCE

Mongolian gerbils (Meriones unguiculatus) are characterized by frequent anomalies of the circle of Willis. Their cerebral vessels lack arterial communication between the cerebral and vertebral system. Therefore ischaemia can be easily produced by unilateral ligation of the common carotid. The ligation-induced neurological signs are correlated with histopathological evidence of cerebral infarction and resemble the syndrome of middle cerebral arterial occlusion in man. Mongolian gerbils present an experimental model for cerebral ischaemia. Many workers have demonstrated a possible role of biogenic amines as norepinephrine, dopamine and serotonin in the pathophysiology of brain ischaemia.

We have tested vasopressin on the gerbil cerebral ischaemia, in order to evaluate its effects on the neurological signs induced by the ligation. The experiments vere carried out in adult Mongolian gerbils (60 - 80 g) of either sex. Mongolian gerbils were selected as previously described (Delbarre G. et al, Soc. Neurosci. Abstr. Vol 5, 333, 1979) and only positive animals (which respond immediately by ptosis resulting from transitory interruption of carotidian supply) were used in the experiment. They were anaesthetized with ketamine (50 mg/kg - IP) and two groups of 8 animals were constituted :

- a control group : saline was administered IP
- a treated group : vasopressin 0,1 U/animal ICV 10 µl 15 minutes before and 1 minute after ligation.

Neurological status was evaluated with the stroke index reported by McGraw et al. (Stroke 7 : 485, 1976) starting one hour after ligation and again at 4, 24, 48, 72 and 96 hours. Like saline, vasopressin 15 minutes before ligation did not affect the neurological status of the gerbils. In contrast, vasopressin after ligation significantly improved the mean stroke index.

Supported by a grant from Fondation Medicale pour la Recherche.

63.8 ANTAGONISM OF MEMORY ENHANCING EFFECTS OF VASOPRESSIN (AVP) BY A VASOPRESSIN ANTAGONIST PEPTIDE (ANTI-AVP). C.J. LeBrun*†, H. Rigter††, J.L. Martinez, Jr.†, G.F. Koob†, M. Le Moal†††, and F.E. Bloom†. † Arthur Vining Davis Ctr. for Behav. Neurobiol., The Salk Inst., San Diego, CA 92138 (USA), ††CNS Pharmacol. Dept., Organon, Oss, Netherlands. ††† Laboratoire de Neurobiologie des Comportements, Universite de Bordeaux II, F. 33076 Bordeaux, France.

The present study examined the effects of a potent anti-AVP [1 deaminopenicillamine-2-(O-methyl) tyrosine arginine vasopressin] [dPTyr(Me)AVP] on the ability of AVP to facilitate retention of a passive avoidance task. Both peptides were tested after s.c. injection. Replicate experiments were conducted in the USA, and Netherlands using the same training procedure and Wistar rats, but different passive avoidance tasks [(Martinez et al., Physiol. Behav. 19: 139-144 (1977); Ader et al. Psychon. Sci. 26: 125-128 (1972)]. On Day 1, the rat was placed in the shock compartment, for 2 min, and then given a pretraining trial in which the rat is placed in the start area and allowed to step through into the shock compartment without receiving shock. On Day 2, two more pretraining trials were given, followed by the acquisition trial, during which the rat received a shock. Immediately following the learning trial, the rat was injected s.c. in the neck [(USA) AVP = 1 µg/rat, antiAVP = 25 µg/rat; (Netherlands) AVP = 3 µg/rat antiAVP = 15 µg/rat]. A retention test was given 24 hours later. The results show (see table) that s.c. injection of AVP facilitated performance in both experiments. Peripheral injection of antiAVP reversed the effects of AVP. AntiAVP alone, produced, in the Netherlands study, an effect similar to that of exogenous AVP. These results confirm the posttrial efficacy of vasopressin in enhancing retention performance. The results obtained with antiAVP indicate that it may be a partial agonist. These data suggest that the peripheral action of AVP are in some way responsible for its behavioral action, since the antagonist is known to block the pressor actions of AVP. Table mean (±SEM). Latency to enter shock compartment 24 hours after training.

	Saline	AVP	AntiAVP	AVP+AntiAVP
Holland	54(10.60) n=60	124(17.39)[a] n=60	106(16.51)[a] n=60	86(13.74) n=60
U.S.A.	28(12.86) n=18	92(24.85)[ab] n=22	65(25.87) n=18	11(5.26) n=19

[a]significantly greater than saline, [b]significantly greater than AVP+AntiAVP, Dunnett's analysis.

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63.9 OXYTOCIN ANTISERA BLOCKS REEMERGENT MATERNAL BEHAVIOR IN EXPERIENCED RAT MOTHERS. J. Caldwell*, C.A. Pedersen*, M. Johnson*, and A.J. Prange, Jr. (SPON: M.C. McNamara). Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

Primiparturient rats (Charles River Breeders) were allowed three durations of postpartum mothering experience before they were isolated from pups for 4-5 weeks. The incidence of full maternal behavior (FMB) during the first two hrs of renewed contact with foster pups (spontaneous rate) was then assessed for each group. Animals that had been allowed 4-24 hrs, 4-5 days, or 7-8 days of postpartum pup contact displayed respectively a 36% (5/14), 67% (6/9), and a 100% (11/11) spontaneous rate of FMB ($p < .002$, Fisher's exact probability, comparing the first and third groups).

Primiparturient animals allowed to mother their offspring for 4-24 hrs and then separated from pups for 4-5 weeks were given ICV injection of either 800 ng of oxytocin (OXY) or saline and then given three young pups. Oxytocin recipients responded at a significantly ($p < .005$) higher rate of FMB (8/10) within two hrs than animals receiving saline (1/9). Other primiparturients allowed brief mothering experience and separated from pups for 4-5 weeks were ovariectomized ten days prior to behavioral testing. Ovariectomized animals receiving 800 ng of OXY ICV responded at a rate (8/11) significantly ($p < .04$) greater than those receiving saline (2/9).

Primiparturient animals allowed 4-5 days of postpartum mothering experience and separated from pups for 4-5 weeks received either 10 µl anti OXY antisera (AOA) or 10 µl normal rabbit serum (NRS) ICV one hr before introduction of foster pups. Recipients of AOA displayed a significantly ($p < .025$) lower rate of FMB (3/9) than recipients of NRS (8/9) in the first two hrs of renewed pup contact.

These observations suggest that: 1) Parturition and/or brief mothering experience produce a prolonged sensitivity to the maternal behavioral effects of OXY that is not ovarian steroid dependent; 2) the high spontaneous rate of reemergent FMB seen in primiparious animals allowed longer periods of postpartum mothering experience depends upon the release of endogenous OXY.

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64.1 HAIR-PIN LOOPS OF MICROTUBULES AT NEURONAL GROWTH CONES. H.T. Tsui & K.L. Klein. Interdept. Prog. Neurosci., Northwestern University, Evanston, IL 60201.

Axons and dendrites contain numerous microtubules. These linear structures appear to play a role in maintaining cell shape and in intracellular transport. They are generally believed to be disassembled and degraded at axon terminals. However, anti-tubulin immunofluorescence of developing neurons in culture showed a fibrous pattern at growth cones although the endings of individual microtubules could not be determined (Spooner B.S. & Halladay C.R. Cell Motility 1, 167-178). In order to understand the overall cytoskeletal organization at the ends of developing neurites, we have examined under the high voltage electron microscope (HVEM) thick sections, whole mounts and Triton-extracted whole mounts of growth cones of chick retinal neurons that have been in culture for 4 to 6 days. In contrast to the generally accepted hypothesis that microtubules terminate at nerve terminals, we present evidence that over 50% of these growth cones have at least one microtubule that forms loops, bending around 180 degrees and back towards the cell body.

Dissociated chick retina cells were plated onto either poly-lysine- and formvar-coated gold grids for whole mount studies or polylysine-coated coverslips for sectioning studies. After 4 to 6 days in culture, the cells were fixed, dehydrated and either critical-point-dried for whole mount studies or embedded in epon-araldite for thick section studies. Detergent extracted whole mounts were prepared using 0.15% Triton X-100 in a microtubule-stabilizing extraction buffer according to Schliwas and van Blerkom (J. Cell Biol. 90, 222-233) before fixation. Thick sections, whole mounts and Triton-extracted whole mounts showed similar organization of the cytoskeleton at neurite terminals. Microtubules spread out, then either terminated or bent around at the growth cone regions. Very often individual microtubules were seen to make a 180 degree turn within a diameter as little as 5 um and return to the main microtubule bundle. Quantitative analysis of the occurrence of these microtubule hair-pin loops were done on Triton-extracted cultures. Of the 93 growth cones examined, 49 showed at least one microtubule that bent back and formed a loop. This unusual disposition of microtubules may be important for the control of neurite growth and bi-directional transport. Our findings also raise the interesting question of how microtubule configuration is controlled to give such elaborate loops.

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64.2 TAU-LIKE MICROTUBULE-ASSOCIATED PROTEINS (MAPs) IN RAT SYMPATHETIC NEURONS CULTURED FOR VARYING PERIODS OF TIME. M.M. Black* and J.T. Kurdyla* (SPON: A. Lamperti). Dept. of Anat., Temple Univ. Sch. of Med., Philadelphia, PA 19140.

The goal of the present studies is to determine whether the composition of tau-like MAPs in cultured sympathetic neurons changes with time in culture. Sympathetic neurons were obtained from superior cervical ganglia of 1-3 d old rat pups. The neurons were enzymatically dissociated and then cultured with cytosine arabinoside to eliminate nonneuronal cells. In previous studies (Black, M.M. and J.T. Kurdyla, J. Cell Biol., in press). 24 proteins with molecular weights ranging from 60,000 to 76,000 were identified as MAPs in 2 week old neuronal cultures. These MAPs have similar properties to the tau class of brain MAPs. Studies by Francon et al., (Eur. J. Biochem 129: 465-471) have shown that the polypeptide composition of brain tau changes during growth and maturation. In the present experiments, we have examined the composition of tau-like MAPs in neurons as they age in culture. Cultures maintained for 7, 14, or 60 d were labeled with [^{35}-S]-methionine and then fractionated to enrich for the tau-like MAPs. The resulting fractions were analyzed by isoelectricfocusing x SDS polyacrylamide gel electrophoresis and fluorography. 24 tau-like MAPs were identified in the cultures, regardless of their age. The tau-like MAPs from 7, 14, and 60 d old cultures had indistinguishable mobilities in isoelectric-focusing x SDS polyacrylamide gel electrophoresis. These data suggest that the composition of tau-like MAPs did not change between 7 and 60 d in vitro. Studies are in progress to characterize the tau-like proteins in cultures less than 7 d of age.

64.3 **REVERSIBLE NOCODAZOLE SENSITIVITY IN YOUNG, NGF ACTIVATED PC12 NEURITES.** Roger Jacobs and John Stevens, Playfair Neuroscience Unit. University of Toronto, and Toronto Western Hospital, Toronto, CANADA M5T 2S8.

PC12 (pheochromocytoma) cultured cells, when activated with nerve growth factor (NGF), are known to produce long branched networks of neurites. We have observed, as have others, that the NGF activated neurites contain microtubules and organelles similar to those found in normal neuronal processes. Additionally, via long term time lapse, we have found that the ontogeny of neuritic branching is similar to that described by Bray (J. Cell Bio. 56:702, 1973) for sympathetic neurons: PC12 processes elongate via growth cone extension and new branches are formed by growth cone bifuraction. Thus, the ontogeny of PC12 process development appears to approximate the normal behaviour of PNS cells in vitro.

We have found that within 45 to 90 minutes after the addition of 1-2 micrograms/ml of Nocodadole (a drug that leads to microtubular dissasembly), to a young PC12 culture (between 6 and 14 days after NGF activation), most neurites constrict to form beaded or varicose processes. These induced varicosites do not move, but correspond to regions of the neurite that were slightly expanded and moving before the drug was added. We believe, therefore, that varicosities represent organelles contained within the neurites. Within two hours of washing out the Nocodazole, the constrictions on the PC12 processes "fill out", restoring a process that appears normal. Thus, we suspect that the microtubular array contained within the young PC12 neuritic processes has been depolymerized and repolymerized within the time span of several hours.

The same experiment carried out on mature PC12 cultures (more than fourteen days after NGF activation) shows a much weaker response. Experiments to date have not yet shown clear recovery. Other agents commonly used to disassemble microtubules, such as Colchicine, produce similar effects in both young and mature cultures (see Black and Greene, J. Cell Bio. 95:379, 1982) but without any sign of reversibility.

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64.4 THE CYTOSKELETAL BASIS OF GANGLIOSIDE-MEDIATED NEURITE OUTGROWTH IN NEURO-2A NEUROBLASTOMA CELLS. David A. Spero* and Fred J. Roisen (SPON: A. Gona). Dept. of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

We have shown previously (Science, 214:577-578, 1981; Anat. Rec., 205:190, 1983) that a mixture of bovine brain gangliosides (BBG) applied to Neuro-2a neuroblastoma (Neuro-2a): 1) markedly increased the degree and rate of neurite formation and 2) stimulated the formation of a complex cytoskeleton consisting of a highly organized network of microfilament bundles. To study further the relationship between neurite outgrowth and cytoskeletal components, we employed cytoskeletal disruptive agents (Colcemid and cytochalasin D) to examine the role that microtubules and microfilaments play in BBG-induced neurite initiation and elongation. Neuro-2a cells were grown in Minimum Essential Medium supplemented with 10% heat-inactivated fetal calf serum. Cells were plated on glass coverslips previously coated with poly-DL-ornithine. Cultures were grown in medium with or without BBG (250 µg/ml) and the appropriate test agent [Colcemid (0.25 µg/ml) or cytochalasin D (2 µg/ml)] for 24 hr in an atmosphere of 5% CO_2 in air at 35°C. Cultures were rinsed briefly in Hanks' balanced salt solution, fixed in 2.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.3) for 2 hr, postfixed in 1% OsO_4 for 30 min, dehydrated in ethanol and processed for scanning and transmission electron microscopy. Neuro-2a cells treated with BBG elaborated long, highly branched neurites. The surfaces of these cells possessed numerous microvillar spine-like projections on their perikarya. Simultaneous treatment with BBG and Colcemid resulted in cells with numerous spine-like projections which did not extend neurites. Cells treated with Colcemid alone did not possess either spines or neurites. In contrast, the simultaneous treatment with BBG and cytochalasin D resulted in cells devoid of spines, but exhibiting anomalous neurite outgrowth consisting of many long, thin, unbranched neurites. These neurites lacked characteristic growth cones and had a tendency to grow in a circular fashion. Cells treated with cytochalasin D alone had smooth surfaces and did not exhibit any significant neurite growth. These findings suggest that, in BBG-induced Neuro-2a cells, microtubules are necessary for neurite elongation and that microfilaments are essential for spine formation and neuritic branching. In this culture system, microtubules appear to play a more central role in providing the forces for neurite extension than do microfilaments.

(Supported by NIH grant NS 11299.)

64.5 **VASOPRESSIN PROMOTES NEURITE GROWTH IN CULTURE.** R. Gruener, R. E. Brinton and H. I. Yamamura. Depts. of Physiology and Pharmacology, Arizona Health Sciences Center, Univ. of AZ, Tucson, AZ 85724.

Vasopressin, a neuropeptide, has been reported to enhance memory functions (De Wied, Nature 232:58,1971; Koob & Bloom, Ann. Rev. Physiol. 44:571, 1982). The mechanism(s) for this effect is unknown. One possible route by which vasopressin (AVP) could influence memory is through the induction of protein synthesis specific for information storage. In an attempt to find a cellular substrate for memory, Crick (TINS 5:44, 1982) has postulated that an increase in dendritic spine formation may permit the de-novo establishment of neuronal circuits which underlie memory engrams. Because neurite production is a specific expression of protein synthesis in CNS neurons, we examined the effects of AVP on this cellular process. Neurons were isolated from the cephalic portion of embryonic Xenopus spinal cords and grown in culture (Kidokoro et al., Dev. Biol. 78:464, 1980). Sister cultures were grown in a defined medium in the presence of 10-200 μM AVP. Neurite production was estimated from the number of loci and total number of neurites in duplicate cultures. Vasopressin-treated cultures showed a marked increase in the number of loci and in total number of neurites when compared to controls. This effect occurred in a dose-dependent fashion. Neurites in AVP-treated cultures were typically longer than in controls. In addition, neurites in AVP-treated cultures were characterized by numerous spines (filopodia) along their entire length. The vasopressin-induced increase in neuritic growth may reflect specific cellular alterations which may be involved in memory processes.

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64.6 INSULIN AND INSULINLIKE GROWTH FACTOR-II STIMULATE NERVE GROWTH FACTOR RECEPTOR ACTIVITY, NEURITE FORMATION, AND GROWTH IN CULTURED HUMAN NEUROBLASTOMA CELLS. E. Recio-Pinto* and D.N. Ishii. Department of Pharmacology and Cancer Research Center, Columbia University, New York, NY 10032.

Insulin and insulinlike growth factor-II (IGF-II) had direct and indirect effects on neurite outgrowth in cloned human neuroblastoma SH-SY5Y cells. With respect to the direct effects, insulin reversibly increased neurite formation when cells were grown in media without serum (-S), a condition under which cells could survive for at least a week without loss in number. The half-maximal response was at 4 nM for porcine, bovine, and synthetic human insulin, and at 0.5 nM for IGF-II. Both values were lowered to 0.1 nM when bacitracin, a protease inhibitor, was present. In media with serum (+S), the anti-nerve growth factor (NGF) antiserum inhibited NGF-stimulated neurite formation, but not that stimulated by ^{125}I-insulin. Moreover, insulin did not compete for binding of ^{125}I-NGF to specific sites.

The indirect effects were due to modulation of NGF activity. In -S, the cells lost virtually all capacity to bind ^{125}I-NGF and respond to NGF with neurite formation; the loss of both functions was prevented by insulin or IGF-II. Moreover, both functions were restored by subsequent treatment with insulin or serum. The indirect effect of insulin and IGF-II on neurite formation could be readily distinguished from the direct effect because at very low concentrations these factors could potentiate the response to NGF. In +S, anti-insulin antiserum inhibited the activity of insulin ordinarily present in serum and decreased the neurite formation response to NGF, and the spontaneous level of neurites.

Insulin and IGF-II stimulated ^{3}H-thymidine incorporation and cell proliferation; the half-maximal response for insulin stimulated cell proliferation was at 0.3 nM. These effects could be dissociated from effects on neurite formation, since, in -S, cell replication was not sustained by insulin despite continuously elevated neurite outgrowth. Ordinarily, peripheral neurons become postmitotic at early developmental stages. Thus, malignancy in human neuroblastoma cells may be due, in part, to inappropriate sensitivity to insulin and insulinlike factors. Thus, at physiological concentrations, insulin and IGF-II have NGF-like effects on neurite formation and increase growth. Rat liver multiplication stimulating activity has been shown by others to have a structure homologous to human IGF-II, and was the source of activity used in these studies. (Supported in part by grants ROl NS14218 from the National Institute of Neurological Communicative Diseases and Stroke, PHS Research Career Development Award 1K04 NS00375, and PHS training grant GM 07182.)

64.7 ISOLATION OF A MONOCLONAL ANTIBODY THAT INHIBITS THE IN VITRO NEURITE PROMOTING ACTIVITY OF A HEPARAN SULPHATE PROTEOGLYCAN COMPLEX. William D. Matthew and Paul H. Patterson. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Several groups have described factors found in conditioned media (CM) which bind to polycationic substrata and stimulate neurite outgrowth from a variety of cultured neurons. Five monoclonal antibodies have been described which precipitate a heparan sulphate proteoglycan (HeS) from PC12 cell CM and, in doing so, deplete CM of its ability to induce rapid neurite outgrowth (Matthew et al. 1982. Soc. Neurosci. Abstr. 8: 83.4). However, when these antibodies are adsorbed to CM coated dishes, they do not block the outgrowth promoting activity. Therefore, it is unclear if the HeS is directly responsible for promoting neurite growth or is merely required to adsorb an associated molecule to the polycationic culture surface. We hope to resolve this issue using a monoclonal antibody which actually blocks the function of the neurite promoting molecule. After generating 138 monoclonal antibodies that recognized the CM factor but did not directly block function, we decided to use a new approach. A mouse was immunized with an inactive HeS to initiate a response to non-functional, immunodominant determinants. Two days later, an immunosuppressant drug was injected. Our strategy was to kill the lymphocytes stimulated by these determinants. Three weeks later, the "tolerized" spleen was immunized in vitro with active CM and then fused to myeloma cells. Using this protocol, we have isolated antibody 4H8 which, when adsorbed to CM coated dishes, blocks the neurite promoting activity. We intend to use this antibody to purify the neurite promoting molecule and determine if the HeS or an associated molecule is responsible for this activity.

Since the 4H8 antibody blocks the function of the neurite promoting molecule in vitro, we feel it is an appropriate probe for localizing the activity in situ. The 4H8 antigen has a distribution in developing tissue consistent with it having a role in neurite growth. Using immunohistochemical procedures, we have detected high levels of antibody binding in neonatal superior cervical ganglia but little or none in adult ganglia. However, when adult rats are sympathectomized with 6 hydoxydopamine, the antigen can be detected in adult superior cervical ganglia and the time course of its appearance parallels neurite regrowth.

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64.8 PERTURBATION OF NEURITE GROWTH BY PULSED AND FOCAL ELECTRIC FIELDS. N.B.Patel* and M-m.Poo (SPON: B.J.Vasquez). Dept. of Physiology and Biophysics, Univ. of Calif., Irvine, CA 92717.

The growth and orientation of embryonic Xenopus neurites in culture are markedly affected by uniform DC fields (J. Neurosci., 2:483, 1982). The present study examined the effects of monopolar pulsed and focal electric fields, which are characteristic of fields associated with endogenous nervous activities, on dissociated Xenopus embryonic neurons plated in monolayer culture. Three types of fields were used: uniform pulsed fields, focal DC fields, and focal pulsed fields. Under pulsed uniform fields, neurites showed preferential orientation toward the cathode of the field in a manner similar to that previously found for DC fields. The extent of neurite orientation depended upon the width, amplitude, and frequency of the (square) pulse, but appeared to be equivalent to the effect produced by a DC field of the same average intensity. The threshold pulse amplitude and frequency required to produce a detectable orientation of neurite growth within 24 hr in a pulsed uniform field was 1 V/cm at 10 Hz with a pulse duration of 5 ms. Focal DC fields were applied near the growth cone of the neurite by passing current through a micropipette. Positive field (current source) was found to deflect the growth cone away from the pipette, while negative field (current sink) attracted the growth cone. The threshold current density needed to orient a growth cone within 15 min was between 0.4 to 4 pA/μm^2 (or 5 to 50 mV/cm) at the growth cone. Pulsed focal currents of pulse amplitude of 8 pA/μm^2 at 100 Hz, or 80 pA/μm^2 at 10 Hz were effective in orienting the growth cone. The latter currents are similar to those which occur at the synaptic cleft during synaptic transmission, suggesting attraction or repulsion of the nerve terminal by the electric field associated with synaptic activity is a plausible cellular mechanism underlying activity-dependent modulation of synaptic efficacy. This work is supported by a grant from NIH (NS-17558-01A1).

64.9 PREFERENTIAL GROWTH OF CNS AND PNS NEURITES ON ASTROCYTES AND SCHWANN CELLS AS COMPARED TO NON-GLIAL CELLS IN VITRO Justin R. Fallon* (SPON: G. McKhann) MRC Neuroimmunology Project, Dept. of Zoology, University College London, London WC1, U.K.

Growing axons in the developing embryo and regenerating nerve are usually in intimate association with glial and other non-neuronal cells, and it has been suggested that such cells play an important part guiding axonal growth. To approach this question in vitro, I have studied neurite outgrowth on highly enriched monolayers of glial and non-neuronal cells. I have shown previously (Soc. Neurosci. Abstr. 8:302 1982) that both CNS and PNS neurites grow vigorously (at rates of .6-1mm/day) and in fine fascicles on rat cortical astrocytes (>95% pure as judged by antibody labelling for GFAP). In contrast, on fibroblast monolayers PNS neurites extend at less than half the rate and tend to form large bundles, while most CNS neurites grow very little. I have extended these studies to show that sciatic nerve Schwann cells (>95% pure on the basis of antibody labelling for RAN-1) also promote vigorous neurite outgrowth from both CNS (E15 retina) and PNS (E20 SCG) explants.

I have also the studied the behaviour of neurites faced with a choice of cells in the same dish. Contiguous monolayers of astrocytes and skin fibroblasts were constructed, and explants of retina or SCG were plated on either side of the border. CNS neurites growing on the astrocytes rarely crossed on to the fibroblasts monolayer, instead they stopped or turned and continued to grow on the glial cells. Few neurites extended from retina explants plated on the fibroblast side. While the majority of SCG neurites remained on the astrocytes, some crossed over to the fibroblasts, but growth rate slowed. When SCG neurites growing on fibroblasts reached the astrocyte frontier, they defasciculated and increased their rate of growth. Neurites of either type did not significantly change their behaviour when they crossed an astrocyte-astrocyte border. In all cases neurite outgrowth was characteristic of the monolayer contacted.

Light and electron microscopic examination revealed that both CNS and PNS neurites grew on top of or between the glial cells. In contrast, the growth cone and distal segment of the PNS neurites were often seen under the fibroblasts, indicating that the growth cones chose to migrate on the culture substratum (or secreted extracellular matrix) and/or the ventral surface of the fibroblast in preference to the dorsal surface.

These experiments suggest that the surfaces of astrocytes and Schwann cells are particularly attractive to both CNS and PNS neurite outgrowth. It seems likely that such interactions play an important part in neural development and repair.

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64.10 NEURITE EXTENSION BY PERIPHERAL AND CENTRAL NERVOUS SYSTEM NEURONS IN RESPONSE TO SUBSTRATUM-BOUND FIBRONECTIN AND LAMININ. S.L. Rogers*, P.C. Letourneau*, S.L. Palm*, J. McCarthy* and L.T. Furcht* (SPON: R. Jacobson). Dept. of Anatomy and Dept. of Lab. Medicine and Pathology, Univ. of Minnesota, Minneapolis, MN 55455.

Laminin (LAM) and fibronectin (FN) are components of extracellular matrices in vivo and mediate adhesion, migration, and differentiation of various cell types in vitro. We have studied neurite extension by peripheral (dorsal root and sympathetic ganglion) and central (spinal cord and retina) nervous system neurons cultured on purified LAM and FN in the presence and in the absence of serum.

Plastic tissue culture dishes were treated overnight with 10-100 μg/ml human plasma FN or mouse EHS tumor LAM (Rogers et al., Dev. Biol., in press). In medium supplemented with newborn calf serum or with FN-depleted calf serum, both peripheral and central neurons attached to, and extended neurites on, LAM. In contrast, only peripheral neurons initiated neurites on FN. Number of neurites initiated, neurite lengths and extent of branching were evaluated and compared with similar measurements of neurite extension on poly-L-lysine-treated surfaces. Although poly-L-lysine provides an adhesive surface for neurite initiation, elongation of neurites was more rapid on the two glycoproteins. Neuronal response to LAM- or FN-treated substrata was markedly reduced by pre-treatment of substrata with antisera to LAM and FN, respectively. The antisera did not diminish neurite outgrowth on poly-L-lysine. In serum-free cultures, neuronal behavior did not differ from that in serum-supplemented medium: both peripheral and central neurons extended neurites on LAM, while only peripheral neurons responded to FN.

Previous studies (L.T. Furcht, Mod. Cell Biol.1:53-117, 1983) have identified tryptic and catheptic fragments of FN containing discrete domains that mediate the multiple functions of this glycoprotein. These fragments include a 27 Kd fragment that weakly binds heparin, a 46 Kd collagen-binding region, a 110 Kd region that contains cell-binding activity but does not bind collagen or heparin, a 58 Kd heparin-binding fragment, and a 31 Kd fragment that contains a free sulfhydryl. Analysis of peripheral neurons cultured on these fragments indicates that the majority of neurite promoting activity resides in the 110 Kd region. Central nervous system neurons did not respond to this portion of the molecule. Additional neurite promoting activity was detected in the 58 Kd heparin-binding fragment but not in the 27 Kd, 46 Kd or 31 Kd preparations. These studies indicate that growing neurites interact with specific molecular features of extracellular environments.

64.11 INTERACTION OF A LATERAL LINE NERVE IMPLANT AND SPROUTS OF INJURED CENTRAL NEURONS IN THE GOLDFISH MEDULLA. S.J. Zottoli and C. van Horne. Dept. of Biology, Williams College, Williamstown, MA 01267

Neurons intrinsic to the central nervous system (CNS) of vertebrates exhibit limited regrowth after injury. Sprouts of injured central axons in the goldfish extend no more than 2-3 mm and few if any project across the lesion site. This limited regrowth may result from limitations imposed by the CNS microenvironment. To test the possibility that the sprouting and elongation of the Mauthner axon (M-axon) and other central neurons of the goldfish may be influenced by a peripheral nerve, we have implanted a piece of lateral line nerve into the medulla oblongata near axons injured by spinal cord transections.

Goldfish, 9-12 cm in body length, were anesthetized and the brain was exposed. The central portion of the cervical spinal cord just caudal to the vagal lobes was cut with microdissecting scissors and one end of an autologous lateral line implant was placed close to the cut axons. Different procedures were used to maximize the chances that the implant and the retracted M-axon would be contiguous. The other end of the nerve was placed in adjacent muscle. After postoperative intervals between 90-120 days (sprouting is initiated by 60 days), both M-axons and non-M-axons were filled with Lucifer yellow. Fourteen M-axons were filled with dye and only 7 sprouted. None of the sprouts entered the implant and there was no indication that the peripheral nerve influenced the direction of sprouting even though M-axon processes were as close as 15 µm to the tip of the implant. Three of 11 non-M-axons that were cut and filled sent sprouts into the implant. HRP backfilling of the lateral line implant in other fish confirmed that many injured central neurons sent processes into the lateral line nerve for distances up to 15 mm, 7 times the distance that non-M-axons were found to sprout within the CNS. In one instance, labeled neurons were found up to 7 mm rostral to the implant and as many as 248 cells were filled.

These results indicate that once sprouts of injured axons contact the implant, they elongate for substantial distances and that the microenvironment presented by the lateral line nerve appears to be more conducive to elongation of sprouts than the CNS. Special thanks is extended to Dr. A. Aguayo, who participated in preliminary experiments. Supported in part by NSF Grant No. BNS-7924655 and by a grant from the Research Corporation.

64.12 INCREASED LOW MOLECULAR WEIGHT MICROTUBULE-ASSOCIATED PROTEIN (MAP) IN PC12 CELLS FOLLOWING LONG TERM EXPOSURE TO NGF. S. H. Green and L. A. Greene*. Dept. of Pharmacology, New York University Sch. Medicine. New York, NY 10016.

In response to NGF, rat PC12 pheochromocytoma cells acquire properties characteristic of neurons. Among these is the elaboration of long, branched neurites. Concomitant with the growth of neurites is a decrease in colchicine-lability of microtubules (Black & Greene, 1982, J.C.B. 95: 379-86) and an increase in a high molecular weight microtubule-associated protein (HMW-MAP) (Greene et al., 1983, J.C.B. 96: 76-83). We now show further that a low molecular weight MAP (MW ≃ 32,000 by SDS-PAGE) is greatly increased in PC12 cells following long-term NGF treatment.

PC12 cells untreated with NGF or treated with NGF for 3 weeks were labeled with [^{35}S]-methionine for 2-3 days. MAPs were prepared either by three cycles of assembly-disassembly with carrier bovine brain microtubules or by a NaCl extraction of taxol-precipitated microtubules (using purified bovine brain tubulin as carrier). Equal amounts of TCA-precipitable radioactivity were loaded on 7.5-15% polyacrylamide gradient gels and visualized by fluorography. Comparison of NGF-treated and -untreated preparations reveals no significant changes in the tau proteins nor in MAPs of MW ≃ 28,000 and ≃ 30,000 which may correspond to MAP1 subunits (Vallee & Davis, 1983, PNAS 80:1342-6). A low molecular weight MAP (MW ≃ 32,000) is much more strongly labeled in the NGF-treated preparations. The increase in relative intensity of this MAP following 3 cycles of assembly-disassembly and following long-term NGF treatment is greater than that of MAP1. This suggests that it is neither a subunit nor a fragment of MAP1. Furthermore, immunoprecipitation of MAP1 fragments does not reveal a polypeptide with a MW close to that of the 32K MAP. We suggest then that changes in the relative amounts of one HMW-MAP and one LMW-MAP accompany NGF-induced phenotypic changes in PC12 cells. Supported by NIH grant NS 16036.

64.13 **EFFECTS OF LOCALIZED NGF WITHDRAWAL ON DORSAL ROOT GANGLION NEURITES IN VITRO**, R. W. Gundersen. Biomedical Research Institute, Univ. of Wisconsin-Parkside, Kenosha, WI 53141.

Initial growth and survival of dorsal root neurons is controlled by nerve growth factor (NGF). Does NGF exert its actions via the entire neuron, cell body, neurite, or growth cone alone? To address this question, dorsal root ganglia from 7 day chick embryos were placed in tissue culture in DMEM supplemented with 10% FCS and 10 ng/ml β-NGF. After 24 hours, the cultures were placed in a perfusion chamber and NGF locally withdrawn by microperfusing individual parts of a neuron with NGF-free medium delivered from micropipettes with 10-20 μm tip diameters, and the neuronal growth photographed and quantified.

When NGF was withdrawn from growth cones, neurite growth rate decreased during the first 45 minutes, and ceased between 45 and 60 minutes. After re-addition of NGF, by micropipette removal, growth rate increased to normal values in 60 minutes. Application of background levels of NGF from micropipettes did not significantly alter neurite growth rate.

Withdrawal of NGF from either the cell body or a segment of the neurite 50 μm distal to the growth cone had no significant effect on neurite growth rate.

NGF withdrawal from growth cones altered their gross morphology, as evidenced by a decrease in the number of lamellipodia and microspikes. However, the number of these extensions which could be identified as being identical between two successive observation periods increased. Upon NGF readdition, these parameters returned to their original values.

The above results indicate that short term growth of dorsal root neurons may only require the presence of NGF in the microenvironment of the growth cone. NGF may be controlling short term neurite growth by affecting growth cone motility. (Supported by NIH grant number NS18214.)

64.14 BLOCKERS OF CALCIUM PERMEABILITY INHIBIT NEURITE EXTENSION AND FORMATION OF NEUROMUSCULAR SYNAPSES IN CELL CULTURE. D.J. Pelto,* B.A. Suarez-Isla and S.I. Rapoport. (SPON: J.C. Conti) Laboratory of Neurosciences, GRC, National Institute on Aging, NIH, Baltimore City Hospitals, Baltimore, Maryland 21224. U.S.A.

Inorganic blockers of calcium permeability, such as cobalt, manganese and lanthanum, were added to cultured trypsin-dissociated retina neurons from chick embryos. They decreased the percent of cells that extended neurites after 24 h in culture, in a concentration dependent manner. Blockers also decreased the rate of neurite growth and the formation of synapses between retina neurons from 8 day chick embryos and rat myotubes, after 24 h in coculture. Fifty percent inhibition of neurite extension was produced at concentrations of 95 μM for cobalt and 250 μM for manganese. Adhesion to substratum and survival of retina cells in culture were not affected at concentrations of inorganic blockers that inhibited more than 90% of neurite extension. Organic channel-blockers, such as verapamil and D-600, prevented adhesion of 8 day retina neurons to substratum before affecting neurite extension. However, nitrendipine significantly inhibited neurite extension before preventing adhesion to substratum.

Growth of neurites in vitro was very sensitive to the extracellular calcium concentration. In medium containing 1.8 mM Ca, 0.8 mM Mg and 10% v/v fetal calf serum, about 16% of cells extended neurites after 24 h in culture. In calcium-free medium, neurite extension and cell substratum adhesion were blocked. However, after addition of 1% or 10% of serum, 6% and 9% of the cells, respectively, extended neurites. In contrast, high Ca concentrations or loading the cells with Ca after 24 h in medium with 25 mM K^+ or 0.1 μg/ml of the calcium ionophore A23187, reduced neurite extension.

With normal calcium concentrations (1.8 mM), inhibition of neurite extension by calcium channel blockers was related to the age of the donor chick embryo. 200 μM cobalt decreased neurite extension by 20% in 6 day retina cells, as compared to 90% in 14 day retina neurons.

Eight day retina neurons, which were densely plated over rat myotubes, innervated 90% of the muscle cells after 24 h of coculture, as determined by intracellular recording. Pretreatment of retina neurons with cobalt or manganese reduced the percent innervation in a concentration-dependent manner, but similar pretreatment of the myotubes had no effect.

These results demonstrate the central role of calcium flux in extension of neurites from primary dissociated neurons, and suggest that synapse formation can be prevented by impairing calcium entry during development and aging.

64.15 FURTHER PURIFICATION AND CHARACTERIZATION OF A NEURITE OUTGROWTH-PROMOTING FACTOR. A. D. Lander*, D. K. Fujii*, D. Gospodarowicz*, and L. F. Reichardt. Department of Physiology and Cancer Research Institute, University of California, San Francisco, CA 94143.

Recently, we have reported the characterization of a factor that promotes neurite outgrowth in vitro (Lander et al., J. Cell Biol. 94, 574-585). The factor is found in medium conditioned by bovine corneal endothelial cells, as well as other cell types, and contains protein and glycosaminoglycan (heparan sulfate) as essential components. When dissociated rat or chick sympathetic or sensory neurons were plated onto polylysine (PLYS)-coated substrata that had been treated with conditioned medium, the neurons extended neurites rapidly and extensively even in the absence of nerve growth factor. Fractionation of serum-free conditioned medium (CM_{SF}) indicated that the active factor behaves as a very large molecular species with a buoyant density in cesium chloride of 1.36-1.37.

We have further purified the factor from ^{35}S-sulfate- and ^{3}H-leucine-labeled CM_{SF}, using ammonium sulfate precipitation, polyethylene glycol precipitation, ion exchange chromatography, and velocity sedimentation. Outgrowth-promoting activity remains associated with ^{35}S- and ^{3}H-labeled material. The ^{35}S-label associated with the factor is degraded by purified heparitinase, confirming the presence of a heparan sulfate proteoglycan. However, our results suggest that the factor exists as a complex with other molecules. Preliminary findings indicate that as many as 3-4 proteins may be associated with the proteoglycan.

Recently, others have reported that the extracellular matrix glycoproteins fibronectin (FN) and laminin (LN) can promote neurite outgrowth (Baron-Van Evercooren et al., J. Neurosci. Res 8, 179-183; Manthorpe and Varon, unpublished). FN is not likely to be the active component of the CM_{SF}-derived factor, since FN-substrata do not promote neurite outgrowth in our assay, and since the CM_{SF}-factor does not bind to gelatin-Sepharose. It seemed plausible that LN might be a component of the CM_{SF}-factor, since we have found LN-treated PLYS substrata to be reasonably effective at promoting outgrowth. However, the outgrowth-promoting ability of LN-substrata is lost following treatment with anti-LN antiserum, whereas neurite outgrowth on CM_{SF}-treated substrata is not diminished after treatment of the substratum with this antiserum, or when the antiserum is included throughout the neuronal culture.

We are beginning to investigate the modes of action of the CM_{SF}-derived factor and LN in promoting neurite outgrowth, in order to see what mechanisms are common to both substances. In this way, we hope to understand better the ways in which a growing axon can interact with its substratum in vivo and in vitro.

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64.16 CHROMOSOMAL, TARGET AND SUBSTRATUM CONTROL OF SPINAL NERVE FIBER GROWTH RATE PARAMETERS IN VITRO. G.M. Procento*, W.L. Muhlach* and E.D. Pollack. Inst. Study of Develop. Disabilities and Dept. Biol. Sci., Univ. of Illinois at Chicago, Chicago, IL 60608.

Nerve fiber growth rate in vitro and its incumbent periodic cycling are primary features of neural development that are under controls imposed by genetic information, target availability and growth substratum. Cyclic extension of spinal nerve fibers in tissue culture as a function of target tissue presence has been demonstrated by us previously (Muhlach and Pollack, Dev. Brain Res. 4,'82). A specific influence of chromosomal dosage on growth rate and cycling can now be shown through the use of a polyploid neural tissue culture model. Further, the nature of the attachment substratum for spinal cord explants appears to affect neurite growth and stability characteristics. Time-lapse cinephotomicrographic analyses have made it possible to compare the relative influences of the genome, substratum and target for nerve fibers in their regulation of neurite elongation.

Neurite extension from spinal cord explants derived from normal diploid or pressure-induced triploid frog tadpoles were filmed for 24 hours and subsequently analyzed for growth rates and periodic cycling of the rates. Triploid and diploid controls were grown on poly-DL-lysine substrata, while other diploid explants were grown under varying target and substrata conditions. Neurites extending from triploid spinal cord explants did so at a mean rate (638μm/24 hr; N=71) approximately 65% greater than for diploid control neurites (380μm/24 hr; N=48). Periodic cycling of growth rates was also altered such that triploid neurites attained higher mean peak velocities (69%) and higher mean minimal velocities (93%) than the diploid neurites. Peak-to-peak interval time, representative of cycle frequency, was reduced, as would be expected, by 56% in triploid neurites (N=20) from those for diploid neurites (N=15). This indicates that a triploid neurite passes through about two cycles of growth for each one that a diploid completes. The available data suggests that the 50% increase in chromosomal dosage in triploid tissue is reflected in alterations of growth rate parameters of a similar order of magnitude.

Just as increasing the chromosomal complement changes growth rate, so does the presence of the natural limb mesenchyme target and attachment substratum affect the rate and cycle frequencies of diploid cord explants. Integration of information on nerve growth rate, growth cycles and neurite density with respect to the controls imparted by genetic and environmental (target and substratum) factors may permit a better understanding of nerve growth regulation. [Supported in part by NIH grant NS 13814]

64.17 EFFECTS OF EXTRACELLULAR MATRIX MOLECULES ON NEURAL RETINAL CELL ATTACHMENT, SURVIVAL AND DIFFERENTIATION. Ruben Adler and A. Tyl Hewitt.* The Wilmer Institute, Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

Cell interactions with other cells and with extracellular matrix components are important for survival and phenotypic expression of many cell types, including neurons. We have tested the influence of substrate-bound factors and extracellular matrix components on the differentiation of 8-day chick embryo retinal neurons. Tissue culture dishes, either uncoated or coated with polyornithine, were derivatized with various materials prior to use. The dissociated cells were cultured for 6-72 hr on these substrata in serum-free medium containing the "N1" supplement of Bottenstein et al.(Exp. Cell Res., 125:183, 1980).

Neuronal survival and neuritic development were dependent upon the composition of the substratum. Neurons did not attach to untreated tissue culture plastic but attached tenaciously to and flattened extensively on polyornithine-treated dishes. However, this attachment was not physiological since there was no neuritic development and the cells showed signs of degeneration within 24 hours. In contrast, on polyornithine-coated dishes derivatized with either bovine serum albumin or chondronectin, cells were rounded and only loosely attached. Dishes pretreated with polyornithine followed by derivatization with fibronectin, fetal calf serum or fibronectin-free fetal calf serum permitted cell attachment and survival, but neuritic development was limited. Laminin treatment of polyornithine-coated dishes, on the other hand, permitted precocious and extensive neurite formation. This effect was dose-dependent and also required polyornithine pretreatment of the substratum, since cells remained rounded and only loosely attached on laminin-coated tissue culture plastic. The laminin-polyornithine effects were similar to those obtained with serum-free schwannoma conditioned medium (SCM) which contains a factor (PNPF) that binds to polyornithine and stimulates nerve fiber formation by retinal neurons (Adler, R., J. Neurosci. Res., 8:165, 1982). When added to the medium within the first 6 hours culture, both laminin and SCM were able to "rescue" cells attached to untreated polyornithine. Laminin is distinct from PNPF, however, since affinity-purified antibodies against laminin suppressed laminin- but not conditioned medium-induced neurite formation.

Together with ongoing investigations using collagen types I-V and various glycosaminoglycans, these studies support the concept that extracellular matrix molecules have profound and specific effects on neuronal survival and differentiation. Qualitative properties of substrate-bound molecules, rather than the strength of cell-substratum adhesiveness, appear critical for controlling neuronal behavior.

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64.18 OPTIMIZATION OF METHODS TO MEASURE BIOLOGICAL CURRENTS USING A CIRCULARLY VIBRATING MICROPROBE. Paul B. Manis*, Philip C. Samson* and John A. Freeman (SPON: D. Buxbaum). Dept. of Anatomy, Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

We recently developed a method to measure low level extracellular biological currents in the nA/cm^2 range using a circularly vibrating microprobe and an ultra-low noise amplifier (Freeman et al., Neurosci., 1982). The extreme sensitivity of our probe allows detection of minute currents associated with single cells and cell processes. We report here technological improvements in the construction of the probe and in the on-line computer analysis of biological currents.

Two probe designs have been developed and tested. One uses two miniature speakers driven in quadrature to move the electrode. This probe may be driven at frequencies between 10 and 500 Hz. Small mechanical nonlinearities in probe motion may be corrected. The speakers generate a phase-coherent magnetic field which must be subtracted from each measurement. We also developed a compact piezoelectrically driven probe which produces no magnetic field. However, the elements must be driven at resonance (200-300 Hz), making corrections for probe nonlinearities more difficult. The two probes are otherwise comparable in terms of ease of construction, cost, and noise.

An inexpensive microprocessor-based system was developed in order to compare the use of a computer to a lock-in amplifier. The lock-in amplifier can determine the magnitude and phase of $\vec{J}$ only, whereas the microprocessor system can compute $\vec{J}, \nabla(\vec{J})$ (a vector pointing towards the current source) permitting servo-controlled mapping of "hot spots". The computer also allows examination of the data during acquisition, displays computed vectors and rejects electrical or mechanical artifacts. Averaging acts as a narrowband filter to reduce noise. Finally, the driving voltage waveforms can be tailored to the specific mechanical configuration to compensate for loading and resonance phenomena.

In order to permit the quantitative correlation of measured currents with structures generating them, we have digitized the microscopic image using a video frame buffer, and have developed software to superimpose polar plots of the current vectors over their measurement location. Image differentiation and the use of a color monitor further enhance image contrast and result in a striking increase in resolution. The combination of these technological improvements greatly increases the temporal and spatial resolution, and the accuracy with which biologically important currents may be measured. The probe has been used to measure currents from growth cones, and injured and regenerating optic nerve fibers.

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64.19 AN IN VIVO ASSAY OF NEUROTROPIC ACTIVITY M.J. Politis and P.S. Spencer
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Previous studies in this laboratory support the contention that distal stumps of transected nerves can attract/support axonal regeneration via diffusible (e.g., neurotropic) factors (Brain Research 253:1, 1982). The present study was undertaken to (a) strengthen this hypothesis, and (b) begin to ascertain the nature of these factors.

The proximal stump of transected rat sciatic nerves was attached to the single inlet end of 6-mm-long, Y-shaped implants made of Silastic tubing. To one of the paired outlet ends was attached an Elvax pellet containing lyophilized homogenate obtained from (A) a distal stump of a transected sciatic nerve obtained 14 days post-operatively (n=6), (B) distal-stump tissue exposed to 80°C (n=4) or trypsin (n=8) prior to lyophilization, or (C) an unoperated sciatic nerve (n=6). The other outlet was attached to a pellet containing no homogenate.

At three weeks post-operatively, a bridge containing well-organized, thinly myelinated axons had formed in the implant lumen in group A, preferentially (n=1) or exclusively (n=5) between the proximal nerve stump and the pellet containing nerve homogenate. No axonal growth was seen in either fork of the implant lumen in groups B and C.

These data lend support to the hypothesis that diffusible substances in distal stumps of transected nerves can attract/support axonal regeneration, and suggest that these "neurotropic" factors are proteins or that their activity is protein-dependent.

Supported by NS 13106.

64.20 STUDIES ON THE FACTORS THAT GOVERN DIRECTIONALITY OF AXONAL GROWTH IN THE EMBRYONIC OPTIC NERVE AND AT THE CHIASM OF MICE. Jerry Silver. Dept. of Developmental Genetics and Anatomy, School of Medicine, Case Western Reserve Univ., Cleveland, Ohio 44106

What are the forces residing at the presumptive chiasm of embryonic mice that control the directionality (i.e., side specificity) of the optic axons? In an attempt to answer this question, the overall trajectories of individual fascicles of axons and the various environments that they encounter along their pathway, have been charted from the eye, through the nerve and into the base of the diencephalon. Serial sections and reconstructive computer graphic techniques were used for the analysis.

The early optic axons (embryonic day 13.5) arrive at the chiasm in a stereotyped topographic arrangement. However, the fiber array at the primitive chiasm is not retinotopically organized nor is it maintained with the same level of spatial precision as it is at the disc. In the distal half of the stalk, the "pioneer" fascicles are organized in a marginally located annulus with inverted retinotopy. However, within the proximal half of the nerve about 17% of the fibers abandon their marginal positions, move randomly across the nuclei rich zone near the stalk's center, and join many other fascicles at the margin but in totally new locations. Thus, although the accuracy of the upside down fiber map deteriorates along the course of the stalk, about 80% of the fibers retain a gross retinotopy as they approach the chiasm. Within 75 µm of the chiasm, the entire contingent of optic axons becomes reorganized into a ventrally located crescent shaped configuration, with fascicles from ventro-temporal and ventro-nasal retina at either side of the crescent and with fascicles from dorsal retina interposed. Because of their gross locations in the crescent, particular clusters of fibers, each largely originating from different retinal sectors, but "contaminated" with fibers from other regions, come in contact with different types of non-neuronal structures at the chiasm. One, a dense, knot-like glial formation that lies along the diencephalic/telencephalic junction, directs all adjacent (ventro-nasal) fibers contralaterally. On the other side of the crescent, a discrete system of lengthy marginal glial processes separated by an anastomotic system of wide-bored extracellular spaces, guides all nearby fibers (ventro-temporal) ipsilaterally. The results suggest that fiber topography as well as local environmental factors may play important roles in guiding axons at the embryonic chiasm. (supported by NIH grant NS-15731)

64.21 GROWTH CONE GUIDANCE IN THE CHICK HINDLIMB.
K. W. Tosney* & L. T. Landmesser (SPON: G. Pilar) Biol. Sci. Group, Physiol. Section, Univ. of Conn., Storrs, CT 06268

The early outgrowth of motoneurons in the chick hindlimb is specific, precise, and responsive to environmental cues (Lance-Jones & Landmesser, 1981, Proc. Royal Soc. 214:1). In order to analyze these features at the level of the growing tip of the neurite, we have labeled identifiable populations of growth cones with HRP and examined them and their relation to the embryonic environment in serial 25u plastic sections.

We find: (1) The morphology, search radius, and trajectory of growth cones vary with their identity and position in the embryo. (2) Motoneurons do not send pioneer fibers into the limb. (3) Growth cones within the pre-muscle masses make few errors in projection, suggesting that pathway guidance is more important than target sampling in forming correct connections. (4) Different populations of growth cones make different but consistent pathway choices in both plexus and limb regions, often by changing their directions abruptly. Choices in each case are customarily made over a definable 100u region. These results suggest that guidance cues are local and specific. (5) At all stages of outgrowth, we see large numbers of dead cells within regions of growth cone activity. This cell death may open a pathway for neurite elongation. Experiments are underway to test this hypothesis and to determine whether the death is related to or indepenent of growth cone activities, such as their release of proteolytic enzymes.

Analysis of surgically altered embryos has shown: (1) When tissues that provide information for the positional differentiation of the limb are removed or transplanted, growth cones still make correct decisions in the plexi. These tissues do not provide the essential cues used in pathfinding. (2) Growth cones are also able to make correct directional decisions in the plexus when their target is absent.

Our results suggest that cues for specific outgrowth lie within the reach of individual growth cones.

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64.22 PATTERNS OF INITIAL WING INNERVATION IN NORMAL CHICK EMBRYOS AND IN EMBRYOS LACKING SOME BRACHIAL SPINAL CORD SEGMENTS.
Margaret Hollyday, Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, IL 60637.

The organization and development of innervation of the chick wing has been studied using orthograde transport of HRP. The single plexus and the adult-like pattern of branching to many major peripheral nerves have formed by stage 28, a stage prior to the onset of naturally occurring cell death. At this stage, injection of HRP into identified spinal segments indicate that axons project to a restricted subset of the peripheral nerves. The projection patterns are consistent with those predicted from the positions of motor pools in the hatchling, indicating that there is a high degree of specificity in initial outgrowth.

Observations of axonal trajectories revealed that axons entering the plexus in an anterior position tended to maintain this position along their growth trajectory, whereas axons arising from caudal wing segments tended to distribute to the posterior portion of the peripheral innervation pattern. In addition, however, axons from a given spinal segment could be seen to diverge from one another at several points along their peripheral outgrowth path. Similarly, axons from a given segment converged at several points with axons originating from other segments. These observations suggest that the normal, specific initial innervation pattern results from interactions of outgrowing axons with growth cues encountered at several points along the outgrowth path.

The peripheral distribution of axons from spinal segments normally having a restricted distribution in the peripheral nerves was also analyzed in embryos at stage 28 in which neighboring spinal segments had been removed at stage 13-14. In many of these cases HRP injections into the remaining segments resulted in the appearance of labeled axons not only in the peripheral nerves labeled by such injections in normal animals but also in other peripheral nerves. Such expansions did not involve large numbers of axons but were seen frequently enough to indicate that abnormalities in the projection pattern from one segment result from removal of adjacent segments. The results suggest that among the cues influencing the outgrowth path of axons from a given segment may be interactions with axons from other segments.

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64.23 MIGRATION AND AXONAL GENERATION OF ANURAN TRIGEMINAL MOTONEURONS.
K. Alley. Case Western Reserve University, Cleveland, Oh 44106

Trigeminal motoneurons in the frog (Rana pipiens) are generated during embryonic stages 13 to 20. These motoneurons innervate different muscle fibers in pre- and postmetamorphic frogs. As part of an ongoing investigation into the differences between the formation of the initial neuromuscular contacts and the subsequent redeployment of these motor axons during metamorphic turnover of the jaw muscles, I have analyzed the earliest events of trigeminal motoneuronal maturation. The goals of the present study are twofold: 1. to chart the migratory pattern of the Vth motor neuroblasts (VMN) after their final mitotic division, and 2. to assess the timing of axonal outgrowth from these neurons.

Light microscopic procedures including tritiated thymidine autoradiography and HRP histochemistry were used to locate and plot the position of the VMN at timed intervals after their generation. Electron microscopy was also used to analyze the timing and events associated with axon formation and extension into the first branchial arch.

Trigeminal motoneurons are produced by the basal plate germinal epithelium of the rostral rhombencephalon. The postmitotic cells migrate in a strictly radial direction from the luminal surface to the intermediate-marginal zone interface. Translocation occurs rapidly. Early born cells require only a few hours to assume their position in the Vth nucleus, while later born cells require up to a day. Axonal outgrowth was first detected at stage 16, during the height of neuron production. However, HRP studies indicate that these axons do not penetrate the external limiting membrane immediately, but seem to wait for the Vth sensory root to make its entry. Axons could be traced into the 1st branchial arch in stage 18 embryos.

From these observations I conclude that the migratory pattern of frog VMN is quite different from the long medial to lateral migration found in the chick (Moody and Heaton, Neurosci.6:1707, 1981). In addition, these observations suggest that the VMN extend axons soon after formation and that their axons are held up at the external limiting membrane. Moody and Heaton (J. Comp. Neur. 213: 327, 1983) have shown a close correlation between the migration of the VMN and the early development of the Vth sensory root in the chick. Perhaps the final extension of the motor axon into the first branchial arch of the frog is guided by the developing trigeminal ganglia and sensory root.

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64.24 THE GROWTH OF MOTOR AXONS IN XENOPUS SPINAL CORD.
Monte Westerfield and Judith S. Eisen, Institute of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

How is the path that axons take in innervating their targets determined during development? We have studied this problem by labeling the axons of primary motoneurons in the developing spinal cord of Xenopus embryos and tadpoles. The axons were lesioned early in development (NF 27) in the clefts between trunk myotomes, and HRP was applied. After a variable survival time, development of the HRP reaction product in whole mounts of the spinal cord and muscles revealed the pathway taken by individual motor axons at various developmental stages.

At the earliest stages studied (NF 27-28) the axons of motoneurons were observed to leave the cord near their cell bodies and innervate nearby muscles. At progressively later stages (NF 35-50) the axon terminals in the muscles became more and more displaced toward the tail relative to their cell bodies. The axons of motoneurons in older animals were found to run caudally in the medial part of the ventral cord for several segments before entering a ventral root.

These results show that the final pathway of motor axons within the spinal cord develops after their initial innervation of muscles. The relative displacement of the axonal terminals may in part be due to differential growth of the myotomal muscles relative to the spinal cord. The addition of later developing longitudinal fibers at the lateral aspect of the cord would account for the medial location of the axons of the older, primary motoneurons. Another possibility, which is being examined, is that the somata of motoneurons migrate rostrally within the cord during development. Supported by the NSF, MDA, MRF, and the Sloan Foundation.

64.26 NEW OPTIC AXONS IN THE GOLDFISH RETINA. S.S. Easter, Jr., B. Bratton, S.S. Scherer, and C.A.O. Stuermer. Div. Biol. Sci., U. Michigan, Ann Arbor, MI 48109.

We have examined electron microscopically the optic nerve fiber layer in the goldfish's retina. This tissue adds new ganglion cells, throughout life, along its annular margin. The axons of each generation of cells are known to cluster together in the optic nerve, tract, and tectum. But in the retina, the axons lie in fascicles arranged like spokes of a wheel centered on the optic disk. These fascicles cross all generations of ganglion cells and pick up axons from all. The more peripheral fascicles merge with other more central ones with older axons. This suggests that the intraretinal axons might not be segregated according to age, and this is the question we have examined.

Retinas were fixed in glutaraldehyde followed by OsO_4, and ultrathin sections were cut perpendicularly to the spokes. The inner limiting membrane is formed by a vitreal basal lamina underlain by Muller cell end feet which separate the neural retina from the basal lamina over most of the vitreal surface. There were bundles of both myelinated axons (MA), (invested by a few loose wrappings of glial cell membrane) and nonmyelinated axons (NMA). The former were larger, and generally lay more sclerad than the latter, which always occupied the most vitread position in the axon layer. Almost all axons contained both intermediate filaments and tubules, transversely sectioned. The most vitread of the NMA contained only tubules. Many of the fascicles of NMA were in direct contact with the basal lamina, through openings between the glial end feet. In most of these cases there was one or more broad processes directly apposed to the basal lamina. These electron lucent processes contained transversely sectioned tubules but no filaments. Quasi-serial sections showed that the processes extended over at least 10's of um, elongated parallel to the spokes.

We believe that the NMA are the new ones because: 1) they are the only ones present in extreme marginal retina, 2) they are continuous with the NMA in the nerve, which we have previously shown to be the new ones, 3) their superficial position is similar to that noted for new axons elsewhere (Xenopus diencephalon: Gaze & Grant; Xenopus spinal cord: Nordlander & Singer), 4) the most superficial ones have tubules only, similar to the new optic axons elsewhere (rat: Peters & Vaughn), and 5) the flattened processes, most superficial, are very likely growth cones.

We conclude that the new axons run superficially in the optic fiber layer, in direct contact with the basal lamina, and just vitread to axons of slightly older cells. Thus the age-specific segregation of optic axons originates in the retina.

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SUBCORTICAL AUDITORY PATHWAYS I

65.1 EFFECTS OF KAINIC ACID ON THE SUPERIOR OLIVARY COMPLEX OF THE RAT. D.A. Godfrey[1], J.L. Park[1]*, J.D. Dunn[2] and C.D. Ross[1], Depts. Physiol.[1] and Anat.[2], Oral Roberts Univ., Tulsa, OK 74171

In an attempt to destroy neurons projecting cholinergic axons to the cochlea and cochlear nucleus, a solution of 5-10 nmoles of kainic acid in 0.5-1 µl saline was stereotaxically injected, over a 5-15 min period, into the right superior olivary complex of four 300-400 gram Sprague-Dawley rats. The rats were sacrificed by rapid decapitation 7 days later and the brains and cochleas frozen within 30 min. Frozen transverse 20 µm-thick sections through the brainstem were alternately stained with thionin or for acetylcholinesterase (AChE) activity, or freeze-dried to permit microdissection for quantitative assay of choline acetyltransferase (ChAT), AChE, or malic dehydrogenase (MDH) activity.

In thionin-stained sections, few if any sizeable somata could be found in the right lateral superior olivary nucleus (LSO), while they were always seen, although not necessarily in the same numbers or sizes as on the left side, in the medial (MNTB) and ventral (VNTB) nuclei of the trapezoid body. In sections stained for AChE activity, the staining in the right LSO was much less than on the left side, that in the right VNTB slightly to moderately less, and that in the MNTB very little on either side. The residual staining of the right LSO seemed localized to small somata and fine processes within it and its hilus regions. Enzyme activities per kg dry wt. of superior olivary complex subregions, right side/left side as mean ± SEM for the 4 rats, were:

	ChAT(µmoles/min)	AChE(mmoles/min)	MDH(moles/hr)
LSO	397±27/560±52	12.7±0.6/28.9±2.0	11.3±0.7/17.4±0.7
VNTB	339±38/366±47	19.5±4.2/32.7±3.0	9.7±0.7/11.0±0.6
MNTB	209±17/361±11	11.5±1.2/16.4±1.3	10.7±0.6/11.7±0.7

The effect on enzyme activities was more marked in LSO than in VNTB, and, in both LSO and VNTB, the kainic acid injection had more effect on AChE and MDH activities than on ChAT, the more definitive marker for cholinergic neurons.

In the large-cell portion of the anteroventral cochlear nucleus, ChAT activities on both sides were similar to those of control rats (in contrast to about 70% loss of ChAT activity on the lesion side of rats with the superior olivary complex surgically destroyed). The AChE-positive bundles containing centrifugal fibers to the inner ear had ChAT activities on both sides comparable to those measured in control rats. ChAT activities of organ of Corti samples from the right cochleas of two of the rats were comparable to those measured in control rats. These results imply that the kainic acid injections into the superior olivary complex of rats did not destroy the neurons giving rise to the centrifugal cholinergic connections to the cochlear nucleus or cochlea.

(Supported by NIH Grant #NS17176 and ORU Intramural funds.)

65.2 DURATION OF INHIBITION SEEN FOR IE NEURONS IN CHINCHILLA LATERAL SUPERIOR OLIVARY NUCLEUS. D.M. Caspary, JoAnn McGee* and Edward Walsh, Dept. of Pharmacology and Surgery, So. Ill. Univ. Sch. of Med., Springfield, IL 62708

The majority of lateral superior olivary (LSO) neurons receive excitatory input from the ipsilateral ventral cochlear nucleus (VCN) while the contralateral VCN inhibits the response to ipsilateral stimulation via secure synapses in the ipsilateral medial nucleus of the trapezoid body. This results in a marked suppression of the ipsilateral excitatory response when binaural stimuli are presented simultaneously. These IE neurons are thought to code for localization of sound in space. Recent studies suggest that glycine may be an inhibitory neurotransmitter synaptically released onto certain LSO neurons upon contralateral tonal stimulation (Moore and Caspary, J. Neurosci., 3:237-242, 1983). The present study investigated the time course of inhibition by varying the time of occurrence of an ipsilateral (excitatory) tone with respect to the offset of a contralateral inhibitory tone (Δt). One hundred presentations of short tone-bursts (3-10 msec) with 0 rise-fall time were presented at the rate of 4/sec; Δt was varied between 0 and 93.8 msec in 10 steps. Percent inhibition was plotted as a function of Δt from the time that the inhibitory tone was turned off until inhibition was no longer observed. A maximum value of inhibition was established by presenting the two tones simultaneously with the contralateral tone between 45 dB and 65 dB above best frequency threshold. Percent inhibition was maximal at small values of Δt and declined rapidly (>10%/msec for many neurons examined) as delay between dichotic tones was increased. Degree of suppression at any given delay was a function of the intensity of the contralateral inhibitory tone relative to the ipsilateral excitatory tone. As the intensity of the excitatory tone was reduced the magnitude and duration of suppression was increased. Rate-intensity plots for monaural control conditions were plotted along with binaural responses at different values of Δt. These findings were compared to the effects of iontophoretically applied glycine at different intensities of ipsilateral acoustic stimulation. The duration of inhibition may involve several different factors including duration of the IPSP and termination mechanisms of an acoustically-evoked inhibitory transmitter. Since iontophoretic application of strychnine, a glycine antagonist, abolishes the contralateral inhibition observed in these neurons, the loss of inhibition with increasing delay may partially reflect transmitter uptake proposed as the main termination mechanism of the action of glycine. This paradigm may be a useful way to more closely examine synaptic mechanisms in the LSO nucleus. (Supported by Deafness Research Foundation, NIH grant 15640, and a gift from the Pearson Family Foundation.)

65.3 FUNCTIONAL ORGANIZATION OF THE BARN OWL'S INFERIOR COLLICULUS. T. Takahashi* and M. Konishi(SPON.:C.R.Hamilton).Division of Biology 216-76, Caltech. Pasadena, CA 91125.

Auditory neurons in the anterolateral part of the barn owl's inferior colliculus (IC) have discrete, spatial receptive fields and are arrayed so that a continuous map of space is formed (Knudsen and Konishi, J. Neurophys.,41:870-884 '78). The remaining posteromedial part of the IC is tonotopically organized and is thought to be the source of afferents to the anterolateral region (Knudsen, In press, J. Comp. Neur.). We present evidence suggesting that the posteromedial part of the IC comprises at least three subdivisions, each of which has a characteristic binaural property and a characteristic set of afferent nuclei.

Most medially, there is an area in which the units are excited only by stimulation of the contralateral ear. This was termed the "EO" area. Stimulation of the ipsilateral ear does not alter the discharge rate. Units driven only by the ipsilateral ear (which would be termed "OE") were not observed. Immediately medial to the EO area is the "EE-phase" area, containing units that are excited by stimulation of either ear, and responding maximally when a noise or tone burst is presented to the ears with a discrete, interaural delay. At a given interaural delay, the discharge rate of EE-phase units depends upon the average binaural intensity and is independent of the interaural intensity difference.Immediately posterior to the EE-phase area is the "EI" area whose units are excited by the contralateral ear and inhibited by the ipsilateral ear. Their discharge rates increase as the intensity in the excitatory ear is increased over that in the inhibitory ear. Increases in average binaural intensity does not alter the spike rate as long as the interaural intensity difference is kept constant. Finally, the tonotopicity demonstrated by Knudsen and Konishi ('78) was confirmed. Units having lower best frequencies are found dorsal to those with higher best frequencies. This tonotopic organization extends across the functional zones described above.

Each of the functional zones are innervated by a different set of pontine auditory nuclei. Horseradish peroxidase injections of the EO area labels somas in the contralateral nucleus angularis (NA) and nucleus olivaris superior (SO) and the ipsilateral nucleus ventralis lemnisci laterale pars posterior (VLVP). Injection into the EE-phase area produces labeled somas in SO of both sides and in the contralateral nucleus ventralis lemnisci laterale pars anterior (VLVA), VLVP, nucleus laminaris (NL) and NA. Finally, injections into the EI area produces labeled somas in VLVP of both sides and in the contralateral SO,NL,NA, and IC.

Projections from each of the functional zones to the anterolateral part of the IC is currently being investigated.

65.4 LOCAL ANESTHETICS DEMONSTRATE THE SEPARATION OF TIME AND INTENSITY PROCESSING BY THE OWL AUDITORY SYSTEM. A. Moiseff, T. Takahashi*, and M. Konishi, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125.

Space-specific neurons in the external nucleus of the owl's (Tyto alba) inferior colliculus owe their discrete auditory receptive fields to selectivity to narrow ranges of both interaural time differences and interaural intensity differences. Neurons of the pontine nuclei, which innervate the inferior colliculus, are sensitive to either time differences or intensity differences, but not both. Based on the binaural response properties that characterize the pontine nuclei and the connectivity among them, we hypothesize that interaural time and intensity differences are processed along separate neural pathways, and that space selectivity emerges through the convergence of these two pathways. We used a local anesthetic, which reversibly blocked activity in selected auditory nuclei, to test the validity of this hypothesis. We present evidence that the sensitivity of space-specific units to time differences can be markedly decreased without drastically affecting their sensitivity to interaural intensity differences.

The first two stages of the "time processing pathway" comprise nucleus magnocellularis (one of the cochlear nuclei) and nucleus laminaris (the avian homologue of the medial superior olivary nucleus). Neurons from n. magnocellularis project bilaterally to n. laminaris. Lidocaine, a local anesthetic, was injected into nucleus magnocellularis or nucleus laminaris through microelectrodes. The anesthetic effectively blocked neural activity to a distance of approximately 300 micrometers from the electrode tip. We placed a second microelectrode into the external nucleus of the contralateral inferior colliculus, where single spacespecific units were isolated. Normally, these units are "tuned" to a narrow range of time differences and a narrow range of intensity differences. When n. laminaris or n. magnocellularis was anesthetized, space-specific units retained their high selectivity to intensity differences, but they lost their selectivity to time differences. As the effect of the lidocaine wore off, the time sensitivity recovered to the normal level.

The ability to selectively modify the time sensitivity of space selective units supports the hypothesis of independent analysis of time and intensity differences.

65.5 RESPONSE PROPERTIES AND DISPROPORTIONATE REPRESENTATION OF FM SENSITIVE NEURONS IN THE INFERIOR COLLICULUS OF THE CF-FM BAT, PTERONOTUS PARNELLII. W.E. O'Neill, Ctr. for Brain Research, Univ. of Rochester Sch. of Med., Rochester, N.Y. 14642.

The auditory system of the mustached bat apparently contains two semi-independent systems for the analysis of information conveyed by the constant frequency (CF) and frequency modulated (FM) components in its biosonar signal. CF signals best convey information about target movements, while the FM components are best suited to ranging, localization and characterization of fine features. Previous studies in the auditory cortex revealed two separate regions containing neurons specialized for processing the CF component (DSCF area) and the FM component (FM-FM area). Neurons in the FM-FM area are sensitive to specific pulse-echo time intervals which encode target distance, and are systematically arranged to represent target range according to place of excitation along the cortical surface.

To define the subcortical pathways subserving this cortical area representing target range, single units were examined throughout the inferior colliculus (IC) of the mustached bat for their sensitivity to CF tones and FM sounds similar in bandwidth to the biosonar signal. Two large areas contain neurons tuned to frequencies within the FM sweeps of the first (FM_1) and second (FM_2) harmonics of the biosonar signal. The area tuned to the FM_1 frequencies lies in the anterior part of the IC, and unit best frequencies (BF) increase only slowly from about 25 to 29 kHz with depth of penetration. The area tuned to FM_2 has unit BFs from 52 to 57 kHz, is located posterior to the FM_1 representation, and is separated from it by a relatively small region where unit BF's increase from 30 to 50 kHz in the anterior-posterior direction, and also with depth of recording site. In the FM_2, and especially FM_1 areas, the thresholds of units to decreasing FM sweeps is equal to or even better than the thresholds to CF stimuli. This is generally not true for units tuned to other frequencies, where the threshold to FM is typically higher than to CF.

FM_1 and FM_2 are the essential components for response of a large cluster of range-tuned units in the FM-FM area of cortex. It appears that two separate areas of the IC having FM sensitive neurons are appropriate candidates for conveying target range information to the cortex via the thalamus. The relative importance of these frequency bands to echo processing is reflected in the disproportionately large area of IC representing them. Exposure of bats to FM_1-FM_2 stimulus pairs after injection of ^{14}C-2-deoxyglucose reveals uptake patterns highly consistent with the electrophysiological maps of frequency representation in these FM bands. The organization of the mustached bat IC is greatly modified from the pattern seen in more "typical" mammals and reflects the strong correlation of acoustic behavior and auditory organization in this species. NSF Grant no. BNS-8023109.

65.6 STIMULUS-DEPENDENT LABELING WITH {^{14}C} -2-DEOXYGLUCOSE IN THE INFERIOR COLLICULUS OF THE MUSTACHED BAT. D.M. Gooler* and W.E. O'Neill (SPON: S. Demeter). Ctr. for Brain Research, Univ. of Rochester Med. Sch., Rochester, N.Y. 14642.

A modification of the {^{14}C}-2-deoxyglucose (2-DG) method for qualitative mapping of functional activity in brain was used to explore the tonotopic organization of the inferior colliculus (IC) in the mustached bat, Pteronotus parnellii parnellii. The mustached bat emits orientation pulses consisting of a sustained constant frequency (CF) component followed by a short downward sweeping frequency modulated (FM) sound, at each of four distinct harmonics (CF_{1-4}; FM_{1-2}).

The purpose of this study was to explore the sensitivity of the 2-DG method for differentiating between regions of the IC activated by different, complex auditory stimuli. While electrophysiological maps of auditory activity are derived from individual recording sites, a map based on evoked 2-DG uptake will show the entire region that is activated by a specific stimulus.

Bats were injected intraperitoneally with 2-DG (1.25 uCi/10 gram body weight) and exposed to open field auditory stimuli for 45 min. Following the experiment, the bat was anesthetized and decapitated. The brain was rapidly removed and frozen to -70°C. Sections (20 u thick) were cut in a cryostat at -22°C, and were placed in contact with Kodak X-TL film and exposed for 14 days. Sections were counterstained with cresyl violet acetate.

In all autoradiographs, the uptake of 2-DG in IC was greater than in other local regions. When exposed to a stimulus composed of FM_2 alone (e.g., 57-45 kHz sweep), autoradiographs of horizontal sections showed a distinct crescent-shaped band of label in the caudal portion of the anterior half of the IC. For a paired FM_1-FM_2 sound (e.g., FM_1 = 30.7-24.7 kHz; FM_2 = 58.8-46.8 kHz), similarly oriented sections show a dense band in the same region as the case with FM_2 alone. In addition, a second band of increased density is found in the rostral portion of the anterior half of the IC. The 2 bands occupy nearly the entire anterior half of the IC. For each band of increased autoradiographic density, cellular density observed in counterstained sections was not different compared to regions showing lower 2-DG uptake.

Evidence from electrophysiological studies of the anterior IC also shows disproportionately large areas representing FM_1 and FM_2 sounds. Also in agreement with electrophysiological evidence are the results of the autoradiographs showing that the FM_1 and FM_2 areas exist throughout the dorsoventral extent of the IC. These results indicate that the 2-DG technique can resolve differences between acoustic stimuli and that results are relevant to electrophysiological study of the IC.

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65.7 THE ASCENDING CONNECTIONS OF THE TORUS SEMICIRCULARIS CENTRAL NUCLEUS IN CHRYSEMYS SCRIPTA ELEGANS. R.H. Browner. Department of Anatomy, New York Medical College, Valhalla, NY 10595.

The central nucleus (CN) of the torus semicircularis is the major auditory nucleus in the reptilian mesencephalon. Ten red-eared turtles were anesthetized with sodium pentobarbital (I.P. 11.00 mg/kg-Fall and Winter). A portion of the temporal muscle was removed and an opening was drilled into the lateral wall of the cranial cavity over the mesencephalon. The meninges were cut and the dorsal dome of the optic tectum was removed to expose the floor of the tectal ventricle. Glass micropipettes (tip diameter of 10-20 um) were filled with 25% Type VI horseradish peroxidase diluted in reptilian ringers. This mixture was injected into the elevated floor of the tectal ventricle where the underlying central nucleus was located. Seven injections were ipsilateral to the surgery, three contralateral in the central nucleus. After pressure injections for 12 minutes the surgical area was packed and sutured and the animal allowed to survive from 2 to 4 days. Following reanesthesia the turtles were perfused and reacted with TMB and BDHC (Mesulam, IBRO Handbook Series, Wiley, 1982) to analyze the anterograde connections. Ascending fibers left the central nucleus laterally and formed the tectoreuniens tract (TRT). At the lateral edge of the mesencephalon, ventral to the optic tectum, the TRT turned rostrally and coursed into the caudal diencephalon in a ventrolateral position. Here the TRT turned medially and slightly dorsally to cross the diencephalon and terminate in the nucleus reuniens (NR) and adjacent neuropil. There was no HRP reaction in the contralateral NR. A second pathway coursed ventral to the cerebral aqueduct to terminate within the contralateral CN. Both laminar nuclei were clear of reaction product. Finally there was a project to the deep layers of the ipsilateral optic tectum.

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65.8 STRUCTURAL AND FUNCTIONAL ORGANIZATION OF MAMMALIAN AUDITORY BRAINSTEM AND PERIPHERY AS REVEALED BY A COMBINED ELECTROPHYSIOLOGICAL AND HRP TRACING METHOD: A.S. Feng and M. Vater* (SPON: G. Neuweiler). Zoologisches Institut der Universität München, Luisenstrasse 14, D-8000 München 2, FRG.

We made extracellular single unit recordings from various regions of the central nucleus of inferior colliculus (IC) and the cochlear nucleus (CN) of echolocating CF bats, Rhinolophus rouxi, followed by focal injections of HRP into specific functionally identified recording sites. After a survival time of 24-48 hrs, individual brains were sectioned and processed according to the tetramethylbenzidine (Mesulam, 1982) and diaminobenzidine (Adam, 1977) methods. In CN injected specimens, the cochlea on the injected side was decalcified, sectioned and processed similarly. From CN injected materials, focal injections into individual bands of neurons with similar best frequencies resulted in retrograde labelling of spiral ganglion cells and fibers in specific regions of the cochlea. These results allowed us to generate a frequency map within the cochlea which can be compared to a similar map obtained with other methods (Bruns, 1976) and to define the "effective" HRP injection size in the CN. Centrally, internal connections between various CN subdivisions were revealed. For instance, there was a 100 µm transverse slab terminal field in the anteroventral cochlear nucleus as well as a columnar terminal field in the dorsal cochlear nucleus following a focal injection of HRP in the posteroventral nucleus showing the frequency representation patterns in each of these divisions. Anterograde CN projections further revealed the functional organizations and terminal bouton types in individual brainstem nuclei of the superior olivary complex, lateral lemniscus complex and the IC. The projection patterns corresponded closely the retrograde labelling patterns shown in IC injected specimens. Furthermore, data from IC injected animals revealed the internal connections between various IC subdivisions. IC projections to the medial geniculate body showed banding termination patterns for various tonal frequencies.

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65.9 ASCENDING PROJECTIONS TO THE INFERIOR COLLICULUS OF THE RAT AND BAT. Richard A. Burne. Bendix Advanced Technology Center, Columbia, MD 21045.

This study was undertaken with the general aim of comparing the organization of ascending auditory afferents to the inferior colliculus (IC) in the bat, Eptesicus fuscus, an echolocating mammal and in the rat, a nocturnal listening animal. The techniques of retrograde and orthograde transport of horseradish peroxidase (HRP) and tritiated L-leucine, respectively, were employed to determine pathways from brainstem auditory nuclei to the IC.

Injections of HRP into the central nucleus of IC in both bat and rat resulted in labeling a prominent number of neurons in the following contralateral regions: cochlear nuclei (dorsal, anterior and posterior ventral), lateral superior olive, dorsal lateral lemniscus and inferior colliculus. Labeled cells also were observed in the following ipsilateral structures: lateral and medial superior olives, dorsal and ventral medial periolivary nuclei, ventral trapezoidal nucleus and ventral and dorsal lateral lemnisci. In some cases with relatively large HRP placements in the IC, a few scattered cells were observed contralaterally in the ventral medial periolivary nucleus of the bat and ipsilaterally in the dorsal cochlear nucleus of the rat.

HRP placements restricted to the pericentral nucleus of the IC yielded labeled cells only in ventral and dorsal lateral lemniscal nuclei. However, tritiated leucine injections in the cochlear nucleus (dorsal and ventral) of the rat resulted in autoradiographic grain labeling over the central and pericentral nuclei of the contralateral IC. A faint distribution of grains also was observed over the ventral region of the ipsilateral central nucleus supporting previous HRP results and suggesting a sparse ipsilateral projection from the cochlear nucleus to the IC.

These findings suggest that the organization of auditory afferents to the IC in Eptesicus fuscus, an echolocating bat that uses primarily a frequency modulated emission, is similar to that found in other non-echolocating mammals. Whether the correspondence in structural organization supports a functional similarity in processing acoustic signals is an issue for future investigation.

65.10 DETERMINANTS OF AUDITORY SPATIAL SELECTIVITY: A COMBINED DICHOTIC AND FREE-FIELD STUDY OF NEURONS IN THE BAT INFERIOR COLLICULUS. Z. M. Fuzessery and G. D. Pollak. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

The spatial selectivity of an auditory neuron is dictated by the directionality of the ears, and by its sensitivity to binaural cues. To directly evaluate how these factors influence spatial selectivity, 60 KHz neurons in the inferior colliculus of the mustache bat (Pteronotus p. parnelli) were examined for both their sensitivity to binaural intensity differences, using dichotic stimulation, and for their spatial selectivity, using free-field stimulation generated by an array of 15 speakers mounted on a hoop which could be rotated about the horizontal axis of the bat's head. Maximum spatial selectivity was found to shift in a predictable fashion as a function of binaural intensity sensitivity. These results were compared with those of a cochlear microphonic study evaluating the acoustic directionality of the ear, permitting a dissection of the contributions made to neuron spatial selectivity by the structure of the ear, and by the central integration of binaural input.

The mustache bat emits a constant frequency echolocation pulse of 60 KHz. Neurons were recorded in a large, anatomically discrete subdivision of the inferior colliculus devoted to processing information at this frequency. At 60 KHz, the cochlea is maximally sensitive to a sound from 20° off the vertical axis and 10° below the horizontal axis, termed the acoustic axis. An area around the acoustic axis in which thresholds are within 5 dB of greatest sensitivity is termed the optimal area. At the cochlear level, the optimal area is roughly circular, extending about 20° radially from the axis.

Three main functional classes of colliculus neurons were studied: E-O cells responding to input from only one ear, E-I cells excited by one ear and inhibited by the other, and E-E cells excited by input from both ears. E-E cells could be facilitated or not by binaural input. E-O cells had an optimal area similar to that of the cochlea, suggesting spatial selectivity was dictated primarily by the ear. Non-facilitated E-E cells had two optimal areas at the acoustic axes of the ears. Facilitated E-E neurons had a single optimal area which shifted systematically toward the vertical midline as the binaural excitatory thresholds approached equality. E-I cells, like E-O cells, had optimal areas centered at the acoustic axis of the cochlea, but these areas were smaller, with size decreasing as the relative inhibitory threshold of the decreased.

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65.11 PHYSIOLOGICAL CELL CLASSES IN THE RAT INFERIOR COLLICULUS EXAMINED BY FACTOR AND CLUSTER ANALYTIC TECHNIQUES. G.R. Farley, E. Javel, B.J. Morley, and D.E. Goldgar*. The Boys Town National Institute for Communication Disorders in Children, Omaha, NE, 68131.

A fundamental issue in auditory physiology is whether response variability in a structure represents continuous variation or reflects different physiological classes. We examine this question for neurons recorded from the inferior colliculi (IC) of rats anesthetized with urethane. Stimuli were 100 ms tone bursts delivered to the contralateral ear, repeated every 400 ms. For each unit, characteristic frequency (CF) was determined, and a rate-intensity function was obtained at CF. Data analysis consisted of two steps.

First, the set of post-stimulus time histograms (PSTHs) from the study was subjected to factor analytic techniques to reduce the dimensionality of the problem. Derived factors appeared to represent time epochs in the PSTHs, including an onset period, a pause period, a build-up period, and an offset period. Factor scores were calculated for each PSTH for use in clustering.

Second, cluster analysis, including hierarchical and K-means techniques, was used to identify classes of cells having similar response properties. Clustering was done on two sets of data: 1) the PSTHs obtained at 20 dB above unit threshold and 2) the PSTHs from the entire intensity series. In each case, the analysis suggested three major clusters: a group of onset units and two groups of primary-like/pause/build-up units, one having high response rates, and the other having moderate response rates.

In order to confirm these findings, other methods for data reduction and clustering are currently under investigation. However, the results from the above analyses do not eliminate the possibility that the differences between primary-like, pause, and build-up units in the IC represents continuous variation rather than distinct classes. A comparison of results from different analyses, and a discussion of their implications for IC physiology, will be presented.

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65.12 FUNCTIONAL CHANGES IN THE INFERIOR COLLICULUS OF ADULT GERBIL AFTER UNILATERAL NEONATAL COCHLEAR ABLATION. L.M. Kitzes and M.N. Semple (Sponsor: E.A. Davis). Dept. of Anatomy, School of Medicine, University of California, Irvine, CA 92717.

Neonatal destruction of the cochlea leads to transneural degeneration of the ventral cochlear nucleus and to changes in the connectivity of the inferior colliculus to each division of the cochlear nuclear complex on the unoperated side (Nordeen, et al., J. Comp. Neurol., 214:144-153, 1983). In the present study, physiological consequences of such destruction were examined by comparing responses of single units in the inferior colliculus of normal adult gerbils with responses in adult gerbils which had been subjected to unilateral cochlear ablation at 2 days of age. All animals were anesthetized with sodium pentobarbital and ketamine hydrochloride. Tonal stimuli, presented through sealed ear pieces, were calibrated from 60 Hz to 30 kHz.

In normal animals, contralateral monaural stimulation was typically excitatory, whereas ipsilateral stimulation was commonly ineffective in eliciting a response when presented alone, or suppressed contralaterally evoked responses under conditions of binaural stimulation. When present, excitatory responses evoked by ipsilateral stimulation had higher thresholds and lower peak discharge rates compared to responses evoked by contralateral stimulation. In neonatally ablated animals, responses evoked by stimulation of the unoperated ear recorded in the ipsilateral inferior colliculus differed markedly from the normal pattern. Excitatory responses were readily evoked by ipsilateral stimulation and, in addition, had lower thresholds and much higher peak discharge rates compared to responses evoked by ipsilateral stimulation in normal animals.

This remarkable physiological change indicates that responses to ipsilateral stimulation in normal animals depend at least in part on the functional integrity of the contralateral ear during development.

65.13 CONNECTIONS OF A LATERAL TEGMENTAL REGION IN THE MIDBRAIN OF THE CAT WITH THE MEDIAL GENICULATE BODY AND THE INFERIOR COLLICULUS: THE NUCLEUS SAGULUM. C. K. Henkel and J. M. Whitley*. (SPON: W. K. O'Steen). Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

Several lines of evidence suggest that the lateral tegmentum is associated with the afferent and efferent pathways of the midbrain and contributes to adjunct systems that interact with processing in the inferior and/or superior colliculus. In the first part of this study, the nucleus sagulum was identified as one of several lateral tegmental sources of subcollicular projections to the medial geniculate body (MG) in the cat using the HRP method. The results of six experiments with large HRP injections in MG showed that neurons wedged in the margin ventral to the inferior colliculus and neighboring the nuclei of the lateral lemniscus have thalamic projections. Labeled cells in the nucleus sagulum were small (14-22 μm) and more numerous ipsilaterally. A string of small tegmental neurons were labeled rostral to the lemniscal nuclei and were continuous with those in nucleus sagulum. In the second part of this study, efferent axonal projections from the region of the nucleus sagulum were studied in autoradiographic experiments. Six cases had tritiated leucine injections involving the nucleus sagulum. Axonal projections to the medial geniculate body ended ipsilaterally in three territories: first, in a ventral region associated with the brachium; second, in a dense field within the caudal part of the dorsal division; and third, in the medial division. These thalamic projections coursed centrally through the midbrain reticular formation. Contralaterally, a projection to the medial division of MG was observed in three cases. In all of the cases, labeled axons followed the central reticular course to hypothalamic and/or ventral tegmental areas. Two other major systems of fibers were labeled at the level of the midbrain in the sagulum cases. A commissural system crossed dorsally in the tegmentum to distribute within the nucleus sagulum and around the dorsal nucleus of the lateral lemniscus and parabrachial region. The major target of the other system of fibers from nucleus sagulum was the inferior colliculus. These fibers ended ipsilaterally in patches within the pericentral nucleus and throughout the external nucleus. In summary, the nucleus sagulum is part of a lateral tegmental system with connections with regions of the inferior colliculus and medial geniculate body that are themselves serially linked. The nucleus sagulum, thereby, may modulate one line of sensory processing at the midbrain and again at the thalamic level.

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65.14 A model of interaural intensity sensitivity. Tom C.T. Yin, Judith A. Hirsch, and Joseph C.K. Chan. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI. 53706.

Most neurons in the cat's superior colliculus (SC) that respond to sounds receive input from both ears and are sensitive to changes in behaviorally significant interaural intensity difference (IID). Many such cells, EO/I, produce a discharge in response to monaural contralateral, but not ipsilateral, stimulation which is supressed when tones are presented to both ears. Neurons of another class, OO/F, require binaural input to trigger action potentials. Since the time interval from stimulus onset to neural response decreases with increasing sound pressure level, the cellular mechanism of IID detection could involve an analysis of the delay between the arrival times of binaural inputs, as Jeffress (1948) proposed. In order to test this experimentally, we compared the sensitivity of single cells to both IID and interaural time disparity (ITD).

Extracellular recordings were made from the intermediate and deep layers of the SC in barbiturate-anesthetized cats. Acoustic stimuli were delivered dichotically. We found the general shapes of the IID and ITD functions to be similar. For example, spike-counts of OO/F neurons are nonmonotonic with respect to both IID and ITD. If a cell fires maximally when contralateral intensity is higher than ipsilateral, then it will also be maximally excited by isointensity stimuli if the contralateral signal is given a sufficient temporal lead. Analogous relationships exist for EO/I neurons. The steepness of the IID and ITD profiles are well correlated as well.

These results are consistent with a model that uses the dependence of response latency on sound intensity to demonstrate how IID sensitivity can be generated by differences in the timing of the arrival of inputs from the two ears. For OO/F cells the model assumes that afferents from each ear cause a transient wave of subliminal excitation at the binaural neuron. Coincident arrival of both inputs evokes maximal discharge. As the two waves of excitation overlap less and less in time spike-count falls. Asymmetry about zero IID is modelled by a temporal mismatch of the responses from the two sides at equal intensity stimulation. EO/I cells differ in that ipsilateral input is inhibitory and that contralateral excitation is suprathreshold. We think it likely that this model is also useful in describing the detection of IID at other auditory loci.

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66.1 RESPONSE PROPERTIES OF PRIMARY AFFERENTS IN THE MECHANOSENSORY SYSTEM OF THE CRAYFISH. M.R. Plummer, J.P.C. Dumont* and J.J. Wine. Neurosci. Prog., Dept. of Biol. and Dept. of Psychol., Stanford Univ., Stanford, CA 94305.

The last abdominal ganglion of the crayfish contains a population of identified mechanosensory interneurons that respond to oscillatory fluid displacements (Sigvardt, K.A., Hagiwara, G., Wine, J.J., J. comp. Physiol., 148:143-157, 1982). We have determined the frequency response characteristics of these interneurons and have found that within the range of 4 - 400 Hz particular identified neurons are selective for low frequencies, selective for high frequencies, or are nonselective. Our present work is an attempt to understand the mechanisms responsible for this frequency selectivity.

The interneurons receive their input chiefly from the animal's tailfan which is covered with numerous cuticular hairs, some of which are sensitive to water currents (Wiese, K., J. Neurophysiol., 39:816-833, 1976). We examined the mechanoreceptive hairs on the rostrolateral part of the telson using S.E.M. and light microscopy and found four morphologically distinct hair types. One hair type (termed 'guard hair' by Wiese) lies parallel to the surface of the telson. The other three types: long feathered hairs (ca. 800 um long), feathered hairs (ca. 300 um long) and smooth hairs (which are somewhat shorter and thicker than feathered hairs) are oriented perpendicular to the telson surface. There are roughly 70 upright hairs on this part of the telson and the ratio of long feathered hairs to feathered hairs to smooth hairs is approximately 1:4:16.

Intracellular recordings from primary afferent axons that innervate these hairs reveal that the frequency responses of some afferents duplicate the frequency responses of certain of the aforementioned interneurons. For example, one type of afferent is excited by low frequencies, supressed by high frequencies and receives what appears to be presynaptic inhibition at intermediate frequencies. We are presently trying to match morphological hair types with afferent response types and are testing the hypothesis that selective connectivity of afferents to interneurons generates the frequency response properties of the interneurons.

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66.2 POST SYNAPTIC INHIBITION MEDIATES SUPPRESSION OF ULTRASOUND AVOIDANCE IN THE CRICKET TELEOGRYLLUS OCEANICUS. T.G. Nolen and R.R. Hoy, Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Tethered, flying crickets avoid bat-like ultrasound by making steering movements away from the source (Moiseff, Pollack, Hoy, PNAS 75:4052). This behavior is mediated by an ultrasound sensitive interneuron (Int-1) (Nolen and Hoy, Neurosci. Abst. 8:529). Int-1's ultrasound response can be suppressed by simultaneous presentation of a tone pulse at the calling song frequency (4-5 kHz) (Moiseff and Hoy, J. Comp. Physiol., in press). We show that the avoidance behavior is also suppressed, at high sound levels of calling song, and that suppression is likely due to post synaptic inhibition of Int-1 by sound pulses of the calling song.

Single, short pulses (1 to 30 msec) of ultrasound (15 to 100 kHz) elicit phasic avoidance steering movements in tethered, flying crickets. Pairing ultrasound with 5kHz pulses suppressed avoidance steering, but only at relatively high sound levels of 5kHz (11 to 15 dB louder than the ultrasound, over a range of ultrasound from 50 to 90 dB SPL), and only if the two pulses overlapped; maximal suppression occurred with simultaneous, two-tone stimulus pulses. At equal sound levels, avoidance steering took precedence over attraction to the calling song. Removal of the contralateral ear showed that suppression could be mediated monaurally.

Intracellular recordings showed that Int-1's ultrasound response was suppressed by 5kHz, which increased the latency to spike, decreased the mean spike rate, decreased the burst duration, and shunted compound EPSPs. Maximal suppression occurred only when the 5kHz pulse was 10 to 15 dB louder than the ultrasound and only when the two pulses overlapped in time.

Sound pulses between 3 and 10 kHz produced an inhibitory post synaptic potential (IPSP) in Int-1, with a best frequency between 4 and 5kHz and thresholds as low as 50 dB SPL. The IPSP was mediated by the ipsilateral ear, had a short latency (31 msec near threshold and 10 msec at higher sound levels) and had a reversal potential at, or just hyperpolarized from, the resting potential. The IPSP lasted the duration of the 5kHz tone pulse and decayed quickly after the stimulus was presented. It had little or no effect on the rising phase, but caused a more rapid decay of unitary EPSPs.

Finally, by recording from Int-1 in a flying animal, we found that 5kHz inhibition was sufficient to prevent the cell from reaching the critical spike rate (about 200 spikes/sec) necessary to elicit the avoidance steering.

These results suggest that calling song suppression of the ultrasound avoidance steering of flying crickets can be explained by post synaptic inhibition of the neuron that initiates the behavior.

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66.3 ANATOMICAL CIRCUITRY OF LATERAL INHIBITION IN LIMULUS. W. H. Fahrenbach* (SPON: C. Bell). Oregon Regional Primate Research Center, Beaverton, OR 97006.

The neuronal plexus under the compound eye of the horseshoe crab, Limulus polyphemus, consists of an open, three-dimensional filigree of fine fiber tracts punctuated at intersections by knots of neuropil. This meshwork is the site of lateral inhibition between eccentric cells (second-order visual neurons), a process that yields contrast enhancement and has been studied for more than 30 years. Specific interconnections in the plexus are largely conjectural beyond results inferred from neurophysiology. This study is based on an unbroken, 1,100-section series, cut at a 50-nm thickness, encompassing the plexus of about 25 ommatidia from an animal with a 13-mm prosomal width. Given the caveat that the animal is immature, that it shows ample neuronal sprouting, and that the reconstruction to date embodies 45 axonal aborizations and 600 synapses in one synaptic knot, the following generalizations can be extracted. Retinula cell axons pass through the plexus without branches or contacts. Octopaminergic efferent fibers receive rare synaptic input from eccentric cell collaterals and in turn have scattered synaptoid terminations on retinula cell axons and eccentric cell collaterals. Eccentric cell axons are devoid of direct synaptic input. Their collaterals travel through the plexus as extremely fine fibers (0.1-0.2 μm). They ramify in synaptic knots and subserve both pre- and postsynaptic functions simultaneously. Arborizations near the axon of origin have a high synaptic density (mean of 5 per μm of neurite length), a high synaptic input:output ratio (up to about 10:1), and a rich branching pattern. Eccentric cell collaterals arborizing at distances of several ommatidial diameters have low synaptic density (2.5 per μm) and input: output ratios (about 1:1), as well as sparse branching. Density of presynaptic sites is largely invariant with distance of the collateral from the parent axon. The structural basis for spatial summation is provided by compact groupings of input synapses, up to 15 per μm, each from a different neuron of origin. Decreasing synaptic input with increasing distance from the parent axon supplies the explanation for spatially graded lateral inhibition. Closely adjacent reciprocal synapses are common and may be responsible for self-inhibition. The simplest hypothesis that combines the known neurophysiology, observed structure, and implied morphogenetic demands, consists of the plexus functioning by decremental conduction of de- and hyperpolarization.

Supported by National Institutes of Health grant EY-00392.

66.4 ANTENNULAR STRUCTURES MEDIATING PHEROMONE RECEPTION IN THE CRAYFISH ORCONECTES PROPINQUUS. Ann Jane Tierney* and C.S. Thompson. Department of Zoology, University of Toronto, Toronto, Canada M5S 1A1.

Our experiments demonstrate that chemical cues are important in sex and species recognition in the crayfish Orconectes propinquus. Males and females perceive the chemicals released from their own and a sympatric species O. virilis, and are attracted only to the chemicals of conspecifics of the opposite sex (Tierney and Dunham, J. Crustacean Biol., 2:544-548, 1982). This response is mediated by the lateral antennular flagella. Experiments were performed in which the medial or lateral antennules were bilaterally ablated from male crayfish. In "medial ablation" animals, behavioral responses to female pheromones were unchanged relative to controls (response reduced 4.7%). However, a highly significant ($P < .005$) reduction in response occurred in "lateral ablation" animals (response reduced 92% relative to controls).

Scanning electron microscopy was used to examine the medial and lateral antennules to identify potentially chemoreceptive sensilla. The flagella are approximately 10 mm long and composed of 25-30 segments. Common to both antennules are rows of feather-shaped setae situated along the distal margin of each segment. These setae are abundant on proximal segments and gradually become more sparse distally. An additional setal type occurs only on the lateral antennule. These setae consist of clumps of 6-9 hairs located ventrally and are present only on the 10 or 11 most distal segments. Two separate hair clumps occur on each segment. The hairs are directed distally and are approximately 150 μm long. The similarity of these structures to other identified arthropod chemoreceptors and their presence on the lateral, but not the medial, flagella suggest that these structures function in pheromone reception.

Extracellular recordings from antennular nerve bundles indicate a possible mechanosensory function for the feather-shaped setae. Axons from the medial antennule fire in response to vibration, touch and deflection. Axons from the lateral antennule respond to similar stimuli, but have a higher response threshold and action potentials are of a consistently lower amplitude. We suggest that the medial antennules are primarily mechanoreceptor structures, while the lateral antennules function primarily as chemoreceptors, and secondarily as mechanoreceptors.

This study was supported by an operating grant to D.W. Dunham from The Natural Sciences and Engineering Research Council of Canada.

66.5 NEUROTRANSMITTER HISTOCHEMISTRY OF NEURONS IN THE ANTENNAL LOBES OF *MANDUCA SEXTA*. S.G. Hoskins and J.G. Hildebrand. Dept. of Biological Sci., Columbia Univ., New York, NY 10027.

In each antennal lobe (AL) in the brain of the moth *Manduca sexta*, several hundred neurons have their somata in 3 superficial clusters (lateral, medial and anteroventral) bordering on a neuropil in which the dendrites of the AL neurons interact synaptically with primary-afferent axons and with each other in glomeruli. The AL neurons have been classified as multiglomerular local interneurons (confined to the AL) and uniglomerular output neurons (which send axons into the protocerebrum) [Matsumoto and Hildebrand, *Proc. Roy. Soc. Lond. B213*:249 (1981)]. Previous neurochemical studies [Maxwell et al., *Comp. Biochem. Physiol. 61C*:109 (1978)] called attention to neurotransmitter candidates in the ALs. We have now begun to use histochemical methods to explore the cellular locations of some of those candidates. In preliminary experiments we have sought neurons that may use acetylcholine (ACh) and serotonin (5-hydroxytryptamine, 5HT) as neurotransmitters.

To reveal possibly cholinergic neurons, we use an enzyme-histochemical method that stains neurons having high levels of intracellular acetylcholinesterase (AChE) activity as specifically shown for known cholinergic neurons in the CNS of the leech [Wallace and Gillon, *J. Neurosci. 2*:1108 (1982)]. We incubate desheathed brains of pharate-adult *Manduca* in a solution of echothiophate iodide to inhibit extracellular AChE irreversibly and then reveal uninhibited intracellular AChE activity by a histochemical procedure. In the AL only a small group of neurons at the ventrolateral border of the lateral cell cluster stain under these conditions. These neurons appear to have uniglomerular dendritic arborizations near the lateral cluster and axons that project to the lateral protocerebrum. As part of our studies of the role of afferent inputs in the development of the ALs, we have also found that output neurons which stain for intracellular AChE activity develop in the stunted, chronically deantennated AL that forms after excision, prior to metamorphosis, of the ipsilateral antennal imaginal disk.

To stain putatively serotonergic neurons, we use antibodies directed against a 5HT-albumin complex (Immuno Nuclear) in immunohistochemical experiments based on a modification of the wholemount method of Bishop and O'Shea [*J. Comp. Neurol. 207*:223 (1982)]. At least 6-10 neurons in the anteroventral cluster of the AL are immunoreactive, suggesting that those neutral-red staining, uniglomerular output neurons [Matsumoto and Hildebrand, *op. cit.*] may be serotonergic.

Thus, we have obtained evidence that 2 different types of AL output neurons may use different neurotransmitters -- ACh and 5HT.

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66.6 PROJECTIONS OF ANTENNAL-LOBE OUTPUT NEURONS IN THE BRAIN OF *MANDUCA SEXTA*. R.A. Montague*, K.S. Kent*, M.T. Imperato*and J.G. Hildebrand. Dept. of Biol. Sci., Columbia Univ., New York, NY 10027.

Each antennal lobe (AL) in the brain of the moth *Manduca sexta* possesses several hundred neurons, which have their cell bodies in 3 clusters (medial, lateral, anteroventral) and dendrites ramifying in the glomeruli of the AL neuropil, and which have been classified as local interneurons and output neurons (ONs) [Matsumoto and Hildebrand, *Proc. Roy. Soc. Lond B213*:249 (1981)]. ONs are projection neurons with axons extending into the protocerebrum and dendritic arborizations confined to one of the AL glomeruli. Based on the form and location of their glomerular arborizations, Matsumoto and Hildebrand described 3 major types of ONs, 2 of which have cell bodies in the lateral (LC) and medial (MC) clusters of the AL. A Type-I cell (ONI) has its dendritic arborization in a single ordinary glomerulus. Type-III cells (ONIII) are sexually dimorphic, having dendrites exclusively in the male-specific macroglomerular complex in the AL. We have now examined in greater detail the morphology of ONI and ONIII cells by means of intracellular staining with cobaltous sulfide [according to Strausfeld and Hausen, *Proc. Roy. Soc. Lond. B199*:463 (1977)] followed by silver intensification in whole-mounts or sections. This approach has permitted us to describe 3 subtypes of ONI and ONIII neurons.

Neurons of the first subtype send axons out of the AL through a medial tract that joins the *tractus olfactorioglobularis*. These axons traverse the protocerebrum in a mediolateral direction, sending out collaterals that ramify in, and terminate mainly within the "lips" of, the calyces of the mushroom bodies and ultimately terminate in the lateral protocerebrum. All ONIII neurons of this kind have exhibited somata in the MC. Neurons of the second subtype have somata in the LC and send axons out of the AL through a different, more ventral tract to project directly (not via the calyces) to the lateral protocerebrum. The third subtype of neurons, also with somata in the LC, have axons that exit the AL in an even more ventral tract and, after entering the protocerebrum, make a "hairpin" turn and ramify diffusely in a region of neuropil rostral to the calyces.

These findings show that AL ONs, which transmit integrated olfactory information to the protocerebrum, do not necessarily project to the calyces, as has been widely believed, and suggest that higher-order processing of olfactory information involves at least 3 centers in the protocerebrum. We are currently examining ONs in chronically deafferented ALs and in ALs of moths with surgically grafted antennae of the opposite sex. Preliminary findings suggest that at least some ON axons (of the first subtype) have normal projections and patterns of termination in animals deantennated (by excision of an antennal imaginal disk) throughout adult development.

These studies have been supported by NIH grant AI-17711 and NSF grant BNS 80-13511.

66.7 INFLUENCES OF AFFERENT INNERVATION ON THE DEVELOPMENT OF AN IDENTIFIABLE GLOMERULUS IN THE ANTENNAL LOBE OF *MANDUCA SEXTA*. K.S. Kent* and J.G. Hildebrand (SPON: E. Holtzman). Dept. of Biol. Sci., Columbia Univ., New York, NY 10027.

A single, identifiable glomerulus in the posteroventral region of each antennal lobe (AL) of adult male and female *Manduca sexta* receives primary afferent inputs from sensory neurons in the ipsi- and contralateral labial-pit organs (LPOs) [Harrow et al., companion abstract] and possibly the ipsilateral antenna. As part of a study of the functional significance and development of the AL glomeruli, we have (a) followed the normal postembryonic development of the inputs from the LPOs to this unique glomerulus, (b) examined the development of this LPO-to-AL projection in ALs deprived of antennal inputs throughout adult development, and (c) studied the development of the AL and its "LPO glomerulus" (LPOG) when chronically deprived of LPO afferents. This system allows investigation of the roles of, and dependency upon, 3 distinct sources of sensory innervation in the development of their mutual target in the CNS.

Using anterograde filling of cut LPO fibers with a $CoCl_2$-lysine complex followed by precipitation of CoS and silver intensification, we observed the development of the central LPO projection beginning soon after initiation of adult development in the pupa. By day 4 of the 18-day adult development, a few fine LPO fibers are growing from the subesophageal ganglion toward the ipsilateral AL but have not reached it. A bundle of fine fibers enters the AL during the next 2 days, and by days 7-8 the LPO projections to both ALs attain adult-like appearance, defining the shape of their target glomeruli. Thus the LPO axons innervate the ALs about the time of emergence of glomeruli [Tolbert et al., *Soc. Neurosci. Abstr. 7*:3 (1981)]. In animals deantennated throughout adult development, an "LPOG" forms in the ipsilateral AL as a discrete, posteroventral region of the "protoglomeruli" (rudiments of glomeruli characteristic of deantennated ALs), and the stained LPO axons entering that AL describe a corresponding, restricted neuropil region possibly more extensive than the normal LPOG. Thus the antennal afferents are not required for the formation of an LPOG, but they may ordinarily influence its final form. Finally, to test for possible influences of the LPO fibers on the development of the AL, we amputated the distalmost segments of both labial palps at the time of pupation. Histological observations suggest that the LPO inputs do not significantly affect the general organization of the AL, the formation of most glomeruli, or the production of "protoglomeruli" in simultaneously deantennated ALs. But the development of the LPOG in the AL may depend on LPO afferents. Recently we have begun to examine the AL interneurons that have arborizations in the LPOG in order to learn how they contribute to the formation of the LPOG and how they are affected by chronic deprivation of LPO afferent inputs. (Supported by grants NSF BNS-80-13511 and NIH AI-17711.)

66.8 CENTRAL PROJECTIONS AND POSSIBLE CHEMOSENSORY FUNCTION OF NEURONS IN A SENSORY ORGAN ON THE LABIAL PALP OF *MANDUCA SEXTA*. I.D. Harrow, P. Quartararo*, K.S. Kent* and J.G. Hildebrand. Dept. of Biol. Sci., Columbia Univ., New York, NY 10027.

In moths like *Manduca sexta*, each of the labial palps, which are paired, modified mouthparts that embrace the coiled proboscis, possesses within its diminutive third, distalmost segment a sensory organ comprising a cavity open to the surface and a number of enclosed sensilla. This "labial pit organ" (LPO) was described in several insect species by O. vom Rath in 1886, who suggested that it is an olfactory organ, but little is known about its function. We have studied the structure and function of the LPO in *Manduca* by histological and physiological methods and the central projections of the axons emanating from it by anterograde filling of the cut axons with $CoCl_2$ complexed with lysine, followed by precipitation of CoS, silver intensification, and examination in cleared wholemounts or sections.

The LPO dominates the third segment of the labial palp. Within the cavity of the LPO and exposed to the environment through its anteriorly directed mouth are numerous sensilla, which resemble the *sensilla basiconica* of antennae. The sensory neurons of these sensilla send their axons through a small nerve that joins the first labial nerve (LN1) to the subesophageal ganglion (SEG). Cobalt-sulfide staining of a cut LN1 reveals profuse terminal processes and a few somata (presumably labial-palp motor neurons) in the SEG as well as a bilateral projection to one particular glomerulus in each antennal lobe (AL). When both LN1s are filled with cobalt, only that glomerulus in the AL receives stained fibers from both ipsi- and contralateral palps. If the third segment of the palp (and with it the LPO) is ablated several days prior to filling the corresponding LN1, then the pattern of stained fibers in the SEG is relatively normal while the projections to the ALs are absent. This finding suggests that the axons projecting bilaterally to the ALs are those of the LPO neurons. Further evidence is obtained by applying cobalt-lysine to the third segment of the palp to reveal the bilateral AL projections and only a few other terminal processes in the SEG. Histological sections and computer-graphic reconstruction of ALs show that the "LPO glomerulus" lies in the posteroventral region of the glomerular neuropil, adjacent to the lateral cluster of AL neuronal somata. Because the AL glomeruli in *Manduca* are principally sites of olfactory information processing, the projection of the LPO neurons to an AL glomerulus supports the hypothesis that these cells are olfactory receptors. We have begun to investigate the function of the LPO neurons by extracellular recording methods. Preliminary physiological evidence suggests that the LPO neurons do respond when presented with certain volatile chemical stimuli.

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66.9 COMPUTER RECONSTRUCTION OF ALL THE NEURONS IN THE OPTIC GANGLION OF DAPHNIA MAGNA. S.J. Sims* and E.R. Macagno. (SPON: K.J. Muller). Department of Biological Sciences, Columbia University, New York, NY 10027

There are two important reasons to undertake a detailed anatomical reconstruction of a neuronal network. One is that the data obtained, such as the branching patterns and distribution of synaptic contacts of each neuron, and the patterns of synaptic connections among the neurons of the network, can be essential to an understanding of the functions of each element within the network and of the network as a whole. The second reason is that detailed information about the structure of the end product tells us what requirements are placed upon the mechanisms responsible for the assembly of the network during neurogenesis. For these two reasons we have undertaken the computer-aided 3-D reconstruction from serial electron micrographs of the approximately 200 neurons in one half of the adult optic ganglion of the small crustacean Daphnia magna. The reconstructions were carried out using the Columbia CARTOS system (Macagno et al. (1979), Ann. Rev. Biophys. Bioeng. 8: 323-351). The Daphnia optic ganglion (OG) is bilaterally symmetric and consists of two regions, the lamina and the medulla. Photoreceptor axons enter the lamina through its distal surface and axons of ganglionic neurons exit proximally from the medulla and enter the supraesophageal ganglion (SEG).

The reconstructed neurons were classified into groups according to (1) which neuropils (lamina, medulla, SEG) their processes innervate and/or (2) the locations of their cell bodies. Individual neurons that innervate each neuropil alone or any combination of them were identified. Seven cells were found which branch only in the laminar neuropil and 17 cells which branch only in the medullary neuropil. Among cells that innervate two neuropils, we found: (a) 82 cells that branch in both laminar and medullary neuropils (55 with cell bodies on the distal surface of the lamina and 27 with cell bodies located at the lamina-medulla boundary); (b) 16 cells that branch in the laminar and SEG neuropils; and (c) 36 cells that branch in the medullary and SEG neuropils. We found also 18 interneurons that have branches in all three neuropils, and 17 which send major branches across the midplane and thus innervate neuropils on both right and left sides. Although cells were reconstructed in only one hemiganglion, inspection of the other half revealed similar cells in approximately mirror-symmetric locations. Within each of these groups, cells were found that branch locally, widely, or in different layers of the laminar and/or medullary neuropils. Current efforts are directed towards elucidating the synaptic connectivity patterns of cells in each of these groups.

66.10 TWO STAGES OF INTEGRATION IN A LEECH VISUAL INTERNEURON E.L. Peterson. Dept. Biology, McGill University, Montréal. H3A 1B1

The medicinal leech has 5 bilateral pairs of eyes each containing 30-50 photoreceptors. The axons of the photoreceptors of the most posterior 3 eyes on one side are electrically coupled to the ipsilateral LV cell, an interneuron in the first segmental ganglion. Light or injected current elicits a spike in the photoreceptor soma, an impulse in its axon in the optic nerve and a small (< 1 mV) EPSP in the LV cell. I blocked chemical synaptic transmission and spontaneous activity with 40 mM Mg saline, then stimulated axons in the optic nerve extracellularly to see how the response of the LV cell varied as a function of its direct input. Weak shocks produced a few individual EPSPs; slightly stronger shocks produced several EPSPs which summated linearly but never surpassed 2-3 mV. At a well-defined threshold a qualitatively different and much larger event (15 mV) appeared in the LV cell. As the stimulus strength was increased further the event grew by discrete steps until it reached 40 mV. The 40 mV event apparently represents the nonlinear summation of 5-10 components, each similar to the 15 mV event. These events arise in the CNS, not in the optic nerve. They are probably spikes that arise locally in individual dendrites but fail to invade the soma actively. Accordingly I call them d-spikes, while noting that they could arise in unidentified interneurons electrically coupled to the LV cell. D-spikes produced by shocking one nerve evidently have a distinct origin from those produced by shocking another nerve, and it is thus possible that the photoreceptors of different eyes contact distinct dendritic regions. Like conventional synaptic potentials d-spikes show temporal and spatial summation at the soma. In normal saline a burst of d-spikes raises the LV cell above threshold thus eliciting an action potential which propagates out the axon. Thus the LV cell has 2 stages of integration of excitatory input, one during which EPSPs summate to generate d-spikes, and the other when d-spikes summate to generate axon spikes. The propensity of an LV cell to produce d-spikes varies from preparation to preparation, and thus the first integration stage may be modulated to regulate the sensitivity of the LV cell. In addition there are inhibitory synaptic inputs to the LV cell which affect the second integration stage but not the first.

66.11 CIRCADIAN CHANGES IN THE FREQUENCY RESPONSE OF VISUAL CELLS IN THE LIMULUS COMPOUND EYE. G.H. Renninger, Biophysics Group, Univ. of Guelph, Guelph, Ontario N1G 2W1, Canada

The response to modulated light of optic nerve fibers from the compound eye of Limulus undergoes changes during the animal's circadian cycle, which is regulated by a clock located in the brain (Batra et al., Soc. Neurosci. Abstr. 7, p. 61, 1981).

Intracellular studies reported here, indicate that these changes in optic nerve response originate in the light-absorbing retinular cells of the photoreceptor unit. The eccentric cell, which receives input from the retinular cells and generates optic nerve impulses, appears to be unaffected by the circadian clock.

Responses of cells to sinusoidally modulated light and, in the case of the eccentric cells, to modulated injected current were observed by means of glass microelectrodes during the day and after the transition to the high-sensitivity nighttime state. These observations were made in intact animals in which subjective night occurred naturally. Light intensity was adjusted so that the mean response level of each cell at night was equal to the daytime level. Because of the increased sensitivity of the eye to light at night, this was achieved by attenuating the light. The average response of the eccentric cells to injected current was 15-20 impulses/s.

The amplitude and phase of the response of eccentric cells in situ to sinusoidally modulated current do not depend on circadian time, and they are in general accord with those found in excised eyes (Knight et al., J. Gen. Physiol. 56, pp. 421-437, 1970).

The response of the retinular cell to modulated light, however, does depend in a significant way on circadian time. During the day, the frequency response resembles the light-to-voltage transfer function reported for the excised eye. At night, the amplitude decreases and the phase lag increases more rapidly for frequencies above 1 Hz. The highest frequency used was 10 Hz. The maximum amplitude was achieved under these conditions at 4-6 Hz during the day and at 0.6-0.8 Hz at night. The increase in phase lag is consistent with an observed increase at night in the latency of the response to light onset. This may reflect in part an increased delay in the phototransduction process at night.
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66.12 IRON CONTAINING CELLS OF BEES. D. A. Kuterbach*, B. Walcott, and P. Brink, Dept. Anatomical Sciences, State University of New York, Stony Brook, N.Y. 11794.

Since intracellular iron granules have been found to be necessary for the magnetic field response of bacteria and since honey bees (Apis mellifera) respond behaviorally to magnetic fields and contain iron, we have been examining the cellular localization of iron in bees. We have previously reported (Science 218:695, 1982) that the oenocytes of mature honey bees which occur in sheets around each abdominal segment contain 0.5µm iron containing granules. These granules were not present in the newly emerged adult bee but rather develop over a ten day period. In a newly hatched bee there were no other tissues that show positive iron staining so that the iron probably comes from an external source.

Other Hymenoptera have also been examined. Both Bombus sp. (Bumble bee) and Scaptotrigona postica (Brazilian stingless bee) have oenocytes with iron positive staining granules as shown by the potassium ferrocyanide technique. In Bombus the granules were larger than Apis but by x-ray microanalysis have the same composition with large amounts of iron. These three species are social bees with a nest to which they return. Ceratina calcarala and Halictus ligatus however are not known to be social, yet they have the same diet as the other species. Adults of these species have oenocytes but are lacking the iron granules.

Physiological examination of sheets of Apis oenocytes was performed to determine if the cells were dye and/or electrically coupled. Isolated sheets of cells were pinned on sylgard and viewed with an inverted microscope. Visually identified cells were impaled with microelectrodes filled with 1% lucifer yellow-CH and the dye was electrophoretically injected (90 nA for 5 min.). The dye filled the impaled cell and spread to adjacent cells but did not fill the fat cells that occur among the oenocytes. In one hour the dye spread to a circular patch of cells with a radius of about 7 cells. Thus there appears to be extensive coupling between the iron containing oenocytes.
Supported by GM 28804 to BW.

66.13 THE EFFECTS OF BEHAVIORALLY RELEVANT TEMPERATURES ON THE NEUROPHYSIOLOGY OF GRASSHOPPER MECHANOSENSORY HAIRS. C.I. Miles* (SPON: J. Palka). Dept. of Zoology, Univ. Washington, Seattle, Wa. 98195.

Grasshopper mechanosensory hairs increase their firing frequency if given stronger stimuli at a constant temperature or if temperature is increased for the same strength stimulus. If the surface temperature of a part of the body changes, it is not obvious that the animal would be able to tell whether the change in firing frequency of the affected hairs is due to a different stimulus or simply to the change in temperature. Furthermore, in nature, microclimates can create steep thermal gradients over very short distances, so it is likely that different hairs will be operating at different temperatures at any given time.

I have monitored the temperatures of the head, thorax, abdomen and feet of freely moving Schistocerca americana placed in thermally heterogeneous environments in the laboratory. Thoracic temperature was found to be the least variable. Animals spent a majority of the time positioned where this was 35^{o}-40^{o}c. The other parts of the body varied around this range to different extents, with the head experiencing a somewhat wider variability and the feet the widest. Temperature differences ranging from 0.5^{o}-10^{o}c could be recorded between head and thorax. Even wider differences were found between thorax and feet.

Electrophysiological recordings were then made from mechanosensory hairs of the head, thorax and feet. The resopnse of an individual hair to a constant stimulus was recorded as the temperature of the cuticle was varied. The response vs. temperature curve for foot hairs is linear up to about 32^{o}c. From 32^{o}-40^{o}c, the foot hairs' responses are largely independent of temperature, and above 40^{o} the hairs' response decreases with further increases in temperature. The curves for thoracic hairs have about the same slope as the linear portion of the foot hair curves, but these continue to be linear through the full range of behaviorally relevant temperatures. Curves for the generally distributed head hairs are also linear, but with lesser slopes. The special fields of wind sensitive head hairs, however, have by far the highest sensitivity to temperature. The behavioral significance of this is being investigated.

Taking into account the range of temperatures which animals maintain when given the choice, then, the foot hairs, which see the widest variability within this range are the most strongly temperature compensated. Thoracic hairs, which are exposed to the least variability are much less compensated, while at least some head hairs are intermediate in both temperature variability and compensation. The neurophysiology of mechanosensory hairs can therefore be adapted to the kinds of temperature variations to which they are regularly exposed.

LIMBIC SYSTEM: HIPPOCAMPUS AND AMYGDALA

67.1 LIGHT AND ELECTRON MICROSCOPIC STUDIES OF RAT HIPPOCAMPAL CELLS CONTAINING CHOLECYSTOKININ IMMUNOREACTIVITY IN AREA CA1. P.E. Marshall*, K.M. Harris and D.M.D. Landis. Neurology Service, Massachusetts General Hospital, Boston, MA 02114

Within the rat hippocampus, cholecystokinin (CCK) immunoreactivity exists in cells whose shape and size correspond to interneurons visualized in Golgi impregnations. As part of our examination of synaptic structure in the hippocampus, we have used light and electron microscopic immunocytochemical methods to determine the synaptic interactions of cells containing CCK.

After aldehyde fixation, the hippocampus was dissected from adult male rats, and vibratome sections were reacted with antiserum to CCK-8 (Immunonuclear). Antibody binding was visualized with avidin-biotin-peroxidase techniques.

207 cells with extensive dendritic and axonal staining were identified in CA1, distributed throughout the septo-temporal axis. 11% were in the alveus and s. oriens; cells in the alveus were multipolar, and s. oriens cells tended to be fusiform. 25% were in the s. pyramidale, and appeared similar to basket cells. 46% were in the s. radiatum and s. lacunosum-moleculare, and 18% were found adjacent to the fissure, all of multipolar shape. These observations are similar to those of other investigators (e.g. J. Comp. Neurol. 203:335; Brain Res. 224:180).

In serial thin sections, cells containing cholecystokinin-like immunoreactivity in s. radiatum had invaginated nuclei and spine-poor proximal dendrites. Large, unstained, irregularly-shaped boutons containing pleomorphic synaptic vesicles formed symmetric synaptic junctions on the immunoreactive perikarya and proximal dendrites. CCK-containing axons formed symmetric synaptic junctions with large diameter, unstained dendrites in s. radiatum. The immunoreactive axons appeared to have pleomorphic synaptic vesicles, but few or no large granular vesicles. No clear instances of CCK-containing axons synapsing with spines have been identified in s. radiatum.

67.2 IMMUNOCYTOCHEMISTRY OF SOMATOSTATIN IN CA1 OF RABBIT HIPPOCAMPUS. D. D. Kunkel*, P. A. Schwartzkroin and A. E. Hendrickson (SPON: A. A. Ward, Jr.). Depts. of Neurological Surgery and Ophthalmology, University of Washington, Seattle, WA 98195.

The neuropeptide somatostatin (SRIF) has recently been localized in several areas of the rodent brain including the hippocampus. Electrophysiological studies of hippocampal pyramidal cell responsiveness to SRIF application have shown both excitatory and inhibitory effects of somatostatin. Utilizing intracellular recordings we have recently demonstrated SRIF excitatory actions on CA1 pyramidal cells (Mueller and Schwartzkroin, this meeting).

In order to study the anatomical correlates of this SRIF effect, light and electronmicroscopic immunocytochemical investigations were carried out on immature (8 day) and mature (30 day) rabbits. The peroxidase/antiperoxidase (PAP) antibody enzyme technique was used. Immunoreactive somatostatin (ISRIF) neurons were found localized in stratum oriens, particularly at the oriens-alveus border. These cells were most frequent in the CA1 subfield; ISRIF neurons were also found within the hilus of the dentate gyrus. ISRIF neurons, located in stratum oriens, are spindle-shaped, with a bipolar dendritic tree. Dendrites run parallel to stratum pyramidale and occasionally ascend into the pyramidal cell layer. Prominent ISRIF processes ascend through stratum pyramidale and proceed towards stratum lacunosum-moleculare. Here they turn and run parallel to the hippocampal fissure. A light immunoreactive band (presumably terminals) is evident within stratum pyramidale.

Electronmicroscopic immunocytochemistry of SRIF revealed highly immunoreactive fusiform somata in stratum oriens. ISRIF dendrites are aspinous and receive numerous synaptic inputs (asymmetric synaptic contacts). ISRIF axons are evident throughout the various laminae of the CA1 subfield; terminals have been found in stratum pyramidale, as well as in stratum lacumosum-moleculare.

From this investigation it is evident that SRIF neurons have a local circuit termination within the hippocampus, although extrinsic projections are possible. SRIF neurons may thus be a significant population of interneurons, the function of which is still unknown. Conceivably, these cells act as excitatory interneurons in local hippocampal circuitry.

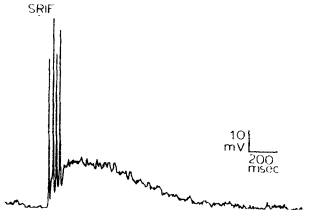
(Suported by NS 15317, NS 00413, NS 17111, and BNS 8209906)

67.3 ELECTROPHYSIOLOGICAL ACTIONS OF SOMATOSTATIN (SRIF) IN RABBIT HIPPOCAMPUS STUDIED IN VITRO. A.L. Mueller and P.A. Schwartzkroin. Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

Intracellular recording techniques were utilized in order to examine the electrophysiological actions of the peptide, somatostatin (somatotropin release inhibiting factor, SRIF), in area CA1 of hippocampal slices prepared from mature (age 1 month) and immature (age 6-10 days) rabbits. SRIF was applied locally to the somata of CA1 pyramidal neurons via pressure ejection (0.1 mM SRIF in 5 mM sodium acetate, pH 6.5; applied pressure 30 pounds per square inch; duration of ejection 20-150 msec; interejection interval 10-30 sec).

Application of SRIF typically evoked a robust depolarization of up to 20 mV amplitude, often associated with a burst of action potentials, in both mature and immature CA1 pyramidal neurons. This SRIF-evoked response appeared to undergo some type of rapid "desensitization," in that the magnitude of the depolarization sometimes decreased greatly from one ejection period to the next. No consistent changes in cellular input resistance were observed. Rarely, SRIF was seen to trigger an episode of spreading depression in immature tissue. Preliminary experiments suggest the SRIF-evoked depolarization persists in conditions in which synaptic transmission has been abolished.

We have recently demonstrated the existence of SRIF-containing neurons (interneurons?) in rabbit hippocampus (see Kunkel, Schwartzkroin and Hendrickson, this volume). Taken together, our results suggest that SRIF may function as an excitatory neurotransmitter in the hippocampus. One can speculate further that SRIF might be the transmitter of the proposed hippocampal excitatory interneuron.



Supported by grants NS 07012 (A.L.M.) and NS 15317, NS 00413, NS 17111 (P.A.S.).

67.4 RESPONSES OF BRAIN TISSUE TO GRADED HYPOXIA: A STUDY USING THE IN VITRO HIPPOCAMPAL SLICE PREPARATION. K. H. Reid, A. Schurr H. L. Edmonds, Jr. and B. M. Rigor, Cellular Neuroscience Lab, Departments of Anesthesiology and Physiology, University of Louisville, Louisville KY. 40292

In vivo, brain responses to hypoxia are a result of a complex interplay between tissue and cerebrovascular responses. The in vitro hippocampal slice preparation provides access to tissue responses in the absence of cerebrovascular changes, since this preparation has no blood supply. Hippocampal slices 400 μm thick were maintained in a linear-flow chamber (Haas et al, J. Neurosci. Meth. 1:323, 1979) with gas interface oxygenation. Oxygen tension was measured continuously in the gas phase close to the slice with a mass spectrometer. The CA1 population spike was used as an index of tissue electrical activity. Schaffer collateral stimulation (2x threshold, 0.1 msec, 1/min) evoked near-maximal (10-30 mV) population spike responses from the pyramidal cell layer. Slices were maintained in 95% O_2, 5% CO_2 until the population spike was stable in amplitude and latency. The gas was then changed to 20% O_2, 5% CO_2, 75% N_2, and responses followed for 20 minutes. The 95% O_2 5% CO_2 was then restored, and after 40-60 min of restabilization a second hypoxic challenge was given. Two distinct types of response to hypoxic challenge were found. The first was a sustained but reduced population spike with a 10-20% increase in latency. This was seen in 9/13 slices on the first exposure to hypoxia, and in 5/11 on the second exposure. The second was a rapid fall in amplitude followed by a recovery, during the hypoxic exposure, to 40-90% of control amplitude. This recovery persisted for 5-30 min before collapsing abruptly to electrical silence. This was seen in 2/13 slices on first exposure, and in 5/11 on the second exposure to hypoxia. On restoration of 95% O_2, the population spike returned, usually reaching control level within 20 min. Latency changes paralleled amplitude changes, but occurred more rapidly. Biochemical data (Duffy et al, J. Neurochem. 19:959, 1972) indicate that hypoxic neurons become electrically silent without loss of ATP. Our results suggest that episodes of controlled graded hypoxia may be useful in studying the transition to this 'dormant' state.

67.5 SEQUENTIAL ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE RAT HIPPOCAMPAL SLICE PREPARATION. A. Schurr, H. L. Edmonds Jr., K. H. Reid and M.T. Tseng* Cellular Neuroscience Lab, Depts of Anesthesiology, Physiology and Anatomy, University of Louisville, Louisville Kentucky 40292.

An essential element of any assay system is a knowledge of its stability. Although the in vitro hippocampal slice preparation is widely used in cellular neuroscience, precise data on its stability and viability are not available. The purpose of this study was to determine the viability of rat hippocampal slices in a linear flow chamber (H. Haas, et al., J. Neurosci. Meth. 1:323, 1979), with a small volume, gas interface oxygenation, and a bathing fluid flow of 0.5 ml/min. The experiment involved two phases. The first explored the properties of the CA1 population response, to identify those which could predict changes in tissue viability. Properties selected were population spike latency, amplitude of rising phase, slope of rising phase, duration of spike latency, amplitude of falling phase, slope of falling phase, and amplitude of the EPSP. The second phase was confirmatory: in 22 experiments responses were recorded automatically every 10 min until complete electrical failure occurred. To test for predictive efficacy (utility as a predictor of impending electrical failure), measures of each property were averaged over a 3 hr period, and successive values compared with the average. Significant deviations ($P<.001$) in any of the measures were detected, and records containing them were not included in the running average. The distribution of significant deviations was used to rank properties in order of predictive efficacy. Lifetimes ranged from 6-19 hr (11.7 ± 4.0, N=22). The property most reliably predicting slice deterioration was the latency of the population spike. Amplitude measures were less stable and slope measures were too unstable to be useful. No specific combination of signal properties was a better predictor than latency alone. In summary, the hippocampal slice preparation, in our hands, is stable for a minimum of 6 hr (90% confidence). Companion morphological studies indicate that structural integrity is preserved at 6 hr. The best single predictor of impending electrical failure is a significant increase in the latency of the population spike.

67.6 SEQUENTIAL ULTRASTRUCTURAL CHARACTERIZATION OF RAT HIPPOCAMPAL SLICES, IN VITRO M. T. Tseng*, K. H. Reid, A. Schurr and H. L. Edmonds, Jr., Depts of Anatomy, Physiology and Anesthesiology, University of Louisville, Louisville, Kentucky 40292

Functional slices of hippocampal formation, as indicated by their ability to respond to electrical stimulation, can be maintained for extended periods. To ascertain the structural integrity of the brain slices samples were removed at 0, 6 and 12 hr after incubation. A small piece of the slice that included stimulation and recording sites was fixed in glutaraldehyde and osmium tetroxide, dehydrated, embedded in plastic, sectioned at a plane parallel to the long axis of pyramidal cells, stained and examined. Pyramidal cells contained a large nucleus filled with finely dispersed chromatin and a prominent nucleolus. Cytoplasmic components included stacks of rough endoplasmic reticulum (RER), ovoid mitochondria, Golgi complexes, microtubules and microfiliments. Some of these cells were quite electron-opaque. Their densely packed chromatin suggested that they were less metabolically active. Microtubules and microfilaments were readily discerned in the apical dentrites. Small capillaries were found both in the neuropil and among the pyramidal cells. Following 6 hr incubation the general cytoarchitecture was well-preserved. Pyramidal cells remained tightly packed, though a slight increase in dark cell variant was evident. Nuclear envelopes were tightly adherent, mitochondria appeared normal and cisternae of the RER were within the normal range. Some disorganization of microtubules near origins of apical dendrites was apparent in most cells. In the neuropil, large clear perivascular spaces were occasionaly noted. By 12 hr, the majority of the pyramidal cell nuclei appeared pycnotic. Scattered microtubular bundles appeared in the electron-opaque cytoplasm. Some cells accumulated vacuoles. Among the neuropil, darkly stained neurites mingled with swollen dendrites. A marked increase in perivascular space was also evident. These ultrastructural analysis indicate that the in vitro hippocampal slice remains viable from 6 to 12 hr. Companion electrophysiological studies strongly support this conclusion.

67.7 FACTORS INFLUENCING THE FIRING PROBABILITY OF PYRAMIDAL NEURONS IN THE HIPPOCAMPAL SLICE. Anita A. Roth* and Raymond Dingledine, Dept. Pharmacology and Neurobiol. Curric., University of North Carolina, Chapel Hill, NC 27514.

Orthodromic electrical stimulation of fibers in stratum radiatum elicits a spatially distributed EPSP on pyramidal cell dendrites and also activates feed forward and feed back inhibitory circuits. We wished to determine the influence of spatial summation and inhibitory circuits on the firing probability of pyramidal cells in response to a single orthodromic stimulus. The dendritic field EPSP was recorded by an electrode placed in stratum radiatum, while an electrode placed in stratum pyramidale recorded the extracellular population spike or, in some experiments, intracellular potentials. Stimulating electrodes were placed in the stratum radiatum at different distances from the recording electrodes. Sigmoid input-output (I/O) curves were generated by plotting population spike amplitude as a function of the initial slope of the field EPSP. For a given field EPSP the amplitude of the population spike was taken as a measure of the firing probability of the pyramidal cell population. Drugs were applied by perfusion and drug effects determined by calculating the lateral shift of the I/O curve (J. Pharmacol. Exp. Ther. 233:502, 1982). For a given size field EPSP a stimulating electrode placed within 200 µm of the recording array was usually less effective in activating the pyramidal cell population than a stimulating electrode placed more than approximately 800 µm away. Thus the I/O curve generated with the near stimulating electorde lay to the right of the I/O curve generated with the far electrode. A laminar analysis of EPSP magnitude showed that the spatial distribution of EPSPs was usually somewhat wider for the far than for the near stimulating electrode, which could partially account for the difference in lateral position of I/O curves. However, a difference in the I/O curves was still obvious even when a microlesion restricted spatial divergence. For a given extracellular EPSP the intracellular EPSP was larger for the far than for the near stimulating site. Morphine, pentobarbital and penicillin had a larger effect on the near than on the far I/O curve. Morphine (20 µM) shifted the near curve to the left. Pentobarbital (0.3-0.5 mM), when it had an effect shifted the near I/O curve to the right, while penicillin (0.5-1.0 mM) shifted the near curve to the left. In summary, both spatial summation of EPSPs and feedforward inhibition influence the position of I/O curves. The selective drug effects on the I/O curve generated by the near stimulating electrode suggest a spatially restricted form of inhibition that is GABAergic and morphine sensitive. This form of inhibition acts to reduce the ability of an orthodromic stimulus to activate pyramidal cells. Supported by DA-02360 and NS-17771.

67.8 INTRACELLULAR FILLING OF RAT DENTATE GRANULE CELLS WITH HRP: ANALYSIS OF MOSSY FIBER COLLATERALS. B.J. Claiborne, D.G. Amaral, and W.M. Cowan. The Salk Institute, La Jolla, CA 92037

It has been known for some time that the axons of the dentate granule cells - the so-called mossy fibers - collateralize immediately below the granule cell layer. It has been difficult, however, to determine the extent and distribution of these collaterals in Golgi-stained material. We have begun, therefore, to investigate mossy fiber structure using in vitro slice preparations and intracellular HRP filling. We have determined thus far that individual mossy fibers give rise to an extensive network of collaterals which extend well beyond the subgranular region and, in many cases, ramify throughout the hilar region of the dentate gyrus.

Transverse slices (350 µm thick) of the hippocampal formation, taken from 35 to 50 day-old rats, were maintained with oxygenated saline in a recording chamber. Electrodes filled with 2% HRP in Tris/KCl were used to impale granule cells generally near the middle of the slice. HRP was injected with depolarizing current pulses (3nA, 250 ms, 2/s for 5 to 10 min) into those neurons that exhibited stable resting potentials of at least - 45 mV. After a further incubation of 2 to 5 hours, the slices were fixed overnight in 1% paraformaldehyde, and 2% glutaraldehyde in 0.1 M phosphate buffer, rinsed briefly, and then reacted with DAB. To facilitate DAB penetration into the 350 µm slices, they were first placed in 1% Triton-X for 1 hr and then preincubated in DAB for 1 hr before reaction with DAB and H_2O_2 for 30 min. Slices were cleared and mounted in glycerol. The filled neurons were examined using brightfield optics and subsequently reconstructed in three dimensions using a Zeiss Universal microscope that is interfaced with a PDP 11-03 computer. Most of the neurons which have been analyzed were located in the suprapyramidal blade of the dentate gyrus, but neurons in the infrapyramidal blade which have been examined appear to have a similar pattern of collateralization.

Each mossy fiber has about 6 or 7 collaterals which are of the order of 0.3 µm in diameter. They are variable in length, but many are as long as 500 µm, and occasional collaterals extend across the entire hilus even reaching the subgranular region of the opposite blade. In a few cases, collaterals have been followed into the granule cell layer, but none have been seen to enter the molecular layer. The majority of the stained collaterals have been found to be contained within the 350 µm slice, but, in a few cases, they extended beyond the borders of the slice. Each collateral has a number of small, rounded varicosities distributed unevenly along its length, and most collaterals have 1, or occasionally 2, larger, irregularly shaped varicosities which resemble mossy fiber expansions in all respects except size; the collateral expansions are only about 2 µm in diameter whereas the mossy fiber expansions, found in the stratum lucidum of field CA3, are between 4 and 8 µm in diameter.

67.9 TOPOGRAPHY OF LATERAL ENTORHINAL NEURONS LABELED FROM SEPTAL AND TEMPORAL DENTATE GYRUS. M.L. Miller*, J.H. Haring and J.N. Davis (Spon: E. Busse). Departments of Medicine (Neurology), Pharmacology and Pathology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have been interested in differences between the afferents to the dorsal and ventral hippocampus. During studies with horseradish peroxidase (HRP), we noted a difference in the pattern of labeling in lamina II of lateral entorhinal cortex (LEC) after injections of each locus. Injections of HRP or WGA-HRP (50-100 µl) were made in either the septal or temporal dentate gyrus of Sprague-Dawley rats (175-225 g). Septal injections were generally well localized in the dentate gyrus and produced labeling in the dorsal part of LEC. LEC neurons in lamina II immediately below the rhinal fissure contained light to moderate amounts of HRP. No labeling was seen in more ventral parts of LEC. Temporal dentate injections were less focal than dorsal injections since the injection traversed a considerable septo-temporal distance and spread more readily into the hippocampus as shown by labeling of lamina III neurons in LEC. Temporal dentate gyrus injections yielded heavily labeled LEC neurons in the ventral two-thirds of lamina II. A few LEC neurons of the dorsal third contained small numbers of HRP granules. This pattern was present at all rostrocaudal LEC levels; no heavily labeled neurons were observed in the region adjacent to the rhinal fissure.

These data show a topographic distribution of LEC axons to the dentate gyrus with septal HRP injections labeling lamina II neurons adjacent to the rhinal fissure while temporal injections heavily labeled more ventral parts of lamina II. The observed differences in both density and intensity of labeling from each locus are most likely attributable to the larger volume of tissue exposed to tracer in the temporal injection cases. The labeling observed in dorsal LEC neurons after temporal injections probably reflects uptake by fibers of passage. These observations of differential LEC labeling from dorsal and ventral dentate gyrus extend the previous observations of lamina II labeling seen after dentate injections at a single septotemporal level. Moreover our data confirm the results of an anterograde degeneration study which suggested a similar spatial arrangement for LEC afferents to the septal and temporal parts of the dentate gyrus. These data together with our previous studies of brainstem afferents emphasize the differences in inputs to the septal and temporal dentate gyrus of the hippocampal formation.

Supported by NS 06233.

67.10 NON-LINEAR SYSTEMS ANALYSIS OF PERFORANT PATH-TO-DENTATE SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS. T.W. Berger, J.L. Eriksson*, D.A. Ciarolla* and R.J. Sclabassi. Depts. of Psychology, Psychiatry and Neurosurgery (R.J.S.), University of Pittsburgh, Pittsburgh, PA 15260.

Characteristics of synaptic transmission in the entorhinal-to-dentate system were examined in New Zealand white rabbits anesthetized with halothane. A pseudorandom series of intervals of electrical stimulation pulses (0.1 msec duration) was applied to the perforant path, and extracellular field potential responses were recorded from the granule cell body region of the dentate gyrus. The stimulation intervals consisted of a Poisson distribution with a mean frequency of 2Hz and a range of 0.2-1K Hz. Stimulation current remained constant at a level just exceeding threshold for a population spike. A total of 4064 pulses was applied in trains of approximately 500 pulses each. Using amplitude of the field potential population spike as the output measure, first-, second- and third-order kernels were computed.

Significant second-order interactions were found that are consistent with what has been described with the use of twin-pulse stimulations of the perforant path. Specifically, stimulation intervals of 10-30 msec resulted in complete or near-complete suppression of the population spike. This high frequency (30-100 Hz) stimulation-induced inhibition decreased at frequencies less than 30 Hz and was typically non-existent at 20 Hz. Stimulation frequencies ranging from less than 20 Hz to typically 3-4 Hz resulted in facilitation of the population spike. This facilitation was greatest at 10-15 Hz and resulted in an augmentation of spike amplitude of 100-200%. In some experiments facilitation was also seen at 4-5 Hz (i.e., the second-order kernel was bimodal in the 3-20 Hz range), though the magnitude of the secondary facilitation was considerably less. At frequencies less than 3 Hz, a depression in population spike amplitude was almost always seen. The depression was on the order of 20-30%, and was observed at frequencies ranging from 1.25-3 Hz. Significant third-order interactions were also found. Computation of third-order kernels revealed that a given stimulation pulse resulted in a population spike amplitude that could not be accounted for solely in terms of the sum of the first- and second-order interactions of the intervals of the two pulses preceding it. For example, within certain frequency ranges, amplitude of the population spike was determined not only by the immediate and preceding frequencies of stimulation, but also by the direction of the change in those frequencies (i.e., lower to higher vs. higher to lower stimulation frequencies).

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67.11 SINUSOIDAL FLUCTUATIONS IN THE THICKNESS OF RAT DENTATE GYRUS AFFERENT LAMINAE. M. A. King, R. L. Reep, B. E. Hunter, D. W. Walker. Department of Neuroscience, University of Florida College of Medicine, and VAMC, Gainesville, FL 32610.

The rat dentate gyrus is frequently used as a model system in investigations of CNS neuroplasticity and toxicity phenomena. Discretely staining bands in sections prepared for Timm's metal sulfide-silver or acetylcholinesterase histochemistry correspond to the segregated axon terminal fields of the major afferents to the granule cells. In principle, the width of these bands can be quantified and used in between-subjects designs to assess the effects of various independent variables on the pattern of afferentation. However, protocols for matching tissue sections, determining sampling frequency, and consistently applying the measurement technique are often based not on empirical anatomical foundations, but upon assumptions that 1)qualitative, visual section matching accurately selects equivalent regions for comparison, and 2)the stain bands parallel the granule cell body layer. In the course of quantitative experiments to determine the effects of chronic ethanol consumption on dentate reactive synaptogenesis, we found that neither assumption is strictly valid. By comparing the relative distance between 7 discrete intra- and extrahippocampal anatomical features in coronal sections through the brains of 47 adult male Long Evans rats, 2 landmarks were chosen to define a region of the dorsal hippocampal formation with minimum interanimal variability. Between the sections containing the landmarks, 10 equally spaced sections were selected for band width measurements. With the aid of a Spatial Data Systems EyeCom II image analysis system interfaced to a MINC 11/23 minicomputer, Timm's stain band widths were measured automatically at 10 equally spaced medial-lateral positions, for each blade of the right dentate gyrus, for each of the 10 sections. Analysis of this 10x10 array of values revealed an unexpected and fascinating pattern in the architecture of the afferent terminal fields: sinusoidal fluctuations in both the M-L and A-P directions contribute to the formation of a series of peaks and troughs in the three dimensional structure, relative to the granule cell layer. The fluctuation in any one band appears to be independent of the others, although a similar "egg carton" effect is seen for total molecular layer width, and the pattern cannot be attributed to blood vessels, histological artifact, or measurement error. This pattern emphasizes the need to consider carefully the fine structure of the dentate gyrus when planning quantitative methods, and raises important questions about the effects of this organization on dendritic input integration and the mechanisms by which this pattern of afferent targeting and distribution might occur.

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67.12 COLCHICINE IS INEFFECTIVE IN DESTROYING DENTATE GRANULE CELLS IN RHESUS MONKEYS. L. Ramirez* and R. Dasheiff (SPON: M. Javid). Division of Neurosurgery and Department of Neurology, University of Wisconsin and VA Hospitals, Madison, WI 53705.

Colchicine is a potent neurotoxin to granule cells of the dentate gyrus in rodents (Goldschmidt and Steward, PNAS 77:3047, 1980 [rats]; McMartin and Schedlbauer, J. Neurobiol. 9:453, 1978 [mice]). Intradentate injection of 2.5 µg will result in cellular changes observable with Nissl stain by the first day. Larger doses and longer postinjection intervals will produce local and remote loss of dentate granule cells and destruction of surrounding pyramidal cells.

The use of colchicine as a selective neurotoxin was investigated in the primate macaca mulatta. Adolescent Rhesus monkeys, weighing 5 kg (1 M, 1 F) were given general anesthesia and placed in a stereotaxic frame. Drugs were dissolved in distilled water and sterilized by passing them through a 0.2-µ Millipore filter. The first subject had 50 µg of colchicine (5λ over 15 minutes) injected at each of four equidistant sites in the left hippocampus. The right hippocampus received 50 µg of DL-N-methylaspartate (NMA) at each site (5λ/15 min). The heart was perfused with formalin/saline 48 hours later and the brain sectioned with a freezing cryostat and stained with cresyl violet. Other than minimal trauma from the injecting needle, there was no evidence of damage from the colchicine. In contrast, the NMA caused local liquifaction necrosis.

The second monkey received 2 intrahippocampal and 2 intraventricular right-sided injections of colchicine, 200 µg in 20λ over 5 minutes. The contralateral side received distilled water. This subject died 5 days later. Autopsy was unremarkable. Both hippocampi showed no cellular damage except along the needle tract. There was a mild proliferation of polynucleated cells around some blood vessels and the periventricular space on the colchicine side. It is concluded that colchicine does not express the same neurotoxicity towards dentate granule cells in monkeys as it does in rodents.

67.13 THE DISTRIBUTION OF ACETYLCHOLINESTERASE IN THE HIPPOCAMPAL FORMATION OF THE MONKEY. I. Bakst* and D.G. Amaral, The Salk Institute, La Jolla, CA 92037.

As part of an ongoing series of studies of the structure and connections of the monkey hippocampal formation, we have stained serial coronal sections through the brains of six macaque monkeys for the demonstration of the enzyme acetylcholinesterase. The tissue was fixed either by 4% paraformaldehyde or by a mixture of paraformaldehyde and glutaraldehyde and the staining was carried out according to two variants of the Koelle, acetylthiocholine method. We have found that the pattern of staining differs in a number of respects from that seen in the rat. In the dentate gyrus, there is intense staining of the inner one third of the molecular layer, with much lighter staining in the rest of the molecular layer except for a thin band of moderate staining at its outer edge. In the caudal half of the dentate gyrus, the deeply stained inner one-third of the molecular layer appears to be further stratified; there is a distinctly denser band of staining just superficial to the granule cell layer. The granule cell layer is unstained but the hilar region is intensely stained at rostral levels where there are two distinct bands of staining, one, just subjacent to the granular layer, being especially intense. In the regio inferior of the hippocampus the stratum oriens is intensely stained, and some of the staining extends into the pyramidal cell layer; the stratum radiatum and the stratum lacunosum-moleculare are also stained and here the staining shows some degree of stratification. By contrast, the alveus, the pyramidal cell somata and the layer of mossy fibers are unstained. The region of field CA2 is especially heavily stained. The regio superior is lightly but uniformly stained in all layers except the alveus. The only staining in the subiculum is in the molecular layer at about the middle of its rostrocaudal extent. The outer cell dense layer of the presubiculum, is lightly stained and this is separated from the unstained molecular layer by a more heavily-stained band in the superficial rim of the cellular layer. All the layers of the parasubiculum show moderate staining but there are distinct, wedge-shaped areas that are devoid of stain at several levels. Layers 2 and 3 of the lateral entorhinal cortex are stained as are layers 2, 4, and 6 of the medial entorhinal cortex. In the lateral half of the medial entorhinal cortex layer 1 is also stained, and there is a tendency for the AChE to accumulate into small, wedge-shaped islands at intervals in layers 1 and 2 of the medial entorhinal cortex, especially at caudal levels.

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67.14 EFFECTS OF SEPTAL LESIONS AND SYMPATHETIC INGROWTH ON HIPPOCAMPAL AND PINEAL HISTAMINE LEVELS. E. Orr*, K. Pace*, J. Howard* and D. Barker. Depts. of Anatomy and Physiology, Texas College of Osteopathic Medicine and North Texas State University, Fort Worth, TX 76107.

Lesions of the dorsal pathway to the hippocampus (fimbria, fornix, cingulum) cause a 50-60% decrease in rat hippocampal histidine decarboxylase (HD) activity and histamine (Hm) levels. A similar decrease in hippocampal HD activity occurs after lesions of the medial forebrain bundle and fornix. This suggests that histaminergic input to the hippocampus from the brainstem is by way of either the postcommissural fornix or the septum. To determine which of these pathways is involved, we lesioned the septal area in rats and measured the change in hippocampal Hm levels. Since the septal ablation induces ingrowth of sympathetic fibers into the hippocampus and enhances sympathetic innervation of pineal, we also investigated the effects of ingrowth and no ingrowth on hippocampal and pineal Hm levels.

Subjects were 30 male hooded rats divided into the following groups: normal, normal+bilateral superior cervical ganglionectomy (Gx), septal lesions (lateral+medial) and septal lesions+Gx. Ablations of the septal area were of the size and location necessary for sympathetic ingrowth. Animals were sacrificed 4 weeks after surgery to allow for ingrowth, and the hippocampus and pineal were dissected for Hm analysis. Hm levels were determined radioenzymatically with labelled reaction products separated by thin-layer chromatography. Septal lesions did not affect hippocampal Hm levels. The levels (ng/g) were: normal=11.5 and septal=11.4. This suggests that histaminergic neurons reach the hippocampus via the postcommissural route. In both Gx groups, hippocampal Hm increased significantly: normal=57%, septal=43%. This effect may be due to changes in vascular Hm stores. Septal lesions decreased pineal Hm by 38%. However, pineal Hm was increased by Gx: 20% in normals and 42% in septals. We interpret this to mean that the degree of sympathopineal innervation is inversely related to pineal Hm levels.

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67.15 TOPOLOGICAL DISCRIMINATION: A BEHAVIORAL PARALLEL TO HIPPOCAMPAL UNIT ACTIVITIES. Lauren K. Gerbrandt[1], Richard M. Pico*[1,3], Gwen Ivey[2] and Joel L. Davis[3] 1)Dept. Psychology, Calif, State Univ., Northridge, CA 91324. 2)Dept. Psychobiology, Univ. Calif., Irvine, CA 92717. 3)Aging and Behav. Biol. Res. Lab., VA Med. Ctr, Sepulveda, CA 91343.

The hippocampus may be essential to the learning of configurations between directly perceived and other, potentially-perceivable,stimulus elements. In rats trained to discriminate reward locations solely in relationship to the topological pattern of four widely-separated extra-maze stimuli, hippocampal single-unit activities are readily observed that are unaffected by the deletion of any single stimulus from the topological cue set; furthermore, the presence of any two stimuli from the original cue set is usually sufficient to activate these units (O'Keefe & Conway, 1978). The objective of the following study is to determine whether similar, stimulus-independent, topological-cue effects can be observed at the behavioral level, using a stimulus-deletion methodology parallel to that used in these unit studies.

In the first phase of this experiment, sham-lesioned (S) and fimbria-fornix lesioned (FF) rats were trained extensively to discriminate 4 constantly-rewarded locations among 8 radial-maze arms. Reward locations were based solely on position in relationship to a constant extra-maze pattern of 4 stimulus elements (2 auditory, 2 visual). The radial maze was rotated in relationship to the topological-cue set after each of 8 daily choices; the cue set was rotated each day in relationship to the room environment. In the final of two 15-day blocks of trials testing the effectiveness of this pretraining, both S and FF groups performed virtually alike at better than 85% correct choices(relative to chance).

The effects of stimulus deletions (1,2,or 3 deletions) from the 4-cue set were then studied. Sham-lesioned rats were not affected by deletions of any 2 stimuli (leaving only a 2-cue set); fimbria-fornix lesioned rats fell to 48% correct choices using a 3-cue set and to chance levels with a 2-cue set. Sham-lesioned rats were suddenly unable to perform above chance using a 1-cue set. It is suggested that the capacity to behave in relationship to topological-cue sets is closely derived from the topological information provided by hippocampal pyramidal cells.

67.16 ARCHITECTONICS AND CONNECTIONS OF THE POSTERIOR PARAHIPPOCAMPAL GYRUS IN THE RHESUS MONKEY, D. L. Rosene and D. N. Pandya. Dept. of Anatomy, Boston University School of Medicine, Boston, MA and Bedford Veterans Administration Medical Center, Bedford, MA.

The posterior parahippocampal gyrus (PPG) in the monkey begins caudal to the rhinal sulcus, is bounded laterally by the occipitotemporal sulcus (OTS) and continues posteriorly as far as the rostral lip of the calcarine sulcus. In sharp contrast to the allo- and periallocortical characteristics of the anterior parahippocampal gyrus, the PPG is mainly composed of proisocortex and neocortex, areas designated as TH and TF by Bonin and Bailey. A reassessment of the cytoarchitectonics of the PPG has identified a more complex series of distinct cytoarchitectonic subdivisions. In the rostral part of the PPG, the presubiculum is bounded laterally by area TH, a proisocortex characterized by its lack of a granular layer IV, the heavy accumulation of pyramids in an undifferentiated layer V-VI and a homogeneous layer II-III. Area TH merges laterally with a second proisocortical area that we have designated area TL. This area is similar to TH except for the emergence of an incipient layer IV and the differentiation of layers II and III. Lateral to area TL, area TF occupies both banks of the OTS and has characteristics typical of neocortex, including a well-developed layer IV and the clear differentiation of layers V and VI. In the caudal part of the PPG, area TH is replaced by area prostriata, an agranular proisocortex that continues posteriorly into the depths of the calcarine sulcus. Area TL is replaced posteriorly by a region that we have designated area TLO since it has features that are intermediate between those of area TL and the caudally adjacent area OA (19).

Injections of radioactively labeled amino acids were placed into the PPG in order to investigate the efferent projections of these different areas. Our initial analysis has identified a number of features of these connections. First, areas TH, TL and TF each send efferents to the entorhinal and presubicular cortices but each projection terminates in a distinct subdivision with a unique laminar pattern. Second, areas TH and TL have strong reciprocal connections while area TF has only limited connections with area TL. Third, areas TH, TL, TLO and TF all project caudally to the ventral peristriate visual cortex and terminate in layer I of area OA and in clusters in area prostriata. Finally, the long corticocortical connections to the association areas of the superior temporal sulcus arise throughout all four areas but those to the association cortex of the posterior parietal lobe and the orbital and lateral prefrontal cortex as well as to the cingulate gyrus originate from only the most rostral parts of areas TH, TL and TF.

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67.17 EXCITATORY PROJECTION OF THE RAT SUBICULAR COMPLEX TO CINGULATE CORTEX AND SYNAPTIC INTEGRATION WITH THALAMIC AFFERENTS. David M. Finch, Edie L. Derian* and Thomas L. Babb. Reed Neurological Research Center and Brain Research Institute, University of California, Los Angeles, CA 90024.

Retrograde and anterograde tracing studies in rat using horseradish peroxidase-wheat germ agglutinin showed a projection from the subicular complex to layer II-III of the posterior cingulate cortex that rivaled afferents from the anterior thalamic group of nuclei (see also Meibach and Siegel, Exp. Neurol., 47 (1977) 264-274). Laminar analysis of field potentials showed that subicular stimulation evoked a negative-positive wave in layer V of the posterior cingulate cortex and a purely negative wave in layers I and II-III. If these potentials reflected active synaptic currents, then they indicate an excitatory-inhibitory sequence of activation in layer V cells and a source of synaptic excitation in or near layer II-III, where the subicular afferents terminate.

Intracellular recordings (with action potential amplitudes of greater than 40 mV) and "quasi-intracellular" recordings (with action potential amplitudes between 20 and 40 mV) from layer V cingulate neurons showed that electrical stimulation of the presubiculum or postsubiculum evoked EPSPs and action potentials. These were followed by shallow IPSPs lasting 20-200 msec. Frequency potentiation of the EPSPs was demonstrated in one case. The responsive cells had spikes and spontaneous discharge patterns that were similar in several respects to pyramidal cells of the hippocampal formation. Intracellular injection of HRP showed that the responsive cells were layer V pyramids. One cell with physiological properties thought to be associated with inhibitory interneurons was recorded in layer V.

Stimulation of the nucleus lateralis and nucleus anterior ventralis of the thalamus also evoked EPSPs and action potentials in layer V cingulate neurons. In one cell it was possible to show that EPSPs evoked by thalamic stimulation and by subicular stimulation summed.

Taken together with other published reports, these results indicate that subicular and thalamic afferents make excitatory synaptic contact onto different regions of the apical dendrites of the same layer V cingulate pyramids; that spatial summation integrates the input from these two sources; and that recurrent inhibition from local interneurons limits the duration of this excitatory influence.

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67.18 ELECTROPHYSIOLOGICAL CHARACTERISTICS OF AMYGDALOID CENTRAL NUCLEUS NEURONS IN THE AWAKE RABBIT. J.P.Pascoe* & B.S.Kapp (SPON: D.Lorenz). Dept. of Psychology, Univ. of Vermont, Burlington, VT 05405.

Recent investigations have indicated that the amygdaloid central nucleus (ACE) and its efferent projections to the brainstem may provide an important anatomical substrate for the mediation of forebrain contributions to cardiovascular/autonomic regulation, particularly during emotional states (Kapp et al., Brain Res., 234 1982; Schwaber et al., J. Neurosci., 2, 1982). Lacking, however, has been comprehensive data concerning the electrophysiological characteristics of ACE neurons. The present experiments were therefore performed in order to characterize the spontaneous activity and sensory-evoked responses of ACE neurons in the awake, restrained New Zealand rabbit, with particular attention given to those ACE neurons which project to the brainstem.

Extracellular single-unit recordings were obtained from 59 histologically verified ACE neurons. Brainstem projection neurons were antidromically identified by stimulating ACE descending fibers coursing through the mesencephalon. The cardiovascular effects of stimulation applied during surgery were used to assure accurate placement of the stimulating electrode in this pathway.

Twenty-four ACE neurons satisfied the usual criteria for antidromic activation, and exhibited a mean antidromic response latency of 9.2 ± 3.5 ms. These neurons exhibited infrequent (n=16, 0.4 ± 0.6 Hz) or virtually no (n=8, $<$ 0.01 Hz) spontaneous activity and were unresponsive to the presentation of simple visual, acoustic and somatic stimuli.

Additional ACE neurons did not meet the criteria for antidromic activation and conformed to one of five profiles: (a) Neurons exhibiting spontaneous activity and sensory characteristics comparable to antidromically activated neurons (n=18), (b) neurons exhibiting infrequent spontaneous activity (0.8 ± 0.8 Hz) and latent (200-250 ms) excitatory responses to the presentation of all sensory stimuli (n=4), (c) spontaneously active (15.2 ± 6.5 Hz) neurons exhibiting a comparatively rapid onset (50-60 ms) excitatory response to the presentation of all sensory stimuli (n=4), (d) spontaneously active (21.3 ± 6.8 Hz) neurons that were unresponsive to sensory stimuli (n=3), and (e) neurons exhibiting spontaneous rhythmical bursting activity (n=6); further analysis of three such neurons revealed that their spontaneous activity was entrained with the inspiratory phase of the respiratory cycle. Neurons exhibiting modality-specific or inhibitory responses to the presentation of sensory stimuli were not observed.

These data indicate the heterogeneous nature of ACE neuronal activity and provide a basis for evaluating the effects of experimental manipulations upon neuronal activity within this nucleus.

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67.19 A DIRECT PROJECTION FROM THE BASOLATERAL NUCLEUS OF THE AMYGDALA TO THE MOTOR AND SENSORY CORTICES IN THE RAT. K. Sripanidkulchai and J. M. Wyss, Dept. of Anatomy, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

The amygdala not only plays an important role in the processing of information within closely related limbic structures, but also participates more directly in sensory and motor system function. Several studies have demonstrated that direct ascending projections of this subcortical region, are directed at the ventral basal complex of the thalamus as well as the midline cortex and the lateral cortex immediately dorsal to the rhinal fissure. Projections from these cortical areas could provide a route for the basolateral nucleus to participate in these functions. Our present results, however, demonstrate that in addition to these indirect projections, a significant direct projection also exists. Injections of the retrograde fluorescent marker fast blue (50-100 nl of 4% in H_2O; 20 animals) or of horseradish peroxidase (10% in H_2O; 5 animals) were made into the lateral motor and sensory neocortex of adult, male, albino rats, via a 0.5 or 1.0µl Hamilton syringe. Following a 1-4 day survival, the animals were sacrificed by transcardial perfusion with saline and the appropriate fixatives. The brains were removed, sectioned at 25µm and a 1 in 6 series was mounted (following a tetramethyl benzidine reaction in the case of HRP injections). The basolateral nucleus was easily identified in adjacent Nissl and AChE stained sections. Cortical regions were identified on the basis of cytoarchitectonics, selective thalamus labeling and in the case of the motor cortex, backfilling of the layer five corticospinal neurons by injections of nuclear yellow into the lateral spinal cord at lower cervical levels. The results clearly demonstrate a large number of neurons within the basolateral nucleus are labeled by the injections into either the motor cortex or somatosensory cortex. These neurons were typically scattered throughout this nucleus but cells outside of the nucleus, in other divisions of the amygdala were not labeled. Injections into the lateral cortex immediately dorsal to the rhinal fissure also resulted in a similar labeling pattern in the basolateral nucleus; however, midline cortex injections did not result in extensive labeling in the amygdala. Posterior cingulate cortex injections resulted in no labeling of cells in the basolateral nucleus, whereas anterior cingulate cortex injections resulted in consistant labeling of only a few neurons in this region. The direct projection of the basolateral nucleus to primary motor cortex is quite intriguing in light of our recent demonstration that the lateral entorhinal cortex projects to this nucleus. Thus this nucleus may provide a rather direct route for hippocampal modulation of motor output.

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67.20 PROJECTIONS FROM THE AMYGDALOID COMPLEX TO MAGNOCELLULAR NUCLEI OF THE BASAL FOREBRAIN IN THE MONKEY (MACACA FASCICULARIS). F.T. Russchen*, D.G. Amaral and J.L. Price. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110; The Salk Institute, LaJolla, CA.

Anterograde tracing experiments in which ^{3}H-amino acids were injected into the various nuclei of the amygdaloid complex indicate that virtually the whole extent of this complex gives rise to fibers which reach the nucleus basalis and other magnocellular nuclei of the basal forebrain. Different amygdaloid nuclei project most heavily to different portions of this area. The most extensive projections originate in the basal, basal accessory and central amygdaloid nuclei.

To determine more precisely which nuclei of the amygdaloid complex give rise to these fibers, injections of WGA-HRP were placed in several locations in the magnocellular basal forebrain. Retrogradely labeled neurons were present throughout the amygdala, but in all cases the highest density of labeled cells was found in the parvicellular part of the basal nucleus, the magnocellular part of the accessory basal nucleus, and adjacent parts of the cortical nuclei and periamygdaloid cortex. Substantial numbers of labeled cells were also found in the central nucleus and in the medial part of the lateral nucleus. Few retrogradely labeled cells could be detected in the magnocellular part of the basal nucleus, the parvicellular part of the accessory basal nucleus, and the medial nucleus. Surrounding the amygdaloid complex there were high densities of labeled cells in the deep layers of the entorhinal and perirhinal cortices. It could also be determined from these experiments that the pattern of anterograde axonal labeling in the amygdaloid complex largely coincides with the distribution pattern of fibers staining for AChE activity. The heaviest labeling is in the basal nucleus.

In an attempt to investigate more closely the appearance of amygdaloid fibers reaching the magnocellular basal forebrain, injections of the lectin PHA-L were placed in the amygdaloid complex of rats and monkeys. In these brains anterogradely labeled fibers, identified immunohistochemically, were found among the large cells of the basal forebrain. These fibers showed varicosities along their length which resemble synaptic boutons. This suggests that although these fibers may also project to other terminal fields, along their course they interact with neurons in the magnocellular basal forebrain.

67.21 THE AMYGDALA: A CONTRIBUTION TO REGULATION OF AGGRESSIVE BEHAVIOR IN DIFFERENT STATES OF CONSCIOUSNESS J. Zagrodzka*, C.E.Washington*, A.R. Morrison. Nencki Institiute of Experimental Biology, Warsaw, Poland and School of Veterinary Medicine, University of Pennsylvania, USA.

Small, bilateral pontine tegmental lesions induce paradoxical sleep (PS) without atonia in cats. (Hendricks et al,'83). Instead of the usual paralysis in PS cats can right themselves, stand and walk. Lesions that unilaterally involved the fibers descending from the central nucleus of the amygdala (CA) to the lateral pons (Hopkins & Holstege,'78) led to aggressive displays during PS. Earlier, Wood('58) had found that lesions in CA evoked aggressiveness in cats. We examined whether removal of a modulatory influence from CA might be responsible for attack behavior during PS without atonia. 12 cats received combined amygdala-pontine or pontine-amygdala lesions, and their sleep and social behavior, i.e. interactions with a conspecific, an experimenter and predation on mice were observed and videotaped. Usually sleep behavior was monitored electrographically. None of 7 lesions placed bilaterally in CA, in the basal nucleus or outside the amygdala produced any changes in aggressive behavior. Lesions confined strictly to the unilateral CA released aggressive behavior toward other cats and increased irritability on handling approximately 3 days postoperatively for a period of 2-3 weeks, while pontine lesion alone produced attack behavior 2-4 weeks after surgery. No changes in predation were observed. Amygdala lesions alone did not alter PS. Pontine lesions added to those in CA at least a month later released aggressive behavior during PS without atonia that would not have been released after such pontine lesions alone. Moreover, the attack behavior, especially biting, in PS after combined lesions was more pronounced and vigorous than after pontine lesion alone. In 2 cases with pontine lesions first, which produced non-aggresssive PS without atonia, CA lesions added later evoked attack behavior and biting during PS. These data indicate that unilateral lesion of CA releases aggressive behavior and that such lesions potentiate the pontine lesion effect. We suggest that besides the system inducing atonia of PS, pontine lesions also damage a system normally modulating aggressive behavior. One source of fibers in this system is CA, especially those damaged that result in released biting in PS. Furthermore, this study, employing small, selective lesions adds to those few others that have demonstrated heightened rather than reduced aggression after amygdala damage.

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67.22 EFFECTS OF AMYGDALOID SEIZURES UPON AGGRESSIVE BEHAVIOR IN THE CAT. M.B. Shaikh*, M. Brutus, H. Edinger and A. Siegel. Dept. of Neurosciences and Dept. of Physiology, University of Medicine and Dentistry of New Jersey, NJ Medical School, Newark, NJ 07103.

That the amygdala plays a central role in the control of aggressive behaviors has been well documented in the literature. Perhaps the clearest demonstration of amygdaloid involvement in hypothalamically elicited aggressive behavior was provided by Egger and Flynn (1963). In a recent analysis, Block et al. (1980), demonstrated that lateral and central amygdaloid nuclei facilitate attack behavior upon electrical stimulation while stimulation of basal, cortical and anterior amygdaloid nuclei suppressed this response.

The purpose of the present study was to examine the effects of amygdaloid seizures upon two forms of aggressive behavior elicited from the hypothalamus in the cat--quiet biting attack and affective defense. Electrodes for stimulation and recording were stereotaxically implanted into the medial and lateral aspects of hypothalamus and amygdala. Initially, threshold values for each form of attack elicited by hypothalamic stimulation were determined over a period of 5-14 days. Then, sites in the amygdala were selected which significantly modulated at least one form of aggression. The experimental paradigm included alternate trials in which seizures were elicited from amygdaloid modulating sites and were followed by a determination of the behavioral threshold. Our results have suggested that the induction of amygdaloid seizures can systematically modify attack thresholds. Concerning affective defense behavior, amygdaloid seizures generated from sites which facilitated this response were followed by a reduction in the behavioral threshold while an elevation in threshold occurred when seizures were generated from sites in the amygdala which suppressed this response. In contrast, an opposing pattern was observed for quiet biting attack. Seizures generated from sites which suppressed quiet attack were followed by a lowering of threshold for this response, while an increase in quiet attack thresholds followed seizures from electrode sites linked to facilitation of quiet attack behavior.

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68.1 SPECIFIC INTERACTIONS OF DIFFERENT SENSORY MODALITIES IN THE PARAFLOCCULUS OF THE RAT. S. Ausim Azizi and Donald J. Woodward Dept. Cell Biology, Univ. of Texas Health Science Center at Dallas, TX 75235

Previous anatomical and electrophysiological studies from this laboratory have demonstrated the existence of visual and auditory cortical inputs to the paraflocculus of the cerebellum.

It was also shown that natural visual and auditory stimuli converge upon single Purkinje cells in the paraflocculus. In this report we describe data indicating that the convergence and subsequent facilitation of spike activity of the parafloccular neurons by the combined auditory and visual inputs is specific to these two modalities and that these inputs may originate mainly in the sensory association cortices.

Recordings were made from the parafloccular neurons of unanesthetized immobilized rats. Visual, auditory and somatosensory stimuli were presented with the use of a computer based system. Retrograde transport of horseradish peroxidase (HRP) and nuclear yellow was used to identify corticopontine projection neurons. These stimuli were moving light bars at specific orientations in the visual field, pure tones (sinusoidal waves 200 Hz to 20 kHz, 65 - 80 db intensity) and light electrical shocks to the forepaw.

Single unit recordings from 32 Purkinje cells and other neuronal elements showed evidence, through histogram analysis, for excitatory and/or inhibitory mossy fiber and climbing fiber inputs following either auditory or visual stimuli but not to electrical shocks to the paw. Some units (15) showed evidence of facilitation when combined auditory and visual stimuli were presented to the animal at the same time. No alteration in activity was observed when either or both of these stimuli were paired with the electrical shock to the paw, indicating that the facilitation observed is modality specific, dependent on correct timing, and not due to a general arousal.

Hydraulic injections of HRP or nuclear yellow into the basilar pons resulted in retrograde labeling of neurons predominantly located in the regions just surrounding the primary receiving areas of the visual, auditory and somatosensory cortices. The delineation of these regions was aided by computer imaging of cell distributions.

These data indicate that specific interactions of inputs from visual and auditory modalities occur in the cerebellum and that this information may originate in the "association cortices". (Supported by NIAAA grant 3901, DA 02338 and the Biological Humanics Foundation.

68.2 THE CEREBELLAR AUDITORY AREA IN THE CAT. Chi-ming Huang and Kuo-long Liu*. Departments of Physiology and Anatomy, University of South Alabama, Mobile, AL 36688.

We studied the topographic distribution of auditory responses in the posterior cerebellum of the cat in order to determine the extent of the cerebellar auditory area and whether it contains subdivisions. Electrophysiological recordings were made from the surface of the cerebellar cortex with silver ball electrodes and in cortical penetrations with glass-coated tungsten microelectrodes. Auditory evoked potentials were present over the posterior vermis including lobules VI and VII of Larsell, but not over the paramedian lobules or the cerebellar hemispheres. Recordings from the tungsten microelectrodes during penetrations through the cerebellar cortex suggested that these auditory evoked potentials were generated locally from the cerebellar cortex. There was substantial variations across individual animals on the topographic distribution of auditory evoked potentials over the cerebellar surface. Positive evoked potentials were usually found in lobule VI whereas negative evoked potentials were found more posteriorly in lobule VII. Peak latencies for both the positive and negative evoked potentials were between 7.7 and 17.7 msec. Within the cerebellar auditory area, the amplitudes of evoked potentials from adjacent cortical points 1 mm apart could differ significantly, suggesting that there may be a microscopic organization within an area of the order of a fraction of a millimeter. We conclude that the cerebellar auditory area in the cat is located entirely within the posterior vermis. It may contain two subdivisions, each characterized by a different polarity of evoked potentials. Within each subdivsion, there may be a pattern of patchy organization in which auditory impulses interact with impulses from other modalities. We also have evidence from single cell recordings and from HRP tracing studies, both of which support the above hypothesis on the pattern of subdivision and micro-organization within the cerebellar auditory area. (This work was in part supported by a grant from the US Public Health Service NS 16935 which we gratefully acknowledge.)

68.3 RED NUCLEUS ACTIVITY RELATED TO FINGER MOVEMENTS. P.R. Kennedy, A.R. Gibson and J.C. Houk. Dept Physiol, Northwestern Univ Med Sch, Chicago, Il 60611.

Magnocellular red nucleus (RNm) neurons were recently shown to discharge in bursts that precede hand movements (Kohlerman N.J., Science, 217:857, 1982). Here we sought to determine if these neurons are also related to individual finger movements. A monkey was rewarded for pressing microswitches mounted on a "piano" device and acquiring visual targets. Single RNm neurons were recorded extracellularly while the monkey performed this task. For 53 RNm neurons sampled during piano performance, the average response rate was 85 pulses per second (pps) while closing thumb switch, 71 index, 61 middle, and 60 ring. Mean spontaneous rate in between switch operations was 30 pps. All 53 neurons had response rates 2 to 5 times their spontaneous rates. Neurons responded differently on individual switches: 31 were highest on thumb, 7 on index, 2 on middle and 4 on ring.

For 30 neurons, discharge during piano performance was compared with activity on a push-pull device requiring movement of the whole arm. Average firing rates were higher on the piano for 24 neurons and higher on the push-pull for 6 neurons. All of the latter showed modulations on the piano that were lower by no more than 25%. Conversely, of the 24 higher rate piano neurons, only 6 showed rates on the push-pull that were within 25%. EMG analysis of 7 forelimb muscles (deltoid, latissimus, pectoralis, biceps, triceps, and long finger flexors and extensors) during performance on the piano showed phasic activity in distal muscles only (he rested his elbow on a cushion). Prominent phasic activity was seen in the 5 proximal muscles during push-pull operation; lesser activity was also seen in distal muscles since the animal was required to grasp the handle. Thus, RNm activity on the piano was unlikely to have resulted from proximal muscle involvement whereas activity on the push-pull arm device could have involved distal movements. RNm activity, therefore, appeared to be related to finger movements.

Photodiode sensors were placed on the monkey's digits to measure their velocity, duration and timing during piano operation. Activity in 9 of 19 neurons showed statistically significant rate/velocity AND duration correlations to movements of a single digit. The average lead time of firing onset to movement onset was 90±26 ms for correlated neurons. There were no lag times. Recording sites were recovered postmortem based on lesions and electrode tracks. 89% of all sites were within, and 11% near, the magnocellular region as defined by retrograde labeling from the spinal cord in 2 control monkeys. The results indicate that most RNM neurons are related to distal movements and poorly related to proximal movements. Some neurons correlate with velocities and durations of single digit movements.

68.4 FUNCTIONAL CEREBELLAR METABOLISM DURING FORELIMB MOVEMENT IN THE RAT. T. Der, E.M. Santori* and R.C. Collins, Dept. Neurology, Washington University, St. Louis, MO 63110.

The functional anatomy for forelimb movement in the cerebellum is not well known. To study this problem we have used electrical stimulation of wrist extensor muscles in rat combined with quantitative deoxyglucose autoradiography.

Male albino rats (300-380 gms) were fasted overnight. Under halothane anesthesia, catheters were inserted into femoral vein and artery. Two 003 gauge insulated wires with exposed ends were inserted into the left forelimb. The negative pole was hooked into wrist extensor muscles at the elbow through a small incision in the skin. The positive pole was inserted into the dorsum of the paw. Animals were restrained on a lead brick and allowed to recover from the anesthesia for greater than 2 hours. Bipolar pulse stimuli (0.1 msec., 5-10 Hz. 12-25 volts) were used to cause a consistent dorsiflexion of the forelimb. The rate of stimulation was varied from 2 pulses per second (pps) to 10 pps in order to determine a dose-response relationship for the metabolic responses. Quantitative regional glucose utilization was determined by the method of Sokoloff et al. (1977).

Repetitive electrical stimulation of left wrist extensors produced increases in glucose utilization in afferent pathways to the cerebellum. At ten pps there was an increase in the medial aspect of lamina III and IV of the dorsal horn (2.24 times control) but no change in the ventral grey. There were increases in ipsilateral cuneate nucleus (1.39 x control) and contralateral inferior olive (1.54 times control).

Metabolic changes occurred bilaterally in the cerebellar hemispheres in the paramedian lobule, posteriorly, and in lobules five and simplex, anteriorly. Within these lobules the greatest metabolic response occurred in the granule cell layer with less response in the molecular layer. The metabolic activity in the granule cell layer of the paramedian lobule was 1.78 x control ipsilaterally and 1.55 x control contralaterally. Anteriorly, in lobule 5 it was 2.15 x control ipsilaterally and 1.38 x control contralaterally. Metabolic activity in simplex lobule was 2.08 x control ipsilaterally and 1.49 x control contralaterally. Glucose utilization in the deep cerebellar nuclei was unchanged by the stimulation.

Five pps produced approximately the same pattern of metabolic changes as described above. These changes were smaller in magnitude compared to the ten pps. With two pps no change in glucose utilization was detected. These results indicate that graded electrical stimulation of forelimb muscle groups can be used to map functional activity in cerebellum. The specific role of muscle afferents, joint receptors and cutaneous sensation in producing this pattern remains to be determined.

68.5 TOPOLOGICAL FEATURES OF CLIMBING FIBER INPUT IN THE ROSTRAL ANTERIOR LOBE OF THE CEREBELLUM OF THE CAT. L.T. Robertson and K. Logan*. Neurological Sci. Inst., Portland, OR 97209.

Anatomical and physiological studies have demonstrated the climbing fiber (CF) projection to the cerebellar cortex is organized in a series of parasagittal strips. Previous work in this laboratory has shown the CF parasagittal organization can be further subdivided into a series of elongated patches, each of which contain a unique representation of the body surface. The goal of this study was to describe the CF topology in rostral anterior lobe using techniques similar to our previous work.

Data were obtained from 36 cats that were anesthetized with Na-pentobarbital. Systematic exploration was made of both the vermal and intermediate zones of lobules III, IV, and Va, using a 300 μm distance between electrode penetrations. Single Purkinje cells were recorded extracellularly and an attempt was made to elicit a CF response by manual tapping or manipulation of body surfaces or by a computer-controlled punctate stimulus. On the basis of force thresholds the units were classified as cutaneous, kinesthetic or nonresponsive. Receptive fields were delineated at the lowest threshold of stimulation.

CF responses were identified in 1375 Purkinje cells, of which 699 were located in the vermal zone and 677 were in the intermediate zone. In the vermal zone the majority of CF responses were classified as cutaneous (52%) versus kinesthetic (20%) or nonresponsive (28%), whereas in the intermediate zone most were kinesthetic (44%) rather than cutaneous (32%) or nonresponsive (24%). The cutaneous input was represented mainly in lobules IV and Va in vermal zone but was found predominately in lobule III of the intermediate zone. The kinesthetic representation was primarily located in Va in both vermal and intermediate zones. The vermal cortex consisted of a medial zone, which contained CF responses that were either nonresponsive (70%) or related to the tail (26%), and a lateral vermis, where 90% of the receptive fields were of the distal area of the hindpaw. The intermediate cortex displayed a more complex mixture of patches representing the distal and proximal areas of the hindlimb (particularly in lobules III and IV), forepaw (mainly in lobule Va), abdomen, face, and portions of the tail and back.

In comparison with the caudal anterior lobe, the rostral anterior lobe has a similar: (1) distribution of receptor modality, (2) distinction between the vermal and intermediate zones, and (3) patchy mosaic topography. However, the rostral anterior lobe has a greater representation of the hindlimb with many receptive fields restricted to the distal paw.

68.6 INCREASED ACTIVITY OF CEREBELLAR NUCLEAR CELLS DURING THE RECIPROCAL INHIBITION AND ANTAGONIST CO-CONTRACTION OF FOREARM MUSCLES IN THE AWAKE MONKEY. Richard Wetts, John F. Kalaska and Allan M. Smith. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

Two mutually exclusive modes of muscular activation are antagonist co-contraction and reciprocal inhibition. Previous work from our laboratory suggested that the cerebellar cortex plays a role in switching between these two patterns of activity. This study was designed to examine the contribution of cerebellar nuclear cells to the control of these two patterns. To produce reciprocal activity in forearm muscles, monkeys were trained to alternately flex and extend the open hand and to hold the wrist against a mechanical stop for 1.5 sec. The same monkeys were also trained to pinch a force transducer between the thumb and forefinger in order to elicit simultaneous co-contraction of agonist and antagonist muscles.

So far, 67 cells have been found which were related to the prehension task. In general, most nuclear cells have a high rate of spontaneous discharge, yet 65 cells (97%) further increased activity during the task. Most of these cells (29) had a large peak of activity at the onset of co-contraction and had a less pronounced increase during the maintained pinch. Of the 67 cells analyzed, discharge frequency was significantly correlated with the rate of force increase for 9 cells, or with the maintained force level (5 cells) or with both parameters (3 cells). Most noteworthy is the fact that the vast majority of nuclear cells were excited during the co-contraction accompanying prehension, whereas a clear decrease in discharge frequency was seen in only two units. Data from the wrist flexion-extension task are also available for 40 of the 67 cells. Discharge frequency was significantly correlated with velocity of wrist movement (14 cells) or with the torque applied to the stop during the maintained wrist position (8 cells). For most cells (73%) the discharge frequency was significantly different ($P < .05$) between the isometric flexion and extension. This pattern of activity was not unexpected since most of the Purkinje cells, which are the major inhibitory input to the deep nuclear cells, also displayed a reciprocal discharge pattern during wrist movement. During the prehension task, the majority of Purkinje cells decreased activity, and so it was expected that most of the nuclear cells would be disinhibited. However, the number of nuclear cells which increased discharge frequency (97%) was surprisingly large in view of the fact that about 37% of the responding Purkinje cells increased their activity during the task. Whatever the explanation for the powerful excitation of nuclear cells during co-contraction may be, this study adds further evidence that the cerebellum plays an important role in the reciprocal versus co-active control of muscles.

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68.7 RESPONSE OF CEREBELLAR CORTICAL UNITS TO MUSCLE STRETCH AND SPINAL CORD STIMULATION. Daniel Bourbonnais*, Charles Krieger*, Allan M. Smith. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada.

The effect of stretching the tibialis anterior and gastrocnemius muscles on the activity of cerebellar cortical neurones of lobules II to V was evaluated in chloralose anesthetized cats (50 mg/kg, i.v.). A ramp stretch of 4 mm at a velocity of 40 mm/s was applied to these muscles separately or simultaneously. Simultaneous stretch of antagonist muscles is unphysiological but was used to reflect the symmetrical pattern of muscle afferent activity during co-contraction of antagonist muscles.

The firing frequencies of 94 of the 204 cerebellar units were increased or decreased by muscle stretch. Twenty-one Purkinje cells, identified by their complex spikes, responded only to the dynamic component of muscle stretch. Only three Purkinje cells which had shown a modulation of activity to muscle stretch were examined with electrical stimulation of the dorsolateral funiculus in the upper thoracic spinal cord. One of these Purkinje cells showed a long latency (20 ms) increase in the simple spike activity and another responded with a single complex spike. The remaining Purkinje cell showed no response to spinal stimulation. The activity of 44 unidentified units also had dynamic responses to muscle stretch. In addition, the activity of 29 other unidentified units was modified during both the dynamic and maintained stretch. The response of 15 unidentified units was tested with spinal cord stimulation. Two units responded to single pulses with variable latencies suggesting a synaptic activation by the spinal stimulation. In contrast eight units responded at fixed latencies and followed without failure double pulses delivered at frequencies exceeding 300 Hz. The latencies ranged from 1.6 to 3.5 which correspond to conduction velocities of about 30 to 75 m/s. It would appear that these units can be identified as action potentials recorded from mossy fiber spinocerebellar afferents. In addition, the majority of these units (7/8) had a dynamic and static response to muscle stretch.

These results indicate that although spinocerebellar afferents convey information about muscle length, the Purkinje cell discharge does not reflect this parameter. It may be that this information is filtered within the glomerular complex or at the level of the Purkinje cell itself. These results are nevertheless consistent with the view that Purkinje cells integrate inputs from different sources and that a specific muscle stretch is not sufficient to obtain a sustained discharge of Purkinje cells.

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68.8 COMPARISON OF RESPONSE PROPERTIES OF DSCT AND VSCT NEURONS TO THE SAME PHYSIOLOGICAL HINDPAW STIMULI. J.H. Kim, T.J. Ebner and J.R. Bloedel. Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Mpls., MN 55455.

The dorsal spinocerebellar tract (DSCT) and ventral spinocerebellar tract (VSCT) are the two major mossy fiber afferent systems projecting directly to the cerebellum from the lumbar spinal cord. Despite apparent differences in their physiological and anatomical properties, both tracts respond to a similar spectrum of peripheral inputs. The purpose of this study was to determine differences and similarities in the way DSCT and VSCT neurons process the same physiologic cutaneous stimuli applied to the hind footpads. In cats anesthetized with alpha-chloralose (60 mg/Kg, IP), DSCT neurons were identified by their antidromic activation from the ipsilateral inferior peduncle. In another group of cats VSCT neurons were identified antidromically by stimulating in the contralateral superior peduncle. Only neurons responding to exteroceptive stimuli with their receptive field on the ipsilateral hind footpad were studied. The stimulus probe consisted of a flat surface contacting the entire plantar surface of the foot. Three different wave forms were used: (1) a step displacement of varying amplitudes (0.2-3.0 mm), (2) constant amplitude ramp displacements of different slopes (5-60 mm/sec), (3) constant amplitude sinusoidal displacement of varying frequencies (1-20 Hz). Interestingly, responses of DSCT and VSCT neurons were remarkably different to sinusoidal inputs. DSCT cells were extensively modulated by sinusoidal stimuli in the frequency range of 1-20 Hz. VSCT neurons were only weakly modulated over the same frequency range. A comparison of the responses of VSCT and DSCT neurons to step inputs revealed systematic differences in their sensitivity to this type of stimulus. The amplitude of the phasic discharge of VSCT neurons at the onset of the step increased with increasing step amplitude, whereas the phasic component of the DSCT neurons' response saturated at relatively low amplitudes. The response characteristics of these two groups of cerebellar afferents to the ramp stimuli were also different. VSCT neurons responded with a phasic peak at a short latency after the beginning of the ramp with little activity in the late phase. In contrast the discharge of most DSCT neurons was increased throughout the ramp, although some showed a phasic response at ramp onset. This increase in discharge was either relatively constant throughout the duration of the ramp or it continued to increase proportionally to the ramp amplitude. These data show that although VSCT and DSCT respond to the same cutaneous stimuli, the characteristics of their modulation by the same input are quite different. This work was supported by NIH Grants RO1-NS 18338 and RO1-NS 09447.

68.9 CONTROL MODEL APPLIED TO THE CEREBELLUM OF THE SHARK
Richard S. Babb, Iona College, New Rochelle, N.Y 10801

Stability in the swimming shark is produced by; the geometry of the shark's body, the inertial guidance provided by the vestibular system, and "critical damping" provided by the vestibulocerebellum. A model system is presented in which the stability of the shark is discussed in terms of levels of control. In order to simplify the problem, stability about the yaw-axis only will be considered.

The classical wind-tunnel studies of Harris (1936) on the shark moving relative to a surrounding fluid, have shown that its static stability is dependent on the shark's geometric form, in particular the configuration of its vertical fins. For small angles of deviation of the body-axis from the swim-axis in the yaw plane, the shark possesses neutral stability, whereas for angles larger than 10^{0} a restoring moment about the center of gravity provides static stability. The region of neutral stability allows higher order stability and control to be effective for small angles. Within this window of neutral stability maneuverability and feedback stabilization can operate. According to the model, the neural basis of feedback would be provided by fibers of the ampula of each horizontal semicircular canal which project to the vestibular nuclear complex, the neurons of which would project via the descending medial longitudinal fascile to the spinal motor neurons. Activity in this three-neuron arc would modify ongoing swimming activity to produce superimposed angular moments and hence angular acceleration. These angular accelerations would be such as to counter any external disturbances which have the effect of exciting the horizontal semicircular canals. Such restoring moments produce oscillations which, in a stable system, converge on the course setting. Oscillations of a negative feedback system can be "critically damped" by adaptive control. Such control is based on superimposing an adaptive section onto the feedback control system. The adaptive section compares an ideal output with the actual output giving a generalized error which modifies one element of the feedback loop thereby modifying the gain. Intentional and actual movement information is fed to the vestibulocerebellum making it a good candidate for the role of the adaptive section, with its known output to the vestibular nuclear complex, which is part of the feedback control system.

Knowledge of the function of the vestibulocerebellum of the shark may help in understanding the homologous structure in humans, the nodulus of the cerebellum.

68.10 In vivo tests of a Multichannel Intracortical Recording Array,
D. J. Anderson, S. L. BeMent* and K. D. Wise* Dept. of Electrical and Computer Engineering, Univ. of Michigan, Ann Arbor, MI 48109. We are applying techniques for integrated circuit fabrication and semiconductor materials micromachining to the development of a multichannel array for chronic recording from closely spaced cortical neurons. Previous work (Wise, K.D., et. al., IEEE Trans Biomed Eng, 17, 238-247, 1970) indicated the feasibility of active probe electronics to improve the signal-to-noise ratio of the neuron-recording electronics interface. Methods for the fabrication of multichannel probes had also been developed (Pochay, P., et. al., IEEE Trans. Biomed. Engr., 26, pp 199-206, 1979). The present study is directed toward fabrication of a multiple-recording site microelectrode with on-board interface amplifiers and multiplexer to combine the multi-channel signals into a single output channel to the external electronics. The project can be broken logically into 3 subprojects. 1) Design and fabrication of the microelectronics and off-chip reconstruction electronics, 2) Determination of the relative physical characteristics of various recording materials, 3) In vivo testing to study the ability of the various probe sites and configurations to record well isolated neural activity.

In vivo tests to date have been on single channel silicon-substrate probes with a shank width of about 75 μm and a thickness of about 20 μm. The recording sites are tantalum conductors which protrude into an opening in the protective dielectric about 5 μm. Successful recordings have been obtained from gerbil cerebellar cortex. We have demonstrated that the probes have the structural strength to penetrate into cerebellum and can isolate single neurons satisfactorily. Work is proceeding to 1) reduce the substrate size 2) improve the electrical characteristics of the recording sites and 3) develop satisfactory methods for the introduction and maintenance of cronic probes in cortex. The amplifier and multiplexer parts of the chip have been fabricated and tested, and design modifications are underway.

This work is supported by NIH Contract NINCDS-NDl-NS-1-2384.

68.PO EXTENSIVE CEREBELLAR REPRESENTATION OF CUTANEOUS TACTILE SENSITIVITY IN OPOSSUM (Didelphis virginiana).
Wally Welker and Georgia M. Shambes. Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

Recent studies of the albino rat revealed extensive cutaneous projections to the granule cell (GC) layer of the cerebellar hemispheres and caudal vermis (Shambes, et al, 1978; Joseph, et al, 1978). Adjacent body parts projected a-somatotopically in a mosaic of columnar GC patches (called "fractured somatotopy").

To determine whether similar circuits exist in a marsupial, we explored the GC layer cerebellar cortex of the opossum using microelectrode micromapping and juxtathreshold cutaneous natural stimulation. We used adult opossums of both sexes anesthetized with sodium pentobarbital supplemented with ketamine hydrochloride. Tungsten ball-tip microelectrodes (5-8 μ tip dia.; impedances of 0.8-1.2 megohms) recorded multiple-unit GC layer responses evoked by gentle stimulation of the skin.

To date we have mapped RF's for 284 active punctures in 7 animals. Results were: (1) Receptive field (RF) maps were obtained for nearly all hemispheric folia and three folia of the caudal vermis (2) Submodality of most RF's was light "touch" cutaneous, but muscle, joint and deep RF's were also found. (3) GC projections were organized in patchy, fractured a-somatotopic columnar patterns. (4) Ipsilateral projections predominated. Most contralateral punctures were medial in all hemispheric folia. (5) Mechanoreceptors from face, snout, mouth and teeth activated the bulk of the GC loci on crus I and crus II. Projections to the paramedian lobule (PML) came from the entire body. The pyramis received hindlimb projections, the rostral uvula received input from hindlimb and forelimb, and the caudal uvula had projections from the face. (6) The largest patches of projections on crus I and crus II were from vibrissae, and on the vermis and PML from forelimb and hindlimb. (7) Adjacent body parts projected to disjunctive GC loci - the overall projections form a patchy mosaic. (8) Individual differences in folial pattern and body representation were apparent.

Except for differences in mosaic pattern and relative size of different projections in rat, gray squirrel and cat (Kassel & Shambes, in preparation), this research suggests that cutaneous inputs to cerebellum are not only functionally important, but that they may exist widely among mammals. Supported by NSF grant BNS 8022321.

69.1 FURTHER EVIDENCE FOR TAURINE OR TAURINE-LIKE SUBSTANCE AS A TRANSMITTER IN FROG SPINAL CORD. A. L. Padjen, P. Metrakos* and C. Karamitsos*. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec.

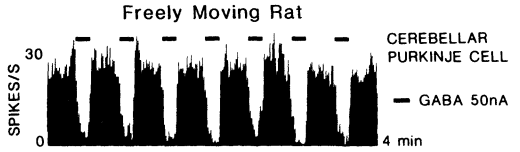
It was reported that AMBD (6-aminoethyl-3- methyl-4H-1,2,4-benzothiadiazine-1,1-dioxide HCl) is a specific blocker of taurine (TAU) and beta-alanine (BALA) receptors in central nervous system (Yarbrough et al., 1981, JPET 219, 604). We have reexamined the effect of AMBD on synaptic and amino acid evoked responses recorded from isolated hemisected frog spinal cord by means of sucrose gap technique (Barker et al., 1975, J. Physiol. 245, 537). When indirect responses were blocked by addition of Mn (2 mM), AMBD (conc 0.01 - 0.5 mM) selectively antagonized TAU and BALA evoked depolarizing responses of primary afferents (dorsal roots) without affecting depolarizations by glutamate, GABA and glycine (the latter usually increased). Depolarizing responses to 0.5 mM TAU or BALA were antagonized 50% by 0.075 mM AMBD and often completely blocked by 0.25 mM AMBD. In normal Ringer, AMBD selectively antagonized dorsal root potential evoked by ventral root stimulation (VR-DRP; threshold at 0.02 mM, 90% block at 0.25 mM); other synaptic potentials (dorsal and ventral root responses evoked by dorsal root stimulation) increased in amplitude and duration due to strong convulsant activity caused by higher concentrations of AMBD.

These results are in agreement with the proposal that taurine or a taurine-like substance is involved in VR-DRP of frog spinal cord (Barker et al., 1975, J. Physiol. 245, 537). (Supported by MRC.)

69.2 A TECHNIQUE FOR MICROIONTOPHORESIS IN THE FREELY MOVING RAT. M.O. West and D.J. Woodward. Dept. Cell Biology, University of Texas Health Science Center, Dallas, Texas 75235.

A limitation of acute iontophoretic studies is determining to what extent experimental results are influenced by anesthetics or by immobility. The implementation of such studies in awake, freely moving animals has been hindered by formidable technical obstacles. Single-barreled glass micropipets have been used to obtain stable recordings from freely moving rats by means of a detachable miniature microdrive (Deadwyler et al 1980. Electroencephalogr. Clin. Neurophysiol. 47:752). We now report on progress in conducting single unit recording and microiontophoresis in freely moving rats with the microdrive adapted to accommodate multibarrel glass.

The adapter, which fits into the center of the microdrive (Biela Engineering, Anaheim, Ca.), contains a bore with the shape of a cloverleaf for holding 4-barrel glass. Pipets pulled to a fine tip (3-10 microns total tip diam.) and filled with drug or salt solutions are press-fitted into the microdrive, which is then attached to a base implanted on the animal's skull. Rotation of the threaded outer cylinder of the microdrive advances the pipet, without rotation, to the desired depth (up to 7 mm). Signals are led to and from drug, balance and recording barrels by means of silver wires and a harness, through which the animal is attached to a 23-lead rotating slip ring assembly.



In preliminary studies, stable unit recordings have been obtained from cerebellum, thalamus, hippocampus and striatum. The number of drug barrels (two) can be increased to three by recording unit activity through a tungsten microelectrode that is affixed to the pipet in "piggyback" fashion. This has the further advantage of allowing various configurations between recording and pipet tips, permitting drug application at selected distances from the soma.

With further development the procedure is anticipated to become a powerful technique, useful for example, in determining the effects of putative neurotransmitters on the behavioral correlates of single brain cells. Supported by NIAAA 3901, DA02338 and the Biological Humanics Foundation.

69.3 IONTOPHORETIC STUDIES OF RESPIRATORY NEURONS IN THE UNANESTHETIZED CHRONICALLY IMPLANTED CAT. M. Denavit-Saubié, A.S. Foutz*, Y. Pavi*, E. Boudinot*, M-P. Morin-Surun* and J. Champagnat*. Laboratoire de Physiologie Nerveuse and LA 204, C.N.R.S., 91190 - Gif-sur-Yvette, France.

We have previously shown that the firing pattern of respiratory-related neurons located in the cat medulla could be modified by local application of exogenous or endogenous substances (1). These pharmacologic manipulations were performed on anesthetized or decerebrated animals. The aim of the present study was to perform local iontophoretic drug applications to respiratory neurons of intact, undrugged animals in which the respiratory system is not depressed by anesthesia or deprived of forebrain afferents. Four adult cats were chronically implanted for recording the EEG, respiratory activity (nasal thermistor), and for cranial restraint. A 10 mm capped opening above the cerebellum allowed penetrations of multibarreled micropipettes into the medulla. A catheter in the jugular vein was used for i.v. drug administration. After habituation to the restraint, up to 40 penetrations were carried out in 3-4 h recording sessions over a period of several months, and histological control was performed post-mortem. Micropipettes were lowered stereotaxically using as a reference a pole embedded in the acrylic mound. In this preparation stable recordings (5-75 min) were obtained and almost one half of units were respiratory-related (35 of 90 neurons in one cat). In these animals respiratory neurons fired more tonically and with a higher discharge rate than in acute preparations. Most neurons were sensitive to L-glutamate application, which showed that the recorded activity originated from the soma. Neurons were sensitive to GABA application, showing that this substance was still effective in the absence of pentobarbital anesthesia (2), which is known to potentiate the effects of GABA. In conclusion the present method allows stable recordings of respiratory-related neurons and iontophoretic application of pharmacologic compounds in intact, undrugged animals presenting natural sleep-waking states. (1) Denavit-Saubié M. et al., Brain Res., 155 (1978) : 55-67. (2) Champagnat J. et al., Brain Res. 237 (1982) : 351-365.

69.4 IN VIVO BRAIN DIALYSIS CONFIRMS IN VIVO VOLTAMMETRIC EVIDENCE THAT ASCORBIC ACID IS RELEASED FOLLOWING DOPAMINE RECEPTOR STIMULATION. L. A. Phebus* and J. A. Clemens. The Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

We reported that in experiments using in vivo voltammetry in the striatum of awake rats, dopamine (DA) agonists increased the current peak at approximately +0.12 volts versus an Ag/AgCl reference. This increase was blocked by DA antagonists. This peak results primarily from the oxidation of extracellular ascorbic acid (AA), with lesser contributions from dihydroxyphenylacetic acid (DOPAC) and DA. The rise in this peak following DA agonists cannot be explained by changes in DA or DOPAC concentrations since these would be expected to decrease. We carried out in vivo brain dialysis experiments to determine the identity of the substance released into the striatal extracellular fluid by DA agonists.

Adult, male, Sprague Dawley rats were anesthetized, and a miniature, graphite paste-tipped working electrode was lowered into the corpus striatum for electrochemical measurements. A stainless steel auxiliary and Ag/AgCl reference electrode were placed in the contralateral cortex. The leads from these electrodes were attached to a miniature electrical connector and the entire assembly was cemented to the skull. Animals were allowed at least 3 days to recover from surgery. Semidifferentiated voltammograms were recorded at 1/2 hour intervals. Drugs were administered i.p. after a stable voltammetric baseline was achieved.

For brain dialysis experiments, minature dialysis loops were lowered into the striatum of anesthetized rats and the cannulae holding these loops were cemented into place. Animals were allowed at least 3 days to recover. In an experiment, saline was perfused through the loop at a rate of 4 microliters per minute and collected in 15 minute samples. These samples were assayed for AA using HPLC.

Pergolide, a DA agonist, produced an increase in the oxidation peak occurring at approximately +0.12 volts. In the in vivo brain dialysis experiments, 6 of 8 rats showed a large increase in extracellular AA following 600 µg/kg of pergolide. We concluded that the increase in voltammetric signal following pergolide was due to a receptor mediated release of AA into the extracellular fluid.

Using the in vivo dialysis technique we also found high concentrations of uric acid in the extracellular fluid of the rat striatum. We believe the oxidation of uric acid contributes to the voltammetric peak at approximately +0.3 volts (vs a Ag/AgCl reference) heretofore ascribed solely to 5-hydroxyindoles.

69.5 PERSISTENT CHANGES IN CNS DOPAMINERGIC ACTIVITY FOLLOWING PRENATAL EXPOSURE TO NEUROLEPTICS. Helen Rosengarten, Kenneth Carr and Arnold J. Friedhoff. Millhauser Labs., New York University School of Medicine, New York, NY 10016.

We have shown previously that the density of striatal D_2 dopamine receptors in pups exposed in utero to neuroleptic treatment is smaller than that in controls (H. Rosengarten and A.J. Friedhoff, Science 203, 1133-1135,1979). We now report that, although by 60 days of age, dopamine receptor density is still low it is no longer significant. However, tail-shock threshold in inducing simple vocalization was significantly lower at this time. Moreover, these rats were also statistically less sensitive to apomorphine when vocalization after discharge was measured and also were more resistant to neuroleptic-inducible dopamine receptor up regulation. These data therefore provide evidence to indicate that the neuroleptic induced changes observed initially by means of receptor binding have, as well, enduring behavioral consequences.

69.6 EVIDENCE OF APOMORPHINE-INDUCED UP AND DOWN REGULATION OF DOPAMINERGIC TRANSMISSION. M. Rodriguez*, R. Castro* and R. Alonso. Dept. Physiology, La Laguna Sch. of Med., Tenerife, Spain.

To further investigate the effects of dopaminergic agonists on motor behaviour, two different patterns of apomorphine administration were tested on male Wistar rats. Experiments were carried out on intact animals as well as on those with unilateral lesions of the nigrostriatal pathway. Lesions were made by injecting rats stereotaxically with 6-OH-dopamine (8 µg in 4 µl 0.9 % saline with 0.1 % ascorbic acid) into those coordenates corresponding to both the substantia nigra and the medial forebrain bundle. Apomorphine (1 mg/Kg in 1 ml 0.9 % saline) was given intraperitoneally either every two hours or two days to a total of four injections. In intact animals motor activity was measured between 15 and 20 minutes after each injection by using an actimeter. In lesioned animals, fifty days after 6-OH-dopamine injections and once denervation supersensitivity was developed, apomorphine was given at the same doses and temporal pattern as described above, and number of rotations toward the contralateral side were monitored. In both intact and previously lesioned rats the effect of apomorphine was apparently dependentupon the interval between injections: both the amount of movements and the number of rotations respectively were increased after each apomorphine injection when given every two days, while the opposite was observed when administration of the drug was made every two hours. It is well known that prolongued administration of drugs affecting neurotransmission may induce up or down regulation phenomena (Kurlan, R., Shoulson, I., Clin. Neuropharmacol., 5:345, 1982), the effect depending of the type of drug. Chronic administration of any drug that reduces neurotransmitter influence at its receptor site can lead to receptor up regulation (Baldessarini, R.J., Tarsy, D., Int. Rev. Neurobiol., 21:1, 1979), whereas chronic use of drugs that increase neurotransmitter action can cause receptor down regulation (Weiner, W.J. et al., Life Sci., 28:2173, 1981). The results reported here indicate that a well characterized dopaminergic agonist such as apomorphine is able to produce, at the same doses, an opposite effect on motor response depending upon the interval between succesive injections. It may suggest a transitory modification of dopaminergic synaptic transmission, probably due to up and down regulation phenomena.

69.7 SEROTONERGIC MODULATION AND SYNAPTIC MEMBRANE PROPERTIES IN THE ADAPTATION OF RAT CORTICAL ADRENERGIC NEURONS AFTER REPEATED ANTIDEPRESSANT TREATMENTS. N.Brunello[1], I.Mocchetti[1+], G.Calderini[2+], B.Di Perri[2+], and G.Racagni[1]. 1 Institute of Pharmacology and Pharmacognosy, University of Milan, Italy and[2] Fidia Research Laboratories, Abano Terme, Italy.

The temporal sequence of changes in cortical noradrenergic neurons after repeated administration with desmethylimipramine (DMI) indicates that a transient increase in the concentration of normetanephrine (NMN), the O-Methylated metabolite of norepinephrine (NE), is followed by the desensitization of the NE dependent adenylate cyclase and the down regulation of beta adrenergic receptors. NMN has been taken as an index of the amount of NE present in the synaptic cleft. After two weeks of treatment these persistent events are paralleled by a decrease in the NMN content, indicating that noradrenergic neurons have reached a new functional state. The maintenance of this reduced functional level seems to be due to transynaptic mechanisms which involve serotonergic neurons. In fact a prolonged treatment with antidepressants elicits an increase in the uptake system for serotonin (5 HT), thus removing a possible modulatory input on noradrenergic system. In fact serotonergic denervation after lesion of the serotonergic system following intracerebral injection of 5, 7 dihydroxytryptamine (5,7 DHT) produced a reduction in the concentration of cortical NMN. Moreover in 5,7 DHT lesioned rats the decrease of NMN concentration after prolonged administration with DMI was markedly attenuated. In addition 5-hydroxytryptophan (5 HTP) given repeatedly was able to potentiate the down regulation of beta receptors.

Chemico-physical properties of the synaptic membranes may also be involved in the modulation of noradrenergic trasmission in rat cerebral cortex after antidepressant treatment. We have recently shown that acute treatment with DMI elicited an increase of phospholipid methylation which disappeared after chronic treatment. This effect was further enhanced by a concomitant administration with a mixture of phospholipids. When animals were treated with DMI and the phospholipid mixture, the incresed efficiency of 5HT uptake complex and the down regulation of beta receptors were already apparent after six days, indicating that changes in membrane composition can influence receptor adaptation to chronic antidepressant treatment.

The results obtained demonstrate that the hyposensitivity of the noradrenergic system produced by a chronic treatment with antidepressants may be indicative of adaptive and integrative changes which bring the noradrenergic neurons to a lower functional level.

69.8 ^{3}H-VERAPAMIL BINDING SITES IN RAT CEREBRAL CORTEX. I.J. Reynolds, R.J. Gould and S.H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Binding sites for tritiated dihydropyridines, such as ^{3}H-nitrendipine, have been demostrated in smooth, cardiac and skeletal muscle and in neural tissue. These sites are believed to be associated with potential-operated calcium channels. Thus, dihydropyridines prevent K^+-stimulated. Ca^{2+}-dependent contractions of smooth muscle, and exert a negative inotropic effect in cardiac tissue. This lab, as well as others, (F.J. Ehlert et al., Life Sci., 30:2191, 1982) has demonstrated a non-competitive interaction between the ^{3}H-nitrendipine binding site and drugs such as verapamil, methoxyverapamil (D600) and lidoflazine. The observation that these drugs increase the dissociation rate of ^{3}H-nitrendipine is taken to indicate an allosteric interaction between the ^{3}H-nitrendipine binding site and the site of action of verapamil. These agents also inhibit calcium dependent processes in smooth and cardiac muscle.

We have demonstrated binding of ^{3}H-verapamil (75 Ci mMol, NEN) to rat cortical membranes. Cerebral cortices were removed immediately following decapitation of male Sprague-Dawley rats (150-200 g) and homogenized in 20 vol of 50 mM sodium phosphate buffer (pH 7.4) containing 1 mM disodium EDTA. The homogenate was spun three times at 50,000 g and resuspended to a concentration of 5 mg/ml (wet weight) in phosphate buffer. Membranes were incubated for 1 hour with 1.4 nM ^{3}H-verapamil at room temperature, filtered, and rapidly washed in 10 ml ice-cold buffer. Tritium was extracted into a scintillation cocktail (Formula 947, NEN) and counted. This technique, using 1 µM D600 to determine non-specific binding gave 1200 displaceable cpm, with total and non-specific binding being 2500 and 1300 cpm respectively. A centrifugation assay performed on the same basis gave a similar number of displaceable counts, but with an inferior signal/noise ratio.

Calcium exerts a complex effect on ^{3}H-verapamil binding. Low concentrations (10^{-7} - 10^{-6} M) added after the EDTA wash increased specific and, to a lesser extent, non-specific binding. By contrast, higher concentrations, (10^{-4} - 10^{-3} M) decreases total binding. In the presence of 10^{-6} M calcium various agents displace ^{3}H-verapamil. These include D600 verapamil, lidoflazine and diltiazem with apparent IC_{50}'s of approximately 15, 30, 560 and 600 nM respectively.

Thus, these agents, which inhibit ^{3}H-nitrendipine binding in an allosteric fashion, also displace ^{3}H-verapamil binding. The observations on the effect of calcium are consistent with the hypothesis that the site of action of verapamil is intracellular (J. Heschler et al., Pflugers Arch., 393:287, 1982).

69.9 THE CHARACTERIZATION OF ^{3}H-NITRENDIPINE BINDING SITES IN RAT BRAIN. R.J. Gould and S.H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

We have previously described the binding characteristics of ^{3}H-nitrendipine binding sites in various regions of rat brain. These binding sites may be associated with voltage-operated calcium channels. A variety of structurally distinct calcium channel blockers interact with ^{3}H-nitrendipine binding sites in a fashion that is not strictly competitive, suggesting several sites of action of calcium channel blockers within the calcium channel. Antischizophrenic drugs of the diphenylbutylpiperidine class inhibit ^{3}H-nitrendipine binding with potencies similar to their potencies at dopamine receptors. This suggests that these drugs may exert therapeutic action via sites associated with ^{3}H-nitrendipine binding. Autoradiographic studies have previously shown distinct localization of ^{3}H-nitrendipine binidng sites to many, but not all, synaptic zones. This is consistent with these sites playing a role in neuromodulation. We have sought to further characterize these binding sites from various brain regions.

First, structure-activity studies reveal that the second, or verapamil-like site has structural specificity similar to that for calmoculin. This suggests that a number of agents believed to be specific for calmodulin may have actions via voltage-operated calcium channels. Second, (3 methyl-^{3}H) 1,4-dihydro-2,6-dimethyl-4-(2-isothiocyanatophenyl) -3,5-pyridine-dicarboxylic acid dimethyl ester (^{3}H-ITC-DHP) binds to rat brain membranes and is irreversible or very slowly dissociates. The t 1/2 for dissociation of [^{3}H]-ITC-DHP is greater than 60 min, while the t 1/2 for dissociation of ^{3}H-nitrendipine is 18 min. The use of this apparently irreversible tritiated ligand allows for preliminary biochemical characterization of ^{3}H-nitrendipine binding sites from various regions of the rat brain.

69.10 INVOLVEMENT OF GABA- AND GLYCINE-MEDIATED TRANSMISSION IN THE CONVULSANT-ANTICONVULSANT EFFECTS OF OPIATES. M. Massotti* and L. Mele* (SPON: S. Mazzari). Laboratorio di Farmacologia, Istituto Superiore di Sanità, Roma, Italy.

Endogenous opioid systems play a role in a variety of physiological functions in central nervous system, other than pain perception. Increasing evidences indicate that opiate agonists and antagonists are furthermore involved in the convulsive phenomena.

Electroencephalographic (EEG) behavioural and binding techniques were consequently utilized to analyze the interactions of naloxone and morphine with GABA and glycine-mediated transmission in vivo.

The administration of a high dose of naloxone (> 40 mg/kg iv) and morphine (> 100 mg/kg iv) elicits behavioural and EEG convulsions in laboratory animals. Such convulsant effect is characterized by the occurrence of high voltage spikes accompanied by tonico-clonic manifestations in the case of morphine and tonic manifestations in the case of naloxone. The intermediate doses of naloxone (5-20 mg/kg iv) elicit a proconvulsant effect in animals previously challenged with drugs which inhibit GABA-(bicuculline, picrotoxin, Ro 5-3663, cardiazol and local penicillin) and glycine- (strychnine) mediated transmissions at post-junctional level. On the contrary, the administration of low doses of morphine (0.25-1.0 mg/kg iv) inhibits the naloxone-induced convulsant and proconvulsant effects as well as the convulsant effect due to administration of the above mentioned GABA and glycine antagonists.

Binding studies show that naloxone modulates, in an inhibitory fashion, the high and the low affinity components of ^{3}H-GABA binding when tested "in vitro" at final concentration 10^{-5}M, and ^{3}H-glycine binding when administered at the dose of 40 mg/kg iv or higher.

The possible relevance of the above data will be discussed. This work was in part financed by CNR-ISS contract n. 82.02048.56 (pos. n. 115.14523).

69.11 ELECTROCONVULSIVE TREATMENT ATTENUATES HALOPERIDOL-INDUCED INCREASE OF ^{3}H-IMIPRAMINE BINDING IN RAT BRAIN. Barkai, A.I.*[1], Kowalik, S., Reches, A.[2], and Fahn, S.[3] (SPON: D.F. Klein). N.Y. Psychiatric Institute and Departments of Psychiatry[1] and Neurology[3], Columbia University College of Physicians and Surgeons, and Department of Neurology, Hadassah Medical Center, Jerusalem, Israel[2].

Binding sites for ^{3}H-imipramine (^{3}H-IMI) in brain are associated with the regulation of neuronal uptake of serotonin (5-HT) and appear to have a function in regulating 5-HT_2 receptors. Cerebral 5-HT_2 receptors bind butyrophenone neuroleptics with high affinity and their density can be modified by repeated electroconvulsive treatment (ECT). To investigate whether chronic haloperidol treatment, ECT, or a combination of both also affect ^{3}H-binding, four groups of rats were treated for 28 days as follows: 1) control; 0.2 ml saline i.p. daily, 2) haloperidol; 2 mg/kg i.p. daily, 3) ECT; 20-30 mamp, 60 Hz, 1.5 sec, 3 times a week, and 4) haloperidol plus ECT. Following a recovery period of 5 days animals were sacrificed and frontal-cortex membranes prepared for ^{3}H-IMI binding assays. A marked increase in the density of ^{3}H-IMI binding sites (Bmax) was seen in the haloperidol-treated rats compared to controls (Bmax 1190±185 vs. 350±40 fmol/mg protein) whereas no significant change in Bmax was induced in the ECT group. Treatment with both haloperidol and ECT resulted in a Bmax of 300±51 fmol/mg protein indicating that ECT acts to attenuate the increase of ^{3}H-IMI binding sites observed after withdrawal of chronic haloperidol. Supported by NIH grant MH 33690 and grants from the Parkinson's Disease Foundation and the Dystonia Medical Research Foundation.

69.12 PHOTOAFFINITY LABELING OF THE IMIPRAMINE BINDING SITE IN HUMAN PLATELET MEMBRANES WITH [^{3}H]-2-NITROIMIPRAMINE. L.P.Wennogle*, R.A.Ashton*[1] and L.R.Meyerson. Dept.CNS Res., Med.Res.Div. of Amer.Cyanamid Co., Lederle Labs, Pearl River, NY and [1]Dept. Chemistry, NYU, New York, NY.

The imipramine (IMI) binding site of human platelets and other tissues is considered to allosterically modify the activity of the serotonin (5-HT) uptake system. [^{3}H]-2-Nitroimipramine ([^{3}H]-2NI) is considered a reversible, "slowly-dissociating," high-affinity probe of the IMI binding site (Rehavi, et.al. 1983). The present study was conducted to exploit the photoactivatible potential of [^{3}H]-2NI and its utility to characterize the molecular components of this allosteric system. [^{3}H]-2NI (20nM) was incubated with platelet membrane preparations in the presence and absence of selected displacers at 0° C. Ultraviolet irradiation of these samples followed by SDS-PAGE with subsequent fluorographic analysis resulted in labeling of one major band of 30K apparent mol.wt. This band was quantitatively chased when photolysis was conducted in the presence of specific 5-HT uptake inhibitors (e.g., fluoxetine, citalopram, femoxetine, indalpine, and imipramine). In the absence of UV light, no photolabeled bands were detected. Drugs specific for other receptors (e.g., α_1, α_2, H_1, H_2, β, muscarinic and D_2) did not inhibit labeling of the 30K band. Several other bands in the gel were photolabeled to a lesser degree, but were not chased by any of the drugs tested. The 30K band is a minor membrane protein (< 0.1% total protein as judged by coomassie staining) labeled to high specific activity. Due to the high selectivity of photoincorporation into this site, and the differential drug displacement that is germaine only to the 5-HT uptake-IMI receptor complex, it is reasonable to associate the 30K band as a polypeptide component of this system. Curiously, when IMI was used (10-100 μM) to inhibit photoincorporation in the 30K band, a dose-responsive enhancement of label into other bands was observed. This effect may be due to membrane altering viscosity changes by imipramine, although IMI-induced conformational changes of the uptake complex cannot be eliminated. Photoaffinity labeling with [^{3}H]-2NI will likely prove to be a valuable tool in the ultimate purification and characterization of the serotonin uptake/allosteric regulator complex.

69.13 BETA-ADRENERGIC/CYCLIC AMP MEDIATED REGULATION OF BENZODIAZEPINE BINDING SITES IN C6 CELLS. M. D. Dibner, R. A. Lampe* and M. A. Bailey.* Neurobiology Group, Central Res. & Dev. Dept., E. I. du Pont de Nemours & Co., Glenolden Lab., Glenolden, PA 19036.

We have previously demonstrated that exposure of C6 glioma cells to fetal bovine serum or to benzodiazepines regulate the expression of benzodiazepine binding sites. The current studies indicate that the regulation of ^{3}H-flunitrazepam (FLU) binding to this receptor (a "peripheral" benzodiazepine receptor) is under the influence of adrenergic agonists. Growth of cells with the beta-adrenergic agonist isoproterenol (10 µM) for longer than one day leads to a significant increase in the Bmax of FLU binding with no significant alterations in the affinity of the receptor for FLU. Thus, 48-96 hr exposure to isoproterenol (changed daily) led to a 30-50% increase in FLU binding sites per cell. The addition of low concentrations of isoproterenol (10 nM) had no effect whereas 100 nM and higher concentrations produced increases in FLU binding. Treatment with isoproterenol did not lead to a significant increase in the amount of protein per cell. Thus, FLU binding site numbers were increased whether expressed per unit cell or per unit protein. Isoproterenol (10 µM) also increased binding sites on cells grown in serum-free defined medium. It appears that these changes are mediated via stimulation of cyclic AMP in the cells. Exposure of cells to 1 mM dibutyryl cyclic AMP for 1-2 days significantly increased FLU binding sites per cell with no alterations in the Kd. Dibutyryl cyclic AMP had no effect after only two hours of exposure. With two days of exposure, lower concentrations (100 µM) of dibutyryl cyclic AMP also increased binding site numbers in serum-free cells, but there was no effect with 10 µM dibutyryl cyclic AMP. In addition to dibutyryl cyclic AMP, cyclic AMP (1 mM) or isobutylmethylxanthine (500 µM) exposure can increase FLU binding sites. It does not appear that these effects are mediated via direct interaction with the benzodiazepine binding site since high concentrations of dibutyryl cyclic AMP or isoproterenol did not directly inhibit FLU binding to intact C6 cells. This effect appears, however, to be mediated via the ß-adrenergic receptor and can be blocked by co-incubation of propranolol along with isoproterenol. Lastly, although in most cases growth of cells with either isoproterenol or dibutyryl cyclic AMP led to a decreased cell growth rate, several lines of evidence indicate that the observed increase in FLU binding sites was not due to changes in the cell cycle or cell density.

69.14 **CHRONIC CLONAZEPAM TREATMENT INCREASES THE DENSITY OF SEROTONIN$_1$ AND SEROTONIN$_2$ BINDING SITES IN RAT FRONTAL CORTEX.** **H.R. Wagner, E. Yablonskaya*, A. Reches, S. Fahn (SPON: E. Housepian). Departments of Pharmacology and Neurology, Columbia University, College of Physicians and Surgeons, New York, New York 10032.**

The benzodiazepine, clonazepam, is an effective drug in the management of reflex myoclonus. Low CSF levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), often occur in myoclonus and increased 5-HIAA levels are associated with clinical improvement. Because of this correlation between serotonin (5-HT) and clinical status in myoclonus, we have studied effects of clonazepam on brain serotonergic function. Male Sprague Dawley rats were treated with clonazepam (5 mg/kg/day, i.p.) for ten days. Two days after the final dose rats were killed and various brain regions were assayed for levels of 5-HT$_1$ and 5-HT$_2$ binding sites, 5-HT and 5-HIAA. ^{3}H-5-HT was used to measure 5-HT$_1$ binding sites; specific binding was defined with 10 uM unlabelled 5-HT. ^{3}H-Spiperone (SPIP) was used to measure 5-HT$_2$ binding sites; specific binding was defined with 1 uM unlabelled spiperone. Chronic clonazepam exposure increased the number of both 5-HT$_1$ and 5-HT$_2$ binding sites. Increases reflected an increase in the maximum number of binding sites (Bmax) with no change in affinity (Kd). Binding increases for both ligands occurred in frontal cortex but not in brainstem. A significant increase in the binding of ^{3}H-5-HT occurred in rats treated chronically with clonazepam for 10 but not 5 days. ^{3}H-SPIP binding was significantly increased in rats treated for 5 days but not following a single drug injection. Specific binding of both ligands to frontal cortex membranes was significantly increased at clonazepam doses of 5 mg/kg/day but not at doses of 2.5 mg/kg/day. Binding changes did not reflect a direct effect of clonazepam on the receptors based on competitive binding studies. Levels of frontal cortex 5-HT and 5-HIAA, as determined by HPLC and electrochemical detection, were unaffected by clonazepam, suggesting that receptor increases were not mediated by transynaptic events.

Supported in part by a grant from the Norman and Barbara Seiden Foundation.

69.15 ACUTE AND REPEATED ADMINISTRATION OF DESMETHYLIMIPRAMINE AFFECTS PINEAL GLAND cAMP-DEPENDENT PROTEIN KINASE ACTIVITY. J.A. Moyer, P.J. Silver,* and E.B. Sigg*. Dept. of Exp. Therap., Wyeth Labs. Inc., P.O. Box 8299, Philadelphia, PA 19101.

Previous experiments have shown that repeated but not acute desmethylimipramine (DMI) treatment reduces the ability of isoproterenol (ISO) administered either in vivo or in vitro to elevate the concentration of adenosine 3':5' cyclic monophosphate (cAMP) in rat pineal glands. This effect is postulated to be due to a decrease in ß-adrenergic receptor number and a reduction in norepinephrine-stimulated adenylate cyclase activity (Life Sciences 24:2237, 1979: Molecular Pharmacology 19:187, 1981). However, additional studies have shown that acute and repeated treatments with DMI result in changes in cAMP responsiveness which are temporally and quantitatively dissociated from the alteration in receptor number (Neuroscience Abstracts 8:377, 1982). Accordingly, we have examined cAMP-dependent protein kinase (cAPK) activity in this model since activation of cAPK and subsequent phosphorylation of specific phosphoproteins is the primary mechanism of action of cAMP.

The concentration of soluble cAPK was determined in male rat pineal glands exposed to constant light following acute (1 injection; 10 mg/kg i.p.) and repeated (9 injections; 10 mg/kg i.p.; 5 days) DMI administration. Total cAPK activity (pmol/mg protein/min; $\bar{X} \pm$ SE) was reduced following both acute (4007 ± 286) and repeated (4191 ± 369) DMI administration compared to saline-treated controls (5450 ± 399). Cyclic AMP independent protein kinase activity, measured in the presence of saturating amounts of cAPK inhibitor, was not found to be different among saline (1260 ± 104), acute DMI (1055 ± 106), or repeated DMI (1407 ± 99) treated groups.

ISO-stimulated (2 µmol/kg i.p.) cAPK activation was also measured in rats treated acutely or repeatedly with DMI by determining cAPK activity ratios (cAPK activity-cAMP/cAPK activity +2 µM cAMP). cAPK activation was apparent in saline-treated rats ($\bar{X} \pm$ SE; basal 39 ± 3; ISO 86 ± 12); DMI treatment alone also activated basal cAPK activity in acute (66 ± 6) and chronic (69 ± 4) DMI groups. Further activation of cAPK by ISO was apparent in the acute (83 ± 4) but not the chronic (66 ± 3) DMI groups. Thus, these changes in cAPK activation paralleled alterations in cAMP responsiveness observed following acute and repeated DMI treatment in previous experiments.

In summary, these results show that the concentration and activation of cAPK is altered following DMI treatment in the rat pineal gland. Therefore, modulation of cAPK may be a locus of regulation for DMI-mediated depression of ß-adrenergic responsiveness.

69.16 INTERACTIONS OF GENTAMICIN AND CALCIUM ON REFLEX ACTIVITY IN THE ISOLATED NEONATAL RAT SPINAL CORD PREPARATION. James M. Tolliver* and Jordan E. Warnick (SPON: N. BROOKES). Dept. of Pharmacol. & Exp. Ther., Univ. of MD School of Medicine, Baltimore, MD 21201.

The injection of aminoglycoside antibiotics in the subdural space at lumbar segment 1 in rats results in an immediate flaccid hindlimb paralysis lasting 3 to 7 hours, depending on the dose and the particular antibiotic injected. Electrophysiologic recordings (membrane and action potentials, ACh sensitivity, spontaneous and evoked transmitter release) at the neuromuscular junction of extensor digitorum longus and soleus muscles as well as standard in vivo twitch experiments did not demonstrate any abnormalities in neuromuscular transmission during the period when hindlimbs were paralyzed. These facts and the observation that the 'H' reflex, but not the 'M' response, is absent during the paralytic state in rats indicates that the paralysis is mediated at the spinal cord level and not in the nerve or at the neuromuscular junction. We therefore elected to further study the effects of gentamicin (GEN) on spinal reflex activity using the isolated hemisected spinal cord of neonatal rats. Spinal cords from 5- to 7-day old neonatal rats were isolated, hemisected and superfused with oxygenated Krebs' solution. Suction electrodes were used to stimulate the dorsal root and record reflex activity from the corresponding ventral root. The frequency-dependent depression of reflex activity was measured as the ratio of the 10th/1st reflex responses evoked by stimulating the dorsal root with a train of 10 pulses at 0.1, 0.2, 0.5, 1.0 and 2.0 Hz. Reflexes were displayed on a storage oscilloscope and recorded on tape for later computer-assisted analysis. Reflex activity was dependent on the external calcium ($[Ca^{2+}]_o$): in the absence of added $[Ca^{2+}]_o$, reflexes were absent but with a gradual increase in $[Ca^{2+}]_o$ from 0 to 10 mM, reflex activity increased reaching a maximum at 5 mM $[Ca^{2+}]_o$. At 10 mM $[Ca^{2+}]_o$, reflex activity was 90% of that observed at 5 mM. In the presence of 5 mM $[Ca^{2+}]_o$, GEN produced a concentration-dependent depression of reflex activity with 2.5 mM causing a 50% depression of monosynaptic reflex amplitude (IC_{50}). Reducing the $[Ca^{2+}]_o$ to 2.5 mM lowered the IC_{50} to 0.8 mM while raising it to 10 mM increased the IC_{50} to 6 mM. Increasing the $[Ca^{2+}]_o$ from 0 to 10 mM decreased the frequency-dependent depression whereas increasing concentrations of GEN (1-8 mM) greatly attenuated this depression. Elevation of the $[Ca^{2+}]_o$ antagonized the increase in frequency-dependent depression induced by GEN. Conversely, reducing the $[Ca^{2+}]_o$ potentiated GEN-induced frequency dependent derpession. These observations indicate that GEN blocks spinal cord reflex activity, that the block is Ca-dependent and that GEN most likely interferes with transmitter release at synapses within the spinal cord. (Supported in part by the U.S. Army Medical Research and Development Command contract DAMD17-81-C-1279.)

69.17 D-AMPHETAMINE INHIBITS MEMBRANE METHYLTRANSFERASE ACTIVITIES. B. H. Rohde and J. C. Flynn. Inst. Research Childhood Learning Disorders, Baylor University, Waco, TX 76798.

Food colorings are reported to exacerbate hyperkinetic behavior in children and D-amphetamine can alleviate this behavior. We have earlier reported that a mixture of common food colorings can inhibit membrane phospholipid methyltransferase (Fed. Proc. 42, 1983), an enzyme system reported to be involved in synaptic transmission (Hirata and Axelrod, Science 209: 1082, 1980). We therefore sought to determine the effects of D-amphetamine on methyltransferase activity in rat brain synaptic plasma membrane fractions (SPM) and in human red blood cell ghosts.

Synaptic plasma membranes were prepared from whole brain of male Long-Evans rats. Human red blood cell ghosts were prepared from blood of adult males. Membranes were incubated with ^{3}H - S-adenosyl methionine (SAM) according to the procedure of Hoffman and Cornatzer (Lipids 16: 233, 1981). A mixture of common food colorings and/or varying concentrations of D-amphetamine sulfate were added to the samples prior to the addition of SAM.

Addition of D-amphetamine caused a consistent decrease in the activity of methltransferase in rat brain SPM at concentrations as low as 1.1 uM. The maximal inhibition was about 30% and the IC_{50} around 10 uM. Addition of food coloring and D-amphetamine together resulted in inhibition equal to that of D-amphetamine alone.

Addition of food coloring to human red blood cell ghosts prior to incubation reduced methyltransferase activity by about 10%; this agreed with data obtained using rat brain SPM. D-amphetamine reduced activity by 14% (5.4 uM) or 19% (21.5 uM); food colorings did not potentiate the inhibition produced by 21.5 uM D-amphetamine. These results suggest that food colorings and D-amphetamine inhibit phospholipid methyltransferases, possibly by a similar mechanism involving the lipid bilayer.

(Supported by Wacker Foundation Grant and Frost Foundation Grant)

69.18 LITHIUM EFFECTS ON NEURONAL MEMBRANES IN RATS OF DIFFERENT AGES. I.J. Wajda, M. Banay-Schwartz* and A. Lajtha. Center for Neurochemistry, Rockland Research Institute, Ward's Island, N.Y. 10035

In our previous studies on lithium we found that chronic administration of lithium produced a number of changes in the brains of treated rats. The binding sites of [^{3}H]naloxone, [^{3}H]dihydromorphine, and [^{3}H]spiperone were reduced in the corpus striatum and the cerebral cortex. The levels of potassium increased significantly by 15-30%, and those of sodium showed a smaller, nonsignificant increase of 10%. The content of glycine, GABA, glutamic acid, lysine, alanine, and valine increased to different extent in several brain regions. Those experiments were performed on adult rats kept on lithium-containing diet for 3-4 weeks or three months. Changes in receptor binding, ionic content, and amino acid composition occurred only when the level of lithium in the brain was close to or higher than 1.0 ueq/g of tissue. Recently the clinical action of lithium salts administered during pregnancy was considered in a number of obstetrical cases. We examined, therefore, two groups of rats (3-day old and 6-week-old pups) kept on lithium diet during the entire gestation period and during extrauterine life. The dams fed lithium diet had high levels of lithium in the brain and plasma; increases in potassium, sodium, and a number of free amino acid levels; and a reduction in [^{3}H]spiperone binding sites. In the three-day-old pups there was no lithium present, and the binding of [^{3}H]spiperone and the levels of ions and amino acids were the same as in controls. Lithium was also absent from the brains of fetuses isolated by caesarean section after decapitation of the lithium-treated dams. In the six-week-old rats kept on lithium diet during the gestation period and during the six weeks of extrauterine life we found high levels of lithium in the brain and plasma, changes in the kinetic constants of [^{3}H]spiperone binding sites, and increases in potassium and sodium levels. The analysis of free amino acids in different regions of the brain showed increases in glycine, GABA, lysine, and taurine. The results indicate that there is a barrier preventing accumulation of lithium during pregnancy and during the lactation period in the offspring. It is only when the pups start the lithium-containing diet that the drug accumulates in the brain and changes in receptor, ion, and amino acid levels can be shown.

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69.19 IMMUNOCYTOCHEMICAL LOCALIZATION OF THE REGULATORY SUBUNIT OF TYPE II cAMP DEPENDENT PROTEIN KINASE (R_{II}) AND OF BINDING SITES FOR THIS SUBUNIT IN THE CENTRAL NERVOUS SYSTEM. P. De Camilli[1],M. Moretti*[1],F.Navone*[1],S.M.Lohomann-Walter*[2]and U.Walter*[2] [1]CNR Center of Cytopharmacol. and Dept. of Medical Pharmacol., Univ. of Milano, Italy;[2]Depts. of Physiol. Chem. Würzburg Univ., West Germany.

We have studied the distribution of R_{II} in the central nervous system of the rat using a specific antiserum directed against rat R_{II}. R_{II} was found to be highly concentrated in the neuronal tissue but was also detectable in nonneuronal cells, in particular in ependimal cells (cilia and basal bodies) and in cells of the choroid plexus. Neuronal R_{II} was present at high concentrations in the gray matter while it was practically undetectable in the major white matter tracts. Its pattern of distribution showed large variations from region to region of the CNS. In the spinal cord, and in the isodendritic core of the brain stem and of the forebrain it was detectable in all neurons. In contrast, it was present at very variable concentrations in individual cells of certain neuronal classes. For instance, it was present at very high concentrations in pyramidal cells of some cortical layers but practically undetectable in the same cells of other layers. In the cerebellum, Purkinje cells (which are rich in cGMP-dependent protein kinase) were unstained. Their cell bodies and dendrites, stood out as negative, unstained images outlined by immunoreactive material which appeared to be localized in axon terminals of other neurons. In order to study the bases for the compartmentalization of R_{II} in neurons (suggested by the absence of R_{II} immunoreactivity from the white matter) we have further extended our previous studies on the existence of binding sites for R_{II} on insoluble components of the neuronal cytoplasm. In agreement with our previous results carried out with heterologous R_{II} (Miller et al., PNAS 79, 5562, 1982), we have found that exogenous rat heart R_{II} binds with high affinity to neuronal dendrites, when incubated with fixed-frozen sections of brain tissue. As also previously showed by us, this binding appeared to be primarily mediated by MAP 2, a major substrate for the catalytic subunit of cAMP-dependent protein kinases. In support of this hypothesis, we have now found that R_{II} binds to a doublet of the same electrophoretic mobility of MAP 2 when incubated with paper blots of SDS gels of total brain homogenates. Binding sites for exogenous R_{II}, as well as endogenous MAP 2, were present at high concentration in dendrites of all neurons (including those negative for R_{II}), at the same concentration and with the same pattern of distribution in the dendritic trees of all cells of a given class. Thus it appears that while presence and concentration of MAP 2 in neurons correlates with their structural characteristics (De Camilli P., Miller P., Navone F., Theurkauf W.E. and Vallee R., unpublished results) the levels of R_{II} in neurons might be dependent upon other factors, possibly upon their synaptic connections.

70.1 SODIUM CURRENTS IN HUMAN MUSCLE UNDER VOLTAGE CLAMP. W.M. Roberts,* R.L. Ruff, and W. Almers.* Dept. of Physiology and Biophysics, SJ-40, Univ. of Washington, Seattle, WA 98195.

The loose patch clamp method (Stühmer & Almers, Proc. Natl. Acad. Sci. 79, 946, 1982), which uses an extracellular pipette to apply voltage steps and measure the resulting membrane currents, was modified to employ pipettes having two concentric barrels. Currents were measured through the center portion (5-15 μm diam.) of a larger (15-25 μm diam.) isopotential patch. This method allows voltage clamp recordings from intact, minimally dissected muscle fibers without enzymatic cleaning of the cell surface. Patch voltage clamp recordings were made from biopsy samples of human intercostal muscle obtained from patients undergoing surgery for reasons not involving muscle disorders.

Sodium channel kinetics were examined in 14 fibers to give the following average values (mean ± S.E.M.): Resting membrane potential -70 ± 3 mV; peak I_{Na} = 9 ± 2 mA/cm^2; reversal potential = +43 ± 2 mV; potential to elicit half-maximal I_{Na} = -52 ± 1.4 mV; potential to cause 50% inactivation in 100 ms = -86 ± 2.8 mV. Recordings were also made from rat omohyoid muscle fibers which yielded similar values for the above measurements, as well as for the voltage dependence of activation and inactivation time constants.

Three striking features of these currents are: (1) The maximal peak inward current was highly variable, with an average value (9 mA/cm^2) that was larger than previously reported for rat extensor digitorum longus (3.8 mA/cm^2) or sternomastoid (6.8 mA/cm^2; Pappone, J. Physiol. 306, 377, 1980), or frog semitendinosus (4.7 mA/cm^2; Hille & Campbell, J. Gen. Physiol. 67, 265, 1976). (2) All fibers showed a marked slow component of inactivation of the Na^+ current. (3) The observed late outward current densities were small relative to the peak inward currents.
R.L.R. was a recipient of an NIH Teacher-Investigator award (#NS00498). Supported by USPHS grant #NS18748.

70.2 Na^+ CONDUCTANCE INACTIVATION IS MODEL DEPENDENT. Dexter M. Easton, Department of Biological Science, The Florida State University, Tallahassee, Florida 32306

The increase of Na^+ current during V-clamp depolarization of excitable membrane is generally thought to involve opening of molecular gates in the membrane conductance channels, while the fall of the current from its maximum is credited to inactivation of conductance. These processes are inferences from the m and h terms in the mathematical model devised by Hodgkin and Huxley (1952) to describe the V clamp currents. A new mathematical theory describes each current, INa^+ or IK^+, as the resultant in each instance of 2 opposing processes. According to this theory, depolarization increases the existing conductance probability of both ion species in both directions, inward and outward through the membrane. There is no "inactivation" term. Instead, as $t \to \infty$, a new steady-state net conductance develops, which is the sum of the oppositely directed conductances, one nearly equal to the other in the instance of Na^+, more unequal for K^+. The Na^+ virtual currents grow at different rates but attain nearly equal (but opposite) levels as $t \to \infty$. Thus, there is an INa^+ peak. In the K^+ system, the levels as $t \to \infty$ are ordinarily sufficiently different that no IK^+ peak occurs. The theory suggests that net movement of an ion species may be attributed to the efficacy of the specific ion rejection/attraction system in relation to the electrochemical gradient. Conductance channels, according to this view, remain open, to an extent depending on the membrane potential. Thus the new theory does not require turn-off of conductance during V-clamp but instead attainment of a new steady state of inward-outward virtual conductance. The theory appears to shift control of charge movement away from the conductance channels. Instead it emphasizes the molecular organization responsible in the first instance for the ion distribution discrepency between inside and outside of the axon.
Aided by computing center and Psychobiology Research Center, FSU.

70.3 THE EXISTENCE OF DISCRETE CHANNEL-ASSOCIATED Ca^{2+} 'DOMAINS' MAY INFLUENCE THE RELATIONSHIP BETWEEN I_{Ca} AND Ca-MEDIATED PHENOMENA. J.E. Chad* and R. Eckert. Department of Biology and Ahmanson Laboratory of Neurobiology, UCLA, Los Angeles, CA 90024.

The properties and distribution of single-channel currents underlying the macroscopic calcium current, I_{Ca}, may be implicated in the kinetics of Ca-mediated processes taking place at the membrane. The dispersion of Ca^{2+} entering the cell is severely limited by buffering that produces steep gradients perpendicular to the membrane surface (Levy et al., 1982, Biophys. J. 37:182a). Assuming that dispersion is limited equally in all directions from the inner mouth of the Ca channel, each channel may control $\underline{a}Ca_i$ predominately within a miniature hemispheric 'domain' centered on that channel. Thus, $\underline{a}Ca_i$ associated with I_{Ca} may be distributed as a mosaic, with individual domains each controlling local Ca receptors, rather than as a laterally uniform layer controlling all Ca receptors. The density and distribution of active Ca channels may therefore be significant in determining quantitative relations between the macroscopic current and the overall response of a Ca-mediated process. Rates of channel cycling as well as domain size and overlap should influence these relations.

Evidence for the existence of functional Ca^{2+} domains appears in voltage-clamp measurements of Ca currents in Aplysia neurons. The apparent efficacy of entering Ca^{2+} in producing Ca-mediated inactivation of the Ca channels decreases with depolarization, contrary to the effect expected for Ca^{2+} reacting with a binding site within the membrane field. The voltage dependence of the apparent efficacy is linear in the range -40 to 0 mV, and has an extrapolated voltage intercept of +16 mV. This is closely correlated with the predictions of the constant field equation for the single-channel current in this voltage range, which also approaches linearity and has an extrapolated intercept of +18 mV. The apparent efficacy of Ca^{2+} in producing inactivation thus appears to depend more directly on the single-channel current than on the macroscopic current, just as predicted if Ca^{2+} accumulates in discrete domains, rather than in a uniform layer.

Dependence of Ca-mediated responses on single-channel currents and their domains might also be expected to produce hysteresis in the relationship between I_{Ca} and a Ca-mediated, saturating response at membrane potentials beyond the peak of the I_{Ca}/voltage curve, for with increasing positive potential more channels are activated whereas the single-channel current diminishes. Hysteresis which may arise in this way has been seen in Ca-dependent transmitter release at the squid giant synapse (Llinas et al., 1981, Biophys. J. 33:323). These examples suggest that spatially discrete, channel-associated Ca^{2+}'domains' may indeed have significance for certain membrane-related phenomena.
Supported by USPHS NS 8364 and NSF BNS 80-12346.

70.4 PASSIVE ELECTRICAL PROPERTIES OF NONUNIFORM TAPERED DENDRITES:A MATHEMATICAL COMPARISON WITH CYLINDRICAL DENDRITES. S.A. Ellias* and J.K. Stevens (SPON: M.A. Dichter). Dept. of Neurol., Mass. General Hosp., Boston, MA 02114 and Playfair Neuroscience Unit, University of Toronto, Toronto, Ont. Canada.

In order to assess the functional consequences of the well documented dendritic taper of many vertebrate neurons, the differential equations for passive voltage propagation were analyzed for idealized cell geometries. These included cells with either one or seven dendrites (all either cylindrical or tapered). All geometries had a 10μ diameter soma. Each unbranched dendrite had a length of 180μ, and a diameter of 1μ initially. The tapered dendrites had an exponential decrease in diameter to a final value of 0.1μ. The model parameters were a specific membrane resistance of 2000 ohm-cm^2, an axial resistance of 100 ohm-cm, a specific membrane capacitance of 1μF-cm^{-2}, a synaptic driving potential of 100 mV, and a synaptic conductance change of 2.0 nanosiemens of 1.0 msec or infinite duration. The transient problem was solved by a Crank-Nicholson procedure; the steady-state by back-solving an equivalent tridiagonal matrix for Kirchkoff's voltage law.

With dendritic taper, the soma voltage, Vs, is more sensitive to the location of solitary inputs in the unipolar and multipolar cells: e.g., in the steady state, Vs varied from 9.7 mV to 5.7 mV (cylindrical multipolar cell), as compared with 26.7 mV to 4.5 mV (tapered multipolar cell). In both cases, the inputs were at 20μ and 180μ, respectively, from the soma. Moreover, all but the most proximal inputs on the tapered dendrite are more effective at the soma than identically placed inputs on the cylindrical cell: e.g., in the steady state, Vs is 9.9 mV for an input at 120μ from the soma in the tapered multipolar cell, while it is only 9.6 mV for an input as close as 20μ to the soma in the cylindrical multipolar cell. These effects are due to the higher local input impedance of the tapered dendrite and the decreased load of each tapered dendrite on the soma. These factors can produce a higher Vs in the tapered dendrite in spite of a higher percentage voltage drop from input site to the soma in this geometry. The transient analysis has the same qualitative results, and demonstrates a faster rise time for Vs in the tapered dendrites for proximal inputs up to 100μ from the soma.

These results show that for one input, tapered dendrite segments can lead to an increased sensitivity of Vs to synaptic location, and an increase in proximal synaptic efficacy with earlier arrival times as compared with cylindrical processes.

Optimal shapes for axons and dendrites will be discussed.

70.5 **SYNAPTIC LOCATION: DISTAL, NOT PROXIMAL, SHUNTING SYNAPSES ARE BOTH EFFECTIVE AND SELECTIVE.**

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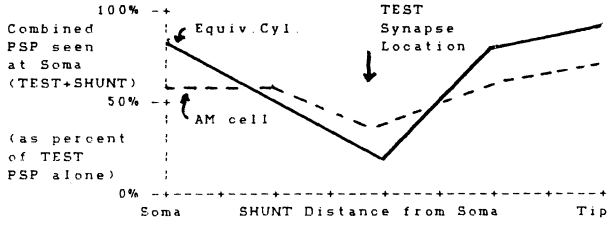
It has been suggested that critically located (such as at a branch point) proximal synapses might effectively shunt most of the synaptic current arriving from more distal synapses in that part of the dendritic tree, thus permitting one synapse to adjust the synaptic strength of whole dendrites, etc. We have tested this intuitive hypothesis in a variety of dendritic tree types and find that it is typically incorrect. Indeed, for the same synaptic conductance change, distal synapses are effective at shunting current developed at more proximal synapses.

Calculations were done using the Rall (1959) steady-state method. The test synapse was simply a 60 mV reversal potential in series with a on-or-off synaptic conductance of 1-10 nS (producing about 1 mV at the soma). The conditioning synapse was a pure shunt tenfold larger with reversal potential at the resting potential, as such, it is equivalent to a dendritic branch attached at the same location.

We examined the following cells: an equivalent cylinder model of cat spinal motoneuron with input to only one branch; models of the AM cell of lobster stomatogastric ganglion, the B4-5 and B8 motoneurons of Aplysia buccal ganglion, the L13 cell of Aplysia abdominal ganglion, and the rat superior colliculus' horizontal cell (see Graubard and Calvin, **The Neurosciences Fourth Study Program**). The graphs below, from the AM cell with 0.25 electrotonic length and the equivalent cylinder of 1.5 length, show typical results:



Thus the most effective place for a shunting synapse is adjacent to the TEST synapse -- but more distal sites for the SHUNT are sometimes quite effective because they too cause a large change in the input resistance seen by the test synapse. Though not as selective as presynaptic inhibition, a distal shunt diminishes those synaptic inputs on its dendrite far more than those on other dendrites (which are more insulated from the shunt's conductance increase). (Supported by NIH grants)

70.6 **THE SOMATIC SHUNT CABLE MODEL OF NEURONS: DERIVATION AND SOLUTIONS.** D. Durand. Addiction Research Foundation, Inst. of Biomed. Eng., U. of Toronto, Toronto, Ont. Applied Neural Control Lab., Dept. of Biomed. Eng., Case Western Reserve University, Cleveland, OH.

The Rall cable model has been widely used to determine the electrical properties of nerve cells. The dendritic tree is replaced by an equivalent cylinder and the soma by a lumped impedance. The specific membrane resistance is assumed constant over the whole surface of the cell. Although this model has been very successful at predicting the voltage decay following the injection of current pulses in some neurons, data generated both in motoneurons and hippocampal granule cells suggest the presence of a shunt at the soma to account for voltage decays faster than expected by the Rall model. This shunt can be interpreted by electrode penetration damage, electronic junctions or a lower specific membrane resistance at the soma than in the dentrites.

A new model was implemented consisting of an equivalent cylinder attached to a soma with a membrane time constant lower than in the dentrites. A variable ε was then introduced and defined as the ratio of the somatic to dentritic membrane time constant. A value of $\varepsilon=1$ corresponds to the Rall model with $\tau s=\tau m$ and $\varepsilon<1$ corresponds to the shunt model with $\tau m>\tau s$. The cable partial differential equation was then solved using this new boundary condition. The voltage decay following the injection of a short current pulse of width W into the cell is given by:

#1 $V(t)=C0.\exp(-t/\tau 0)+C1.\exp(-t/\tau 1)+...+Ci.\exp(-t/\tau i)+...$

where Ci are the coefficients and τi are the equalizing time constants determined by:

#2 $\tau i=\tau m.(1+\alpha i^{**}2)$

τm is the membrane time constant and αi the solutions of the following transcendental equation:

#3 $\alpha i.\beta i.L.\cot\alpha i.L=\alpha i.L.\coth L=k$ where:

L and ρ are the electronic length of the neuron and the somatic to dendritic conductance ratio. βi is defined by:

#4 $\beta i=(1-\varepsilon.(1+\alpha i^{**}2))/\alpha i^{**}2$

If I is the current injected and Rn the input resistance of the neuron, the coefficients Ci are then given by:

#5 $Ci=2IRn(1-\exp(-W/\tau i))(\rho+1)(\tau i/\tau m)/(\beta i+2\varepsilon+k+(\alpha i.\beta i.L)^{**}2/k)$

The inverse problem of estimating $L,\tau m,\rho,\varepsilon$ and Rn from the measured values of $C0,C1,\tau 0,\tau 1$ is solved by reducing the system of equations #1 to #5 to a system of 2 equations (#5 for i=0 and 1) with 2 independent variables L and τm. Initial values for these variables are fed into a computer program and an algorithm is then used to minimize the error between the measured and calculated values of coefficients and time constants. These equations were successfully tested on a compartmental model. Supported by NIH grant RO1NS16660-02 AND THE ADDICTION RESEARCH FOUNDATION.

70.7 ELECTROTONIC ANALYSIS OF MOUSE VENTRAL HORN NEURONS IN CULTURE: A.C. ANALYSIS. P.B.Guthrie and G.L.Westbrook. Lab.Developmental Neurobiology, NICHD/NIH, Bethesda, MD 20205.

The complex morphological structure of most neurons produces a complex electrical structure. In order to model interactions between synaptic inputs on different processes of a neuron, a detailed description of both the morphological and the electrical structure is necessary. An a.c. (alternating current) analysis should provide a more detailed electrical description of the neuron than other methods of electrotonic analysis.

Whole-cell patch electrode recording was used in experiments analyzing the a.c. properties of mouse ventral-horn (VH) neurons in dissociated cell culture. The low-impedance, low-capacitance patch electrodes generated lower noise and gave higher input impedances (suggesting that less damage was done to cells) than standard microelectrodes. As a result, the use of small currents (0.01-0.1nA) and small voltages (1-5mV) prevented activation of non-linear membrane properties. Patch electrodes could be unambiguously balanced to greater than 500 Hz (allowing a single electrode to be used for both current-passing and voltage-recording). For a.c. analysis, patch electrodes proved to be significantly better than standard microelectrodes. To further improve the signal-to-noise ratio, 1uM TTX was added to the bath medium to suppress spontaneous activity.

Impedance and phase shift (frequency range 1-500 Hz) were determined by using either single-frequency sine wave currents or complex sinusoidal currents consisting of up to 50 linearly summed sine waves. Voltage output and current-monitor output were averaged for 16 repeat cycles of the complex stimulus. Magnitudes and phase-shifts were obtained using a discrete fourier transform. Both a.c. methods gave similar results. The characteristic frequency (F_0) of the Bode plots (impedance vs. frequency) compared closely with the membrane time constant (tau_m) determined with step currents or with short impulse currents (range : F_0=36Hz,tau_m=5msec to F_0=6Hz,tau_m=20msec). Of interest interest, at high frequencies the phase-shift appeared to approach a maximum of less than 90° (range : 50° to 80°). This might be attributed to reflection at the ends of dendrites.

We have extensively modified SPICE2 (an electronic-circuit analysis computer model) to develop a compartmental neuronal modelling system : NEUROS. We are presently using NEUROS to model the response of HRP-filled VH neurons to step currents and to sinusoidal currents.

70.8 MEASUREMENT OF COMPLEX IMPEDANCE BY SINE-WAVE ANALYSIS IN TRIGEMINAL GANGLION NEURONS. B. Gimbarzevsky*, R. M. Miura* and E. Puil. Department of Pharmacology, Faculty of Medicine, and Department of Mathematics, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

An objective of our in vivo investigations has been to devise a methodology for estimation of certain electrical properties of neurons, not subject to the uncertainties associated with conventional methods using bridge-balance. We have found that the spherical, pseudo-unipolar cells of the trigeminal root ganglion present simple electrical behaviour; their intracellular voltage responses indicate a circuit comprised of a resistance and capacitance in parallel. Estimates of membrane input resistance and complex input impedance were obtained from neurons at their resting membrane potential (≤ -60 mV) using single microelectrodes (3M KCl or 5% Lucifer Yellow in 2M LiCl). These neurons were injected with small sinusoidal currents in the form of a multispectral current excitation function (MSCEF) such that $MSCEF(t) = I\ \sin(Bt^{e^{Ct}} + D)$ or $I\ \sin(Bt^{C} + D)$ where I is the amplitude of the current employed and B, C, D are empirically assigned constants. Complex impedance was calculated on-line using a pair of PDP-11 processors which were employed (1) to generate the MSCEF and sample the voltage response of the neuron and (2) to compute the total complex impedance from the ratio of the fast Fourier transforms (FFTs) of the intracellular voltage response and MSCEF(t). The amplitude of MSCEF was adjusted for a peak-to-peak displacement of membrane potential of 2-5 mV. Electrode capacitance was observed to be negligible and thus complex impedance was obtained after subtraction of the electrode resistance from the total complex impedance. When the input resistance of the neuron was calculated from a 10-20 mV displacement in membrane potential caused by the injection of a pulse of current via a balanced bridge, and from a 'flat', low frequency portion of the membrane transfer function, nearly identical values were obtained. Because it became necessary to monitor electrode resistance at frequent intervals during the intracellular recordings, a second method based on a least squares fit of the theoretical transfer function to the experimental data was developed for estimation of electrode resistance, neuronal input resistance and neuronal input capacitance. Application of the conventional and FFT methods to various networks of resistors and capacitors, and to neurons, revealed the greater accuracy, and especially precision, of the FFT methodology. Another important advantage is that estimates of neuronal input resistance obtained with FFT methodology contain a smaller contribution of voltage-activated channels.

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70.9 PASSIVE MEMBRANE PROPERTIES OF THE MAUTHNER CELL. W.D. Crank* and D.S. Faber (SPON: C. Smith). Div. Neurobiology, SUNYAB, Buffalo, N.Y. 14214.

The membrane resistivity, Rm, of the goldfish Mauthner cell (M-cell) has previously been estimated to be about 60-120 Ωcm^2 (Furshpan & Furukawa, J. Neurophysiol.,25:735, 1962), which is surprisingly low compared to other neurons. To more accurately determine Rm of the M-cell, a combined histological and computer modeling study was undertaken. The objective was to base the Rm estimate on accurate, detailed knowledge of cell shape and size.

M-cells were reconstructed from 30μm serial sections after iontophoretic HRP fills at the soma. Each reconstructed cell was divided into about 50 branches which were outlined on the digitizer tablet of a computer. Each branch was divided into many short (0.5 to 1.0μm) segments. Assuming circular X-section, each segment was taken to be a truncated cone. Total cell surface area was computed by summing edge surface area of all such segments from all branches. A resistive model of the cell was formed by representing each segment with axial and surface membrane resistances. Resistance values depended on segment dimensions and assumed resistivities.

Analysis of branch diameters showed that neither the lateral nor ventral M-cell dendrites follow the 3/2 power relationship, which implies that an equivalent cylinder representation is not justified. M-cell surface area averaged 2.65×10^{-3} cm^2. Multiplying by an input resistance value of 167 kΩ (Faber & Korn, J. Neurophysiol., 48:654, 1982), we estimate an upper bound for Rm of 442 $\Omega\ cm^2$. The resistive model made possible a more accurate estimate of Rm by taking into account the cell's cable-like behavior. For different assumed membrane resistivities, maps of input resistance over the entire cell were computed. Computed input resistance at the soma agreed with the experimental value when Rm was about 200 Ωcm^2 (n=3 cells). Our Rm estimate is higher than that of Furshpan and Furukawa, but is still low compared to other neurons.

From this Rm estimate and the 0.39 msec time constant of the M-cell (Fukami, Furukawa, & Asada, J. Gen. Physiol., 48:581, 1965), we estimate M-cell specific capacitance to be about 2.0 $\mu F/cm^2$. This value is within the range expected of a biological membrane (Cole, Membranes, Ions, and Impulses, 1968). Previous estimates were significantly higher.

One might suspect the low Rm to be due to coupling with the fibers forming electrotonic junctions on the M-cell. However, uncoupling with Co^{++} injections does not markedly affect input resistance (Faber, Kaars, & Zottoli, Neurosci.,5:433, 1980). Therefore, the low Rm may be an inherent property of the membrane itself. (Supported by NIH grant NS15335)

70.10 CABLE PROPERTIES, SURFACE HETEROGENEITY AND CIRCUITRY OF CULTURED MURINE SPINAL CORD NEURONS MEASURED USING MULTIPLE PATCH ELECTRODE EXTRACELLULAR RECORDINGS. R.P. Rand and B. Middagh*. Brock University, St. Catharines, Ontario L2S 3A1 Canada.

We have previously demonstrated that the patch electrode can be used as an extremely sensitive, non-destructive extracellular electrode of high spatial resolution. It measures to a large extent membrane patch capacitive current. In co-cultures of DRG and Raphe cells 'minispiking' in the submicroscopic architecture of neural processes could be detected and, rarely, the coupled activity of interconnected cells could be seen (Brain Res. 249; 371). We have extended these initial studies to the rather well characterized murine spinal cord tissue cultures in an attempt to measure the cable properties of neural processes,the possible heterogeneity of membrane conductance over the surface of neurons and the nature of the circuitry in this system. Unlike the former system 'minispiking' is rare. Simultaneous recording from two moveable patch electrodes provides an easy measure of conduction velocity of neurites, derived from multiple pairs of recording sites on the same neuron. When derived from the high spontaneous activity of these cultures the spatial-temporal relationships of the signals in these paired recordings often suggest that signal origins are multiple and the connectivity too complex to derive unambigious circuits. We have grown these cells in microcultures of 5-7 cells but the spontaneous activity is still too complex. Although often the measured patch current is a simple time derivative of the simultaneously measured membrane potential, at different sites on the same cell its time course can be qualitatively different. We are attempting to determine the contribution of intrinsic cable properties, surface heterogeneity of membrane conductance and possible artefacts derived from the patch technique to this difference.

70.11 DIGITAL ANALYSIS OF RELATIONS BETWEEN OLFACTORY RECEPTOR MORPHOLOGY AND UNIT FIELD POTENTIALS G. D. Adamek and R. C. Gesteland. Nose City, Northwestern Univ., Evanston, IL 60201

Unit field potentials recorded from olfactory receptor neurons of terrestrial vertebrates have more complex waveforms than any reported from elsewhere in the nervous system. The symmetry of the epithelial anatomy and the regular changes in cell morphology with developmental age permit identification of the processes contributing to the field potential. Knowing these we can infer the mechanisms of impulse initiation and propagation. We use ultra-low noise metal filled electrodes fabricated from patch-type glass blanks to record single units extracellularly. Waveforms are digitized at sampling intervals of 20 us using a processor with dual-bus architecture for direct memory access. In this work we examined how these waveforms varied with electrode position and between spontaneous and odor-evoked events, and as a function of regenerating epithelium thickness. We also found extracellular noise changes with durations comparable to those of action potentials. These are probably local membrane conductance increases which are subthreshold for action potential initiation. These have not been reported before. Action potential waveforms recorded from shallow penetrations are simple and of low amplitude. The receptor dendrites lie in this region. Below this, multiphasic, notched waveforms are seen. The relative amplitudes of the peaks and notches change systematically as the electrode tip traverses the layer occupied by the receptor somata. The depth of the soma of a cell and its dendrite length depend upon the developmental age of the cell. The most recently differentiated neurons lie deepest and have the longest dendrites. As new neurons are added just above the basement membrane, the somata of older cells are displaced upward and the dendrites shorten. Our results show that spike waveform can be used as a measure of the maturation state of the receptor. In some cells the waveforms of spontaneous potentials are different from those evoked by odors. odor stimulation. These cells are spontaneously active with wide variations in average frequency. Waveform information can be used to determine the true response by discounting the spontaneous events. During regeneration the epithelium gets thicker with age. Waveforms become complex with shorter durations as thickness increases. Since regeneration occurs in patches, the waveforms can be used to infer the developmental age and epithelium thickness in the region of the recording electrode. Each of these observations shows that morphological information normally obtainable only by tedious dye-marking and histological procedures can be inferred from voltage records.

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71.1 DIFFERENCES BETWEEN MYELIN-ASSOCIATED GLYCOPROTEIN FROM CENTRAL AND PERIPHERAL NERVOUS SYSTEMS DETECTED WITH MONOCLONAL ANTIBODIES. L.S. Marton* and K. Stefansson* (SPON: E.M. Stadlan). Department of Neurology and The Brain Research Institute, University of Chicago, Chicago, Illinois 60637.

Myelin-associated glycoprotein (MAG) (approximately 100,000 daltons) is a minor component of myelin. Originally it was found to be associated with myelin of normal brains. Immunocytochemical studies using polyclonal antisera have shown MAG to be located in the periaxonal region of mature myelinated axons and in the cytoplasm of oligodendroglia just before and during myelination. (Sternberger, N.H. et al. PNAS 76:1510, 1979) Subsequent biochemical investigations have demonstrated the presence of MAG in the peripheral nervous system (PNS). Our work with monoclonal antibodies indicates that CNS and PNS MAG may be different.

We have raised and characterized a rat monoclonal antibody (mab) that in Western blots binds to MAG purified from human hemispheric white matter. In tissue sections the mab stains brain and spinal cord myelin, and it stains peripheral nerve myelin very intensely. No staining of tissues outside of the nervous system was found. We have also studied a patient with monoclonal gammopathy and neuropathy whose monoclonal antibodies bind to MAG on Western blots and show the same immunohistochemical staining pattern of human central and peripheral myelin as the rat mab. (Stefansson, K. et al, Acta Neuropath. 59, 255, 1983) When we used Western blots to compare binding of the rat mab and the patient's mab to chloroform-methanol extracted homogenates of cerebral white matter and peripheral nerve we found some differences between the antibodies. Although both the patient's serum and the rat mab gave intense specific staining of CNS MAG, only the rat mab bound to peripheral nerve MAG. There was insignificant staining with the patient's serum. In addition, we were unable to block binding of the rat mab to CNS MAG with the patient's serum and vice versa. These results indicate that the rat and patient mab's recognize different antigenic determinants and furthermore they imply that PNS MAG may be distinct from CNS MAG.

71.2 IMMUNOBLOT IDENTIFICATION OF MYELIN BASIC PROTEIN IN GOLDFISH CNS MYELIN. B.I. Roots[1], D. Agrawal*[2], G. Weir[1]* and H.C. Agrawal[2]. [1]Dept. of Zool. Univ. of Toronto, Erindale College, Mississauga, Ont., L5L 1C6. [2]Dept. of Ped., Wash. Univ. Sch. Med., St. Louis, Missouri, 63178.

Goldfish were acclimated to 5°, 15° and 30°C and myelin was subsequently isolated. The myelin isolated from the brain and spinal cords exhibits a characteristic multilamellar structure with distinct major dense lines and intraperiod lines. When the proteins of brain myelin from fishes acclimated to these temperatures were analyzed by SDS-slab gel electrophoresis, there was a progressive increase in the proteins with a molecular weight of 13.5K with the increase in temperature (5°C-30°C). In order to establish that the 13.5K protein is, in fact, related to myelin basic protein of rodents, proteins of goldfish and rat brain myelin were separated by SDS gel electrophoresis, transferred to nitrocellulose sheets, and immunostained with anti-serum to human large basic protein (LBP) by the immunoblot technique. Since this protein in goldfish myelin was recognized by anti-serum to LBP, it is reasonable to conclude that this protein in goldfish myelin has antigenic sites similar to rat and human brain myelin basic protein. Surprisingly, the three proteins migrating to the position of 21K, 18K and 15.5K basic proteins were also immunostained. In addition, a number of high molecular weight proteins were also immunostained with anti-LBP serum. Furthermore, when proteins of goldfish myelin were immunostained with anti-PLP serum, no protein migrating to the position of proteolipid protein was seen. These observations induce us to postulate that the presence of PLP may not be a prerequisite for the presence of the intraperiod line. The significance of these observations in relation to the formation of the multilamellar myelin sheath will be discussed.

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71.3 APPEARANCE OF THE GLIAL FIBRILLARY ACID PROTEIN AND PHOSPHORYLATION OF THE NEUROFILAMENT PROTEINS IN THE DEVELOPING RAT NERVOUS SYSTEM. Michael J. Noetzel, Betty I. Roots and Harish C. Agrawal. Washington Univ. School of Medicine and University of Toronto, Erindale College, Ont., Canada L5L 1C6.

We have systematically studied the appearance of the glial fibrillary acidic protein (GFAP) in the nervous system of rats from birth to 37 days of life. Filaments were prepared from spinal cord and whole brain according to the method of Chiu, et al. (J. Neurochem. 27:147, 1981). Neurofilament preparations isolated from newborn spinal cord and brain were examined by electon microscopy; morphlogically distinct neurofilaments were clearly observed at birth. Filament proteins were separated by SDS-slab gel electrophoresis, transferred to cellulose nitrate sheets and immunostained with monospecific antiserum to bovine GFAP. This protein was present in spinal cord and whole brain preparations at birth. These studies were extended to determine if GFAP is phosphorylated at any stage of development. Rats (ages 0, 7, 14 and 36 days) were injected intracerebrally with ^{32}P-orthophosphate and sacrificed 24 h later. The filaments were isolated as described above. To determine if GFAP was phosphorylated, the filament proteins were transferred from gels to cellulose nitrate sheets and immunostained with anti-GFAP. The immunostained sheets were fluorographed. The glial fibrillary acidic protein per se was not found to be phosphorylated at any stage of development. In contrast, in filaments isolated from rat spinal cord, proteins migrating to the position of fodrin (Levine and Willard, Jour. Cell Biol. 90:631, 1981) and each of the individual neuronal specific neurofilament proteins (NFP: 210K, 145K and 70K) were clearly phosphorylated at all ages. The significance of phosphorylation of each of the individual NFPs in the developing nervous system and its possible role in the assembly of neurofilaments will be discussed.

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71.4 SCHWANN CELLS CONTAIN A PROTEIN SIMILAR TO THE CNS ASTROGLIAL FILAMENT PROTEIN. S.-H. Yen*[1] and K. L. Fields*[2]. (SPON: W. T. Norton). Dept. of Pathology[1], Neurology[2] and Neuroscience[2], Albert Einstein College of Med., Bronx, NY 10461.

An antiserum raised against the 49,000 dalton major protein subunit of human glial filaments (GF) binds to some elements in the peripheral nervous system. The GF related antigens in the peripheral nerve (PNS-GF) are detected by immunofluorescence in rat sciatic nerve, vagus nerve and bovine splenic nerve. They are distributed as single fibers or bundles of long thin fibers and are more abundant in unmyelinated nerves than in mixed nerves. In the rat sciatic nerve they develop postnatally after day 12 and they remain in the nerve after transection. In teased nerves the immunofluorescent positive elements appear to be Schwann cells closely associated with unmyelinated axons. The PNS-GF are insoluble in Triton X-100 but could be extracted by 2% SDS. Electrophoresis of sciatic or splenic nerve cytoskeleton on SDS polyacrylamide gels shows that each preparation contains a protein band at the same molecular weight as the glial filament protein of rat brain astrocytes. Bovine splenic nerve preparations appear to have a higher concentration of PNS-GF than the sciatic nerve judging by Coomassie blue stain. Immunoblots of peripheral nerve cytoskeleton preparations show that the anti-GF antiserum reacts strongly with the protein band that comigrates with CNS-GF. The antiserum, in addition, binds weakly to a 58,000 dalton protein, which is more abundant than the PNS-GF band and comigrates with vimentin. Both the 58k and the GF (50k) bands react with the Pruss anti-IF monoclonal antibody. Incubation of the anti-GF antiserum with the cytoskeleton preparation of cultured rat astrocytes or chromatographically purified rat brain glial filament protein removes the antibody that binds to Schwann cells in tissue sections or the protein bands in SDS gels. The results demonstrate that Schwann cells of unmyelinated nerves contain a protein of immunological and biochemical properties indistinguishable from the CNS astroglial filament protein.

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71.5 PROLIFERATON AND SPECIFIC PROTEIN PRODUCTION IN OLIGODENDROCYTES AND OTHER GLIA FOLLOWING TRAUMA. S.K. Ludwin*. Dept. of Pathology, Queen's University, Kingston, Ontario.

Gliogenesis in the developing mammalian brain has been well studied, but it has long been felt that mature oligodendrocytes were post-mitotic cells, and did not proliferate in the mature central nervous system. In experimental models of demyelination and remyelination, oligodendrocytes have been shown to divide. In addition, oligodendrocytes during remyelination have also shown the presence of both myelin basic protein (BP) and myelin associated glycoprotein (MAG) in their cytoplasm, recapitulating the pattern seen in normal development. In order to determine whether the oligodendrocyte proliferation and specific production of proteins were a specific response to demyelination or remyelination or represented a more general response to central nervous system injury, small pieces of cortex were removed from adult male mice, and the proliferation of glial cells was studied by light and electron microscopic autoradiography. In some of the animals the fragments of tissue removed were replaced by explants of fetal and adult tissue. The brains were examined for the presence of both myelin oligodendrocyte proteins (myelin basic protein MBP and myelin associated glycoprotein, MAG) and astrocyte specific protein (glial fibrillary acid protein, GFAP). Three days after the induction of trauma a brisk mitotic response was seen in all the animals. Astrocytes and microglia showed marked uptake of tritiated thymidine. Labelled oligodendrocytes, indicating recent division, were found easily not only adjacent to injured tissue, but also in apparently normal tissue away from the site of the lesion. Although medium oligodendrocytes were most frequently labelled, occasionally dark oligodendrocytes also contained label. Mitosis occurred in intrafasicular (white matter) and grey matter oligodendrocytes, as well as in the perineuronal satellite oligodendrocytes. The frequency of labelling diminished the further away from the lesion, but labelled cells were seen across the corpus callosum into the white matter of the opposite hemisphere, as well as in the basal ganglia. Concomitant with the astrocytic proliferation, was a marked increase in the production of GFAP. In contrast only very occasional oligodendrocytes demonstrated staining with BP. MAG staining was seen in oligodendrocytes after injury, but the amount did not appear to be that much greater than that seen in normal animals. The results tend to suggest that oligodendrocyte proliferation can occur following nonspecific injury, but that the production of proteins specifically related to myelin is not necessarily stimulated by the same factors as those causing proliferation.

71.6 Complement Activation by Myelin: The binding and Activation of C1 in serum by isolated myelin results in formation of myelin membrane-associated membrane attack complex (MAC) of complement. M.L. Shin*, B.A. Silverman*, P. Vanguri*, and D.F. Carney* (SPON: E. Glaser). Dept. Path., Univ. Md. Sch. Med., Balt., MD. 21201.

Many pathological conditions of the central nervous system involve damage to and removal of myelin membrane. Extensive phagocytosis of myelin by macrophages and astrocytes and release of myelin constituents into cerebrospinal fluid and into systemic circulation following CNS injury have been well documented. However, very little is known about the initiation of this membrane damage and mechanism of disposal of the damaged tissue. We are interested in the interaction between complement and myelin membranes and its possible role in the disposal of damaged myelin in vivo because activation of complement generates both opsonin(s) and membrane attack complexes (MAC, C5b-9). Myelin isolated from brains of rat, mouse or human directly activates serum complement, while non-myelin membranes from the same brain do not. Activation of complement is shown by C1 transfer from myelin, cleavage of C3, and C5 consumption by myelin. Such activation generates C3b, an opsonic fragment, as well as inflammatory peptides such as C3a and C5a. We are further interested in whether activation of early acting complement components by myelin leads to the generation of MAC and the formation of myelin membrane-associated MAC, since activation and consumption of complement proteins does not always result in membrane damage (Joiner et al., J. Exp. Med. 155:797-919). Purified rat myelin was incubated with fresh human serum or with heat inactivated human serum for 60 min at 37°C. Myelin was then washed with 0.3M NaCl, 0.02M EDTA buffer, and solubilized with 1% Triton X-100 and 0.25% DOC. Aliquots of this solution were placed on a 40%-5% linear sucrose gradient. Gradients were centrifuged at 36,000 rpm for 16 hrs in a Beckman ultracentrifuge with SW 50.1 rotor, and fractionated. Each fraction was dialyzed and tested for the presence of MAC antigen by ELISA with Rabbit IgG against human MAC neoantigen, kindly supplied by Dr. Bhakdi. Detection of MAC in similar gradients of zymosan activated human serum or untreated human serum served as positive and negative controls. In this control gradient, fractions were assayed for MAC as well as the functional activities of C5. A single peak of MAC was observed at the base of the gradient of myelin incubated with human serum. MAC antigen was not detected in any fractions from gradient of myelin incubated with heat inactivated serum. MAC-containing fractions were further analyzed for subunit structure of MAC by SDS-PAGE, electroblotting and immunostaining. The results showed the presence of subunit structure of MAC, C5-C9. The lane stained with anti-C9 revealed bands corresponding to both C9 dimer as well as monomer.

71.7 MYELIN DEVELOPMENT IN HUMAN INFANT BRAIN (VICTIMS OF SUDDEN INFANT DEATH SYNDROME), S. E. Poduslo and Y. Jang*. Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Infants in Maryland who are victims of Sudden Infant Death Syndrome (SIDS) are required to undergo autopsy. Brain tissue from these infants was obtained through the SIDS Institute at the University of Maryland School of Medicine. Ten samples were evaluated (including two controls), with ages ranging from 24 to 350 days. Gray and white matter samples from each age were dissected for analysis. Myelin and other membrane subfractions were also isolated from each brain. Lipids were extracted from all the samples and were analyzed. No abnormalities in lipid composition were found with any of the SIDS samples.

It was found that the lipid composition of whole gray matter showed little change during development. Total galactolipids showed a slight increase (1.5% to 3.0% of the total lipids), while phosphatidylcholine declined (32% to 25% of the total lipids). The cholesterol and plasmalogen content remained relatively constant. The developmental changes in the lipid composition of whole white matter were more interesting. There was very little white matter present in whole brain during the younger ages examined. The amount of lipid as a per cent of the total wet weight increased from 4% to 9% during development. Total galactolipids increased from 12% to 23%, with the increase primarily in cerebrosides (9% to 19%). Total phospholipids decreased (65% to 54%), with the decrease mostly in phosphatidylcholine (23% to 15%). The plasmalogens also increased (10% to 14%).

Even though there was little white matter present at the younger ages, it was possible to isolate myelin from the tissues. The yield was quite low as expected. The yield of myelin increased during development from 0.5 to 5.8% of the total wet weight. Surprisingly the lipid composition of the myelin samples obtained even from the younger infants was quite similar to adult myelin. Galactolipids were 22% of the total lipid, cholesterol, 23%, and phospholipids, 52%. There did appear to be a slight increase in plasmalogens (11% to 16%) and a possible decrease in phosphatidylcholine (20% to 14%).

Thus myelin was isolated from infant brains during the first year of life. While the yield of myelin increased during development, the lipid composition was similar to adult myelin. Since the control samples fit quite well into this developmental pattern, it is concluded that there are no abnormalities in the lipid composition of myelin obtained from victims of Sudden Infant Death Syndrome.

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71.8 SAXITOXIN BINDING TO AMYELINATED MUTANT RAT BRAIN AND SPINAL CORD, A. L. Oaklander*, R. G. Pellegrino* and J. M. Ritchie*† (SPON: P. S. SPENCER). Institute of Neurotoxicology, Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461, and †Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT 06011.

Myelin deficiency (md) is an X-linked mutation in the Wistar rat characterized by failure to develop CNS myelin, tremors that worsen with age, and death at about 25 days of age. In this study, the md mutant was used to determine the effects of myelination on saxitoxin-binding capacity during postnatal development of the rat CNS. Saxitoxin (STX) is a toxin which binds specifically to voltage-sensitive sodium channels. ^{3}H-STX binding was assayed in the brain and spinal cord of 6- and 21-day-old md rats and their normal littermates. These timepoints encompass the period of most active myelination in the rat CNS.

Maximum binding values (fmol/mg wet weight) were as follows:

	BRAIN		SPINAL CORD	
	md	control	md	control
6 DAY	18	17	40	40
21 DAY	71	57	53	64

No significant change in apparent K_d was observed between md and control.

No statistically significant differences in STX-binding capacity were detected between md rats and normal littermates. This suggests that the presence or absence of myelin does not influence voltage-sensitive sodium channel density in rat CNS. A statistically significant increase (p 0.01) in STX-binding capacity was detected between 6 and 21 days in both brain and spinal cord. Thus, increased STX-binding capacity is associated with postnatal neural maturation in the rat CNS. In addition, at 6 days, STX binding is significantly greater (p 0.01) in spinal cord than in brain. This is consistant with the caudorostral axis of CNS maturation.

In summary, STX-binding capacity in the rat CNS appears to increase with maturation, but seems unaffected by amyelination.

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71.9 THYROID ABNORMALITIES IN MYELIN DEFICIENT JIMPY MICE. R.P. Skoff and K.M. Liu*. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

In a previous report (Skoff et al, Life Sciences, 24:2099, 1979), we reported morphologic abnormalities in the pituitaries and thyroid glands of jimpy-Tabby mice. As the animals were examined shortly after birth, we were unable to determine whether these changes were due to the jimpy gene or to the Tabby gene which is closely linked to the jimpy gene. To determine which gene is responsible for the abnormalities, we have studied crossover animals which express the jimpy gene but lack the Tabby marker gene (+jp/Y). Jimpy males (+jp/Y) were compared to normal males (++/Y), Tabby males (Ta+/Y), Tabby-jimpy males (Tajp/Y) and female carriers of the jimpy gene (Tajp/Ta+). The mice examined ranged in age from 14 to 22 days postnatal. At these ages, the animals exhibit tremors characteristic of the jimpy mutation and show the tabby coat color due to the Tabby gene. A light and electron microscopic quantitative analysis was performed on 7 jimpy males, 7 normal males, several Tabby-jimpy males and female carriers. The results of this analysis show that the thyroid glands of the jimpy-non-Tabby animals are similar to that of the newborn jimpy-Tabby mice. In the jimpy thyroids, the follicular cells are columnar and sparsely distributed around the colloid. In contrast, the follicular cells of the normal animal are cuboidal and somewhat evenly spaced around the follicle. The number of follicular cells surrounding the colloid of the jimpy mice is half the normal values. The follicular cells themselves are very densely stained and some of them appear pyknotic in the jimpy. At the ultrastructural level, large extracellular spaces are present between the jimpy follicular cells. Another striking difference is that almost every follicular cell in the jimpy is accompanied by a small capillary. Quantitative analysis reveals a twofold increase in the number of vessels associated with a follicle. However, other parameters of the thyroid gland are normal including the size of the gland, the number of follicles and the surface volume of the colloid. Thus, the development and basic organization of the jimpy thyroid appears normal. The results of the present study demonstrate for the first time that the jimpy mice exhibit abnormalities outside the Central Nervous System. Assays of thyroid hormone levels are in progress to determine whether a physiologic abnormality is also present. (This research supported by NS 15338.)

71.10 ACYLATION OF PLP AND DM-20 IN THE QUAKING MOUSE. M.M. Garwood* and H.C. Agrawal (Spon: T.W. Jasper). Dept. of Pediatrics, Wash. Univ. Med. Sch., St. Louis, MO 63178.

Myelin isolated from quaking mouse brains has been reported to have a severe deficiency of PLP. This study was designed to examine, in vitro, the synthesis and acylation of PLP and DM-20 in the whole brain and myelin of quaking, littermate control, homozygous dominant control and albino mice, using ^{35}S-cystine and ^{3}H-palmitic acid. Proteins of the whole brain and myelin were separated by SDS-gel electrophoresis, the gels were stained with Coomassie blue and the radioactivity determined by liquid scintillation spectrometry or fluorography. Evidence will be presented that a band migrating exactly to the position of PLP is apparently absent while DM-20 is present in the whole brain extract and myelin of the quaking mutant. These findings were confirmed by transferring the proteins from acrylamide gels to cellulose nitrate sheets, followed by immunostaining with antiserum to rat brain myelin PLP by the immunoblot technique.

Since PLP has been shown to be acylated in vivo (Agrawal, H.C., et al., J. Biol. Chem., 257:4588, 1982) and in vitro (Townsend, L.E., et al., J. Biol. Chem., 257: 9745, 1982) by ^{3}H-palmitic acid, brain slices from the four groups of animals were incubated with ^{3}H-palmitic acid and ^{35}S-cystine, and the myelin proteins and Folch-Lees proteolipid protein fraction from the whole brain were subjected to SDS gel electrophoresis. The gels were sliced into 1 mm sections and the radioactivity was determined. DM-20 was clearly labeled in quaking brain as well as in the brains of the three control groups. However, there was little labeling of PLP in brains of quaking mice, whereas PLP from the three control groups was well labeled under identical conditions. The significance of these results will be discussed.

Supported by NIH Training Grant, 2T32NS-07027-05 and NIH Grant, NS19414.

71.11 MODULATION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE) IN GUINEA PIGS BY POST-SENSITIZATION ADMINISTRATION OF AN ANTI-T CELL MONOCLONAL ANTIBODY. R.A. Sobel*, B.W. Blanchette*, J.L. Hanzakos* and R.B. Colvin*. (SPON: L.R. Wechsler). Dept. of Pathology, Massachusetts General Hospital, Boston, MA 02114.

EAE is a well characterized, T-cell mediated, neuroimmunological disease model. To investigate the role of T cell immunoregulation in vivo in EAE, adult (>500g) strain 13 guinea pigs (GP) were sensitized with GP spinal cord and complete Freund's adjuvant. Ten days after sensitization, 12 GP were given a single intraperitoneal dose of 3.4 mg 8BE6, an anti-GP pan-T cell monoclonal antibody (E. Shevach, NIH). In preliminary studies, this dose caused a transient decrease in circulating T cells and suppressed delayed hypersensitivity skin reactions. Controls received 3.4 mg MOPC 21, the parent mouse myeloma (5 GP), normal saline (3 GP), or no treatment (2 GP). CNS tissues were analyzed in luxol-fast blue-H.& E.-stained paraffin sections and 4 micron cryostat sections stained with monoclonal antibodies to T cells and IgM, using avidin-biotin immunoperoxidase techniques. 10/10 MOPC 21, saline, and untreated controls and 4/12 8BE6-treated GP developed acute, paralytic EAE, 11-21 days after sensitization ($p<.002$) and had typical mononuclear infiltrates composed of many T cells and few IgM+ cells. The other 8BE6-treated GP were well or showed mild neurologic signs during the acute period. On observation for up to 6 months, 6/8 of these GP developed relapsing or progressive neurologic deficits. They had large demyelinative plaques and persistent parenchymal and meningeal T and IgM+ cell CNS infiltrates characteristic of chronic EAE. Five other GP were treated with 8BE6 from days 11-13 at the earliest detectable onset of neurologic signs. These GP exhibited a rapid neurologic deterioration within hours after treatment and showed loss of T cell staining, necrosis of inflammatory cells, and prominent acute inflammatory cells and fibrin deposits in CNS sections. These experiments demonstrate: 1) a new model of chronic EAE (previously only occurring consistently in GP sensitized as juveniles) produced by post-sensitization modulation with an anti-T cell monoclonal antibody and 2) acute neurologic deterioration associated with T cell lysis following anti-T cell monoclonal antibody therapy given after the onset of neurological signs. The findings illustrate the importance of T cell immunoregulation in the different forms of EAE. These studies have important implications for understanding the pathogenesis and for potential therapy of neuroimmunological diseases using monoclonal antibodies. (Supported by NIH CA-20822 and the Kroc Foundation).

71.PO PROCEDURE FOR ISOLATION OF GANGLIOSIDES FROM SMALL- AND MEDIUM-SIZE SAMPLES IN HIGH YIELD AND PURITY. R.W. Ledeen, M.C. Byrne*, J.R. Sclafani* and D.A. Aquino*. Depts. Neurol. and Biochem., Albert Einstein College of Medicine, Bronx, NY 10461.

Now that gangliosides have been shown to induce neurite growth and to manifest other biological properties in neural cells, greater attention is being paid to the purity of ganglioside preparations. This aspect was not dealt with in detail among the several published methods for ganglioside isolation; our study has found some of these to yield preparations with considerable levels of phospholipid and peptide contamination. Lipophilic peptide contaminants are a particular problem in gangliosides isolated from CNS white matter and peripheral nerve. We have found that both purity and yield can be significantly enhanced by a procedure employing Sephadex LH-20 chromatography as the first step, which removes much of the peptide. A key feature of this method is mild acidification (0.05 N HCl, final conc.) of the mixture after thorough homogenization of the tissue in 10 vols of chloroform (C)-methanol (M)-water (W) 5/5/1. This insured complete extraction, especially for white matter and nerve, presumably by dissociating gangliosides from (basic) proteins. The supernatant resulting from centrifugation is applied to a 17 g column of LH-20 (16 x 585 mm) packed in the same solvent (no acid). This removes most of the low MW contaminants as well as the acid. The ganglioside-containing fraction is then passed through DEAE-Sephadex A-25 and the eluted gangliosides treated with base as described (J. Neurochem. 21:839,1973). Base and salt are removed from this mixture by a second Sephadex LH-20 column, 18-19 g packed in M-W 95/5 (16 x 500 mm). Before packing, the LH-20 is thoroughly washed with M-W 95/5 containing 0.3 M KOH and then M-W 95/5. The sample in 4 ml of this solvent, after centrifuging, is applied to to column and eluted with the same solvent. Some of the residual peptide contaminants are also removed in this step. Repetition of this chromatography may be carried out to insure a completely salt-free sample. The gangliosides are finally purified on a small silica gel column of Iatrobeads or Unisil as described (Methods in Enzym. 83:139,1982). Bovine brain gray and white matter gave ganglioside mixtures with 16.6 and 47.4 µg peptide per mg ganglioside, resp. These levels were considerably below those in gangliosides isolated by 3 other procedures; the same was true of phospholipid contamination. Using [^{3}H]ganglioside tracer which was added to the original tissue homogenates, recoveries of 82% and 86% were obtained for gangliosides from bovine gray and white matter, resp. The method has been successfully applied to peripheral nerve samples weighing as little as 0.2 mg and to brain samples of 0.5 g or less. It has also been applied to myelin and cell cultures. [Supported by USPHS grants NS 04834 and NS 03356].

71.PO NODE-LIKE COMPLEXES IN THE SPINAL CORD OF MYELIN-DEFICIENT MUTANT RODENTS. J. Rosenbluth. Depts. of Physiology and Rehabilitation Medicine, N.Y.U. School of Medicine, New York, N.Y. 10016.

Nodes of Ranvier in the CNS are known to have an "undercoated" axolemma surrounded by a widened extracellular space containing a matrix material. Astrocytic microvilli have also been shown to surround the node in some cases. Based on these characteristics, several examples of node-like specializations associated with astrocytic processes have been identified in fiber tracts chronically demyelinated by EAE (Soffer & Raine, Brain Res., 186:301, 1980) or a glial toxin (Blakemore, personal communication) as well as in a region normally lacking myelin, the retina (Hildebrand & Waxman, Brain Res., 258:23, 1983). Equivalent aberrant nodal specializations have also been seen in the amyelinated spinal roots of dystrophic mice (Rosenbluth, J. Neurocytol., 8: 655, 1979) at sites of contact with Schwann cells.

In the present study, two mutants having grossly deficient CNS myelin, the jimpy mouse and the myelin-deficient rat, have been examined in order to determine whether such node-like complexes occur here as well. Survey of the spinal cord by electron microscopy of thin sections in both cases shows examples of axons that display a dense undercoating, usually extending only part way around their circumference, and a coextensive widened extracellular space. A matrix material is often visible in this space, usually in the form of a discontinuous, fuzzy lamina just outside the axolemma. Astrocytic processes surround the specialized region but usually do not form microvilli. No such complexes have been seen where axons are directly apposed to each other.

Since peripheral myelin is relatively normal in these mutants, the deficiency in the CNS myelin presumably arises from a defect in the oligodendrocytes. Thus, axons can form complexes resembling nodes of Ranvier in the CNS,even in the absence of contact with normal oligodendrocytes, where they are associated with astrocytic processes. Freeze-fracture studies have not so far shown corresponding E face particle accumulations in the axolemma. This could merely reflect the rarity of such node-like complexes or could indicate that undercoating of the axolemma in aberrant locations is not necessarily associated with intramembranous particle accumulation.

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CELL AND TISSUE CULTURE: BEHAVIOR OF NEURAL, GLIAL, MUSCLE, AND MODEL CELLS

72.1 A REPLICA PLATING TECHNIQUE FOR DETECTING MUSCLE CELL VARIANTS THAT ARE DEFICIENT IN THE ACETYLCHOLINE RECEPTOR. R.A. Black*, R. Brown*, and Z.W. Hall (SPON: J. Bixby). Div. Neurobiol. Dept. Physiol., Univ. Calif., San Francisco, CA 94143.

Genetic variants of established cell lines offer a powerful tool for defining physiological roles and the pathways of assembly and maturation of particular proteins. Application of this approach to problems of synaptic differentiation in muscle cells has previously been limited by the availability of suitable cell lines and by the difficulty of selecting appropriate mutants. Mutant selection is complicated by the fact that many of the proteins of interest are synthesized only after the cells have withdrawn from the cell cycle and have fused to form myotubes.

We have attempted to establish a system in which muscle cell variants can be selected using C_2, a mouse cell line whose properties in culture resemble those of primary muscle cells (Inestrosa et al, Exp. Cell Res. in press). The system is based on a replica plating technique for animal cells devised by Raetz et al (PNAS, USA 79, 3223 (1982)). As an initial test we have attempted to select variants that lack normal levels of the acetylcholine receptor (AChR).

We screened for variants by plating myoblasts at clonal density in a tissue culture dish and overlaying them with a polyester cloth. As the cells proliferated, each colony partitioned between the cloth and the dish. After several days, the cloth was removed and placed in medium which induces myotube formation. Myotubes were found to form on the cloth and to synthesize AChRs, which were detected by autoradiography of the cloth after incubation with ^{125}I- α-bungarotoxin. The cloth was subsequently stained to make the colonies visible, and AChR-deficient clones were located by comparing the stained cloth with the autoradiogram. Myoblasts from clones of interest were then recovered from the original plate.

In an initial screen with 2500 colonies derived from cells mutagenized with ethyl methanesulfonate, six colonies were identified as deficient in AChR. These were recloned and, after growth in fusion medium, the amount of AChR in cell extracts was quantitated by binding of ^{125}I- α-bungarotoxin. All had less than 25% of the wild-type level of AChR; control clones which were AChR-positive in the screen had levels of AChR 70-100% that of the wild-type cells. Of the six variants, five fused poorly, suggesting that they were defective in some aspect of differentiation. The sixth showed excellent fusion. Further characterization of this mutant is in progress. (This work was supported by Fellowship DRG-612 of the Damon Runyon-Walter Winchell Cancer Fund and by grants from NIH and MDA.)

72.2 SERUM AND SUBSTRATUM-MEDIATED INFLUENCES OF NEURITE GROWTH. S. Varon, S.D. Skaper, G. Davis*, I. Selak and M. Manthorpe. Dept. Biol., Sch. of Med., Univ.of Calif. San Diego, La Jolla, CA 92093.

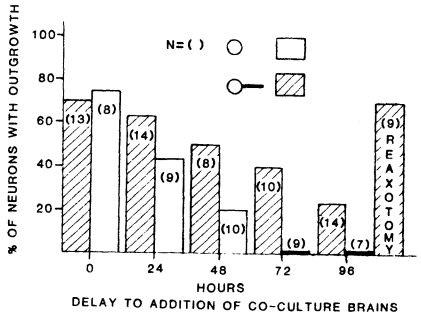
Modulation of neuritic growth involves both influences exerted directly on the growth cone by factors present in its humoral environment and influences mediated by or presented from the surfaces (cellular or extracellular) with which the growth cones come into contact. The classical example of a humoral influence is Nerve Growth Factor (NGF). The importance for neuritic growth of surface interactions has been well-established, and there is evidence for soluble biological macromolecules which promote neuritic outgrowth only after they have become surface bound.

In the present study, we have compared neuritic behaviors of selected neural tissues under the influence of i) the appropriate neuronotrophic agent, ii) serum-containing and defined serum-free media, and iii) three different culture substrata. Using first explant cultures of embryonic day 8 (E8) chick dorsal root ganglia (DRG) in the absence of NGF, serum-containing cultures displayed neuritic outgrowth that was negligible on collagen, sparse on polyornithine (PORN), and considerably more developed on PORN coated with a PORN-bindable neurite-promoting factor (PNPF-PORN) obtained from RN22 schwannoma cell-conditioned medium. The substrata also affected the outgrowth and/or morphology of ganglionic nonneurons. In the serum-free N1 medium neuritic outgrowth was markedly enhanced while retaining the differential influences of the substrata. In the presence of NGF, serum and substratum effects on DRG neurite outgrowth were masked by the massive response to NGF. Similar results were obtained with chick E11 sympathetic ganglia.

The more general occurrence of this serum-inhibitory effect on neurite growth was shown using monolayer cultures of chick E8 ciliary ganglia (CG) and PC12 cells, a clonal line of rat pheochromocytoma. Ciliary ganglion neurons, supplied with their trophic factor (CNTF), showed little, if any neurite growth on either collagen or PORN in the presence of serum, but extensive neurite development (especially so on PORN) in N1 medium. Serum delays the onset of neuritic growth using the PNPF-PORN substratum; however, there still is extensive neuritic growth at 24 hrs of culture. PC12 cells, cultured on PORN in the presence of serum, will extend neurites slowly (7-10 days) when NGF is added. In the N1 medium, however, neurite development with NGF occurs far more rapidly (1-2 days). Presentation of serum to such PC12 cells results in the rapid (15 min) retraction of all neurites. The serum susceptibility of PC12 neurites to serum decreases with increasing time in culture. This PC12 neurite response to serum has been used to define a bioassay for the inhibitory factor(s). Preliminary characterization indicates a slightly acidic protein species of 50-100,000 molecular weight.

72.3 COMPETENCE TO RESPOND TO NEURITE OUTGROWTH PROMOTING FACTOR(S) IN CELL CULTURE DECLINES WITH TIME, IS PROLONGED BY RESIDUAL AXON, AND COMPLETELY REGAINED BY REAXOTOMY. D. A. Bodnar and S. B. Kater. Department of Zoology, University of Iowa, Iowa City, IA 52242.

Isolated neurons of the snail Helisoma respond to a brain derived factor by producing extensive neurite outgrowth (Wong et al., 1981, J. Neurosci., 1:1008-1021). Identified neurons co-cultured simutaneously with brains (to condition the media) can initiate outgrowth either from spherical somata of isolated neurons or from neurons with a residual axon stump (see Haydon and Kater, these abstracts). There is a steady decline in the percentage of neurons producing neurites when co-culture brains are added at later times. However, the presence of residual axons slows this decline. For instance, brains added at 48 hours evoke only 20% of neurons plated as spheres to grow neurites, as compared to 50% of those with residual axons. Responsiveness to co-cultured brains is completely regained by reaxotomy.



This data set indicates that there is a requirement of temporal proximity of signals from axotomy and environmental factors in order to initiate neurite outgrowth.

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72.4 THE PC12 CELL LINE AS A MODEL FOR STUDY OF INTRACELLULAR TRANSGLUTAMINASE. J.C. Byrd, J.P. Schwartz, E. Costa. Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Transglutaminase (TGase), an enzyme which catalyzes the formation of isopeptide bonds between protein molecules, has been recently implicated as a mediator of several important cellular functions, including receptor-mediated endocytosis, regulation of plasma membrane rigidity, increased cellular adhesion, and mediation of RNA synthesis. Thus far these effects have been primarily studied by examining the effects of TGase inhibitors on these various processes in fibroblast or epithelial cell lines.

We have found the pheochromocytoma cell line PC12 to be a useful model system in which to study the role of TGase in neural crest cells. TGase activity in these cells is quite low (approximately 0.2 pmol/mg/min, using ^{3}H-putrescine as a substrate), and can be inhibited still further by a variety of well-known TGase inhibitors (dansylcadaverine, Ki = 260 μM; methylamine, Ki = 650 μM; ethylamine, Ki = 10.25 mM; and bacitracin, Ki 235 μM). Alternatively, TGase activity can be increased 20-fold by treatment with sodium butyrate. This increase in activity requires protein synthesis, reaches a maximum after 2 days of treatment, and is dose-dependent, with maximal stimulation at butyrate concentrations of 6 mM. Admixture studies suggest that this increase in activity is not due to the presence of an activator in the treated cells or an inhibitor in the control cells. While TGase activity in control cells is approximately equally divided between the soluble and particulate fractions, the TGase activity in butyrate-treated cells appears to be principally located in the soluble fraction.

In addition to these enzymatic changes, PC12 cells exhibit morphological changes in response to butyrate. Within approximately 12 hrs after treatment the cells begin to cease dividing, become flattened, and the amount of protein per cell increases about 2-fold during this time. This treatment does not alter cell viability.

We are currently using the PC12 model to study the many putative roles of intracellular transglutaminase, including the following: regulation of internalization of nerve growth factor and epidermal growth factor (PC12 has receptors for both), the role of isopeptide bonds in regulating the proliferative state of the cell, and the role of TGase in the regulation of protein synthesis.

72.5 QUANTITATION OF THE IN VITRO NEURONAL RESPONSE TO EXOGENOUS GANGLIOSIDES. K. C. Leskawa* and E. L. Hogan (SPON: J. Blackburn). Department of Neurology, The Medical University of South Carolina, Charleston, SC 29425

Many laboratories have reported that the inclusion of a ganglioside mixture (sialoglycosphingolipids from bovine brain) in tissue culture media stimulates the extension of neurites from cultured neuroblastoma cells. The effect of the individual gangliosides comprising this mixture remains to be critically assessed. We have purified to homogeneity individual gangliosides (possessing the gangliotetrose oligosaccharide backbone) from bovine brain and applied them at varying concentrations to mouse neuroblastoma cells (N2A) in vitro. After 48 hours of incubation, the cells were stained and examined with a Zeiss Videoplan, employing a program to measure neurite length, degree of sprouting (arborization) and other parameters. All the individual gangliosides tested promoted neurite extension in a dose-dependent fashion. Asialo-ganglioside GM1 was without significant effect however, which suggests that the presence of sialic acid (N-acetyl neuraminic acid) is important in eliciting this cellular response. With increasing concentrations of GM1 (5 to 500 ug/ml), average cellular neurite length increased significantly, whereas the number of neurites per cell decreased. Trisialosyl ganglioside GT1b exhibited slightly different effects. Neurite length did not increase to the magnitude seen with GM1, but promoted a significant increase in the number of neurites per cell (sprouting) as well as the degree of neurite branching (arborization). These results suggest that the in vitro neuronal response to an exogenous ganglioside mixture may be a combination of specific responses to each individual glycolipid comprising the total.

The technical assistance of James H. Nicholson, Dept. Pathology, is gratefully acknowledged.

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72.6 MODIFICATION OF HYDROPHOBIC SURFACES BY FLAMING PERMITS ADHESION OF DISSOCIATED MAMMALIAN CNS TISSUE. M. H. Hightower*, J. H. Lucas*, G. W. Gross and L. E. Czisny*. (SPON: L. Uphouse). Dept. of Biology, Texas Woman's University, Denton, Texas 76204.

Insulation of materials for multiple electrode plates (MEPs) must fulfill strict requirements of (1) optical clarity, (2) long-term stability under warm saline and after repeated sterilization, and (3) allow convenient electrode deinsulation. Suitable materials such as a polysiloxane resin (DC648, Dow Corning) in use in our laboratory are generally highly hydrophobic and do not permit cell adhesion. We have determined that such hydrophobic surfaces can be modified (i.e. made hydrophilic) by brief exposure to a gas flame.

Surface areas of water droplets were used as a quantitative measure of surface wettability. Flamed glass (FG) and flamed DC648-insulated (FI) glass plates exposed for 5 sec to the outer regions of a propane flame demonstrated average 350% and 850% droplet area increases respectively over unflamed controls (UC). Droplet area increases on FI and FG plates fell to less than 150% above UC plates after 20 min exposure to dry heat (100°C), autoclaving (130°C) or boiling. FI and FG plates under culture medium at 37°C for 20 d revealed gradual decreases in the hydrophilic effect (avg. 4.5%/d).

Dissociated spinal cord tissue from 11-14 d mouse embryos was seeded (10^6 cells/ml) onto FI and UC surfaces. Visual observations at 24 h revealed little cell adhesion to UC plates. Attachment to FI plates was much greater and characterized by small clumps and single cells. Polylysine coating of FI plates (PFI) improved adhesion greatly. PFI plates demonstrated the highest proportion of adhered cells generally attached singly. Process extension and interconnection were most extensive on PFI. A similar superiority of PFI could be observed after 14 d in culture. Polylysine coating of unflamed DC648 was ineffective. Adhered cells were removed from FI, PFI and UC plates at various times post seeding and counted. After the first 4 h cell adhesion to UC surfaces remained approximately 7.5%. Adhesion on FI plates increased linearly until by 26 h more than 25% of the cells had adhered. PFI plates showed a greater initial adhesion (>30% by 10 h) but no further significant increase by 26 h.

This surface modification has permitted recording of vigorous, spontaneous activity from mammalian CNS neurons grown on FI and PFI MEPs (Gross and Lucas, JEPT 9 1982). Recordings for periods up to 75 d have been obtained (Gross and Lucas. Neurosci. Abstr. 1983).

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72.7 NEURONAL RESPONSES TO PROCESS AMPUTATION VIA LASER MICROBEAM CELL SURGERY: ELECTROPHYSIOLOGICAL AND ULTRASTRUCTURAL CHANGES. J.H. Lucas*, G.W. Gross and M.L. Higgins* (SPON: J. Kirkpatrick) Department of Biology, The Texas Woman's University, Denton, Texas 76204

Cell surgery with a laser microbeam offers unique advantages of precision and control to the study of trauma on the single cell level. Responses to process amputation of single neuronal cells in vitro were monitored using standard techniques of intracellular recording. Surgery was performed on cultured spinal neurons from 12-14 day mouse embryos. Precise transections of primary cell processes were effected by the application of multiple, low-energy pulses from a UV laser microbeam (337 nm) focused to a diameter of 2.2 μm. Cumulative energy densities at the target ranged from 0.5 to 3.0 $\mu J/\mu m^2$. Resting potentials prior to surgery ranged from 48 to 56 mV with an average of 52.7 mV. The average culture age at the time of surgery was 24 days.

A variety of responses to neurite amputation was observed. In most cells, a loss of potential occurred following transection. In 37.5% of these the loss was irreversible. Complete recovery of resting potential was observed in 30% of the cells and partial, temporary repolarization in the other 31%. Overall depolarization rates ranged from 0.14 to 1.68 mV/sec with an average of 0.48 mV/sec. Rates of repolarization ranged from 0.04 to 0.49 mV/sec with an average of 0.22 mV/sec. Depolarization rates varied inversely as a function of target distance from the perikaryon. Target distances did not affect repolarization rates.

LM and TEM have revealed that multiple shot laser microbeam transections are preceded by process constriction in the target area. Local ultrastructural changes during pinching included loss of microtubules, mitochondrial disruption and increased tautness of the plasma membrane. In some cases loss of neurofilaments was also observed. Cells failing to transect completely usually recovered their prelasing dimensions.

We hypothesize that laser transection is initially a photobiological event involving absorption by NADP and NADPH which causes calcium release from mitochondria followed by subcellular contractile events and cytoskeletal collapse. At present we believe that recovery of resting potential, prevention of damage spread and cell survival after amputation depend on the rapidity of membrane resealing. Future experiments utilizing various pharmacological agents and manipulation of culture conditions will test these hypotheses.

Supported by NIH grant NS15167.

72.8 CHARACTERISTICS OF NEURONAL MONOLAYER NETWORK ACTIVITY RECORDED IN CULTURE WITH MULTIMICROELECTRODE SURFACES. G. W. Gross and J. H. Lucas*, Dept. of Biology, Texas Woman's University, Denton, Texas 76204.

With techniques described previously (Gross and Lucas, JEPT 9, 1982), we have been able to monitor vigorous, spontaneous activity from monolayer circuits of dispersed mouse spinal neurons for as long as 75 days. Under present culture conditions, an average of 20 out of 32 photoetched electrodes carry spontaneous activity with mean signal-to-noise ratios of 3:1. This ratio may exceed 20:1 if a neuron covers a recording crater. Glial cells usually do not cover these craters but tend to move around this surface irregularity. The signal-to-noise ratios as well as the number of active electrodes increase from the earliest observation times of 6 days to reach a peak at about 40 days in culture. This is followed by a gradual decline over the next 30 days. However total loss of activity has so far always resulted from contamination or equipment failure and culture life spans of over 100 days can be anticipated. Although spontaneous activity has been recorded from apparently isolated cells, most activity is complex and depends on interconnections made over the entire 0.5 x 1mm recording matrix. Functional connections have been confirmed by laser microbeam cell deletion and process transection during recording. Almost all types of firing patterns have been observed, with high frequency bursting being the most commonly observed pattern that becomes increasingly prominent as the culture ages. Average bursting cycles are 2 sec at 30 days and increase to 8 sec at 60 days. Tonic discharges occur, especially in younger cultures, in random or widely varying cyclic patterns.

We are presently testing a transparent metal oxide as our thin film conductor. This substantially improves optical monitoring of circuits over the previously used opaque gold conductors without sacrificing electrode stability and durability. However, transmission electron microscopy indicates that about 20% of the putative neuronal fibers at 30 days in culture are too small to be resolved by phase contrast microscopy. Although an unprecedented correlation between morphology and spike activity is, in principle, possible with this approach, the C-fiber content of cultured monolayers will complicate the interpretation of long-term optical and electrophysiological data.

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72.9 HISTOCHEMICAL AND ULTRASTRUCTURAL ANALYSIS OF CILIARY GANGLION NEURONS IN CULTURE. G. Crean and G. Pilar. Physiology Section, The Biological Sciences Group, The Univ. of Connecticut, Storrs, CT 06268

The endoplasmic reticulum of chick ciliary ganglion cells in vivo undergoes a typical organization during development. At St 34 the ribosomes are present as monosomes and polysomes. Scarce endoplasmic cisternae can be seen in the cytoplasm. At St 40, when peripheral synapses are formed, the ribosomes are organized in a highly structured rough endoplasmic reticulum (RER) localized in the periphery of the cell body. Since these changes did not occur in neurons deprived of the target it was hypothesized that contact with the target is important in development of ultrastructural characteristics of the neuron, which coincides with increase in enzymes in cholinergic metabolism (Landmesser and Pilar, 1978). Experiments were done to ascertain the influence of the target upon the ultrastructural characteristics of cultured neurons. Ciliary neurons were isolated at St 34 and 40 and histological studies were done at different times after plating, in culture medium containing 10% horse and 10% embryo extract serum. St 34 neurons, one day after plating, have dispersed ribosomes. After 5-7 days they develop a peripherally organized RER. When co-cultured with muscle the development of the reticulum was similar to that of neurons alone. RNA staining follows a similar pattern. St 40 cells when isolated and cultured lose the organization of the RER and fail to regain it. St 40 neurons extend neurites within 24 hours similar to St 34 neurons, but within 7 days all cells die in these culture conditions.

In an attempt to correlate the RER organization with the synthesis of a cholinergic enzyme, acetylcholinesterase (AChE) was localized histochemically after designated periods in culture. Preincubation in prostigmin and iso-OMPA were used to determine the specificity of the reaction. At the light microscopic level 75% of the cultured cells stain specifically for AChE. The stain covers the entire cell body. The remaining cells appear unstained. There was no difference in staining characteristics after the different times in culture, thus the AChE staining does not correlate with the presence of RER.

Since neurons in vitro make synaptic contact among themselves (Crean et al, 1982) it is possible that these putative contacts (non specific) are sufficient to trigger the fine structural maturation of the St 34 neuron we have observed. Alternatively the neurons may develop in vitro following an independent program for cytological development. The ability of neurons to survive for long periods in vitro seems to be related to the capacity of the neuron to develop an organized RER. Supported by U.S. Army Research Office and NS 10338.

72.10 DISPERSED CELL CULTURES FROM THE DENTATE GYRUS OF NEONATAL RATS. B.D. Boss, T.P. Condon*, E. Lanz*, and W.M. Cowan. The Salk Institute and Clayton Foundation for Research/California Division, P.O. Box 85800, San Diego, CA 92138.

To study the morphology and development of dentate granule cells in vitro, we have grown dissociated cells from the dentate gyrus of 4 or 5 day old rats in a variety of media and on different substrates. We have found that the cells survive optimally when first plated in a serum-containing medium and then switched to the chemically-defined medium N2 of Bottenstein and Sato (PNAS 76:514-517, 1979) with the elevated K^+ (25 mM) level recommended by Lasher and Zagon (Brain Res. 41:482-488, 1972). Under these conditions, dentate granule cells have been maintained for periods of up to 6 weeks.

During the first few days in culture (in the presence of serum), the granule cells are widely scattered, extend a number of neurites, and assume a complex multipolar form quite different from that of normal granule cells in vivo, but not unlike the ectopic granule cells seen in the reeler mouse (Stanfield and Cowan, J. Comp. Neurol. 185:393-422, 1979). This suggests that the normal morphology of dentate granule neurons is largely shaped by local factors within the stratum granulosum and the overlying molecular layer. After switching to N2 medium, the granule cells remain multipolar but become clumped together, and their processes form a complex network of fine fascicles. In co-cultures of hippocampal and dentate neurons maintained under the same serum-free conditions, interneurons become more prominent and many can be stained, as in the intact dentate gyrus, by antibodies against either cholecystokinin or glutamic acid decarboxylase (Ribak, Vaughn and Saito, Brain Res. 140:315-332, 1978). The increasing prominence of such cells suggests that they are not only favored by the culture conditions but also grow and differentiate in vitro.

Glial cells also appear to differentiate in serum-free medium. In the presence of serum they generally form a complete monolayer, but in N2 medium their rate of proliferation (as judged by ^3H-thymidine autoradiography) is appreciably slowed and they only underlie the clumps of neurons and fascicles of neurites. Under these conditions, most of the glial cells become stellate in form with many short, radiating processes that can be heavily stained with anti-glial fibrillary acidic protein and with a monoclonal antibody against S-100.

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72.11 MAINTENANCE OF IMMUNOCYTOLOGICALLY IDENTIFIED PURKINJE CELLS FROM MOUSE CEREBELLUM IN MONOLAYER CULTURE. Andrée Weber+, Udo Sonnhof+ and Melitta Schachner, Department of Neurobiology, University of Heidelberg, Fed. Rep. Germany.

PC1 antigen, a Purkinje cell-specific intracellular constituent recognized by monoclonal antibody, is localized in histological sections of cerebella from adult mice in Purkinje cell bodies, axons and dendrites (Weber and Schachner, Cell and Tissue Res. 227: 659-676, 1982). To investigate under which conditions Purkinje cells can be maintained in culture, PC1 antibody was used to identify Purkinje cells by indirect immunolabelling procedures in monolayer cultures of trypsin dissociated cerebellar cells from embryonic and early postnatal mice. PC1 positive cells were found to express other Purkinje cell-specific markers, such as PC2, PC3 (ibid.) and UCHT1 (Garson et al., Nature 298: 375-377, 1982), the neuronal marker L1, tetanus toxin receptors and the PC4, M1 and Thy-1 antigens. The glial markers, glial fibrillary acidic protein and O4 antigen were not expressed by these cells. Survival of Purkinje cells was best when cerebella were taken from mice not older than one day of age and cultured in chemically defined medium which facilitates the survival of neurons (Fischer, Neuroscience Letters 28: 325-329, 1982). PC1 antigen developed in vitro on the same time scale as in vivo, i.e. it was first detectable at postnatal day 3 to 4. At this stage cell bodies had a diameter of 13-14 µm and few processes. Dendrite-like arborizations, extension of usually one thin and long (0.5-1.6 mm) axon-like process with collaterals and cell body diameter of up to 18-19 µm developed with time in culture until the final form was reached by the equivalent of postnatal day 16 approximately. This form was reminiscent of the one described for Purkinje cells in certain cerebellar mouse mutants and in experimentally agranular cerebella. Ultrastructural features of these cells correlate with those described for their in vivo counterparts. Electrophysiological measurements showed that the cultured Purkinje cells were capable of spontaneous action potential discharges and excitatory and inhibitory synaptic potentials. These observations document that under the present culture conditions Purkinje cells are synaptically connected to other neurons.

72.12 PRIMARY CULTURE OF RAT CEREBELLAR NEURONS IN MEDIUM SUPPLEMENTED WITH BOVINE PAROTID GLAND EXTRACT. G. Merkel*, G. Patrick, V. Bergdall* and C. Phelps*. Indiana Univ. Sch. Med., Depts. of Microbiol. and Anatomy, Fort Wayne Ctr., Fort Wayne, IN 46805

Before studying the effects of lead on the physiology of cerebellar neurons and glial cells grown in primary culture, bovine salivary glands were reexamined as an inexpensive source of neuronal and glial growth factors.

Bovine parotid glands were minced in PBS, pH 7.2, containing DNase, RNase, and PMSF. The minced tissue was homogenized in a blender and centrifuged. The resulting supernatant constituted the crude extract (CE). The CE was subjected to precipitation with ammonium sulfate (AS) (30%, 55%, 75%, and 85% saturation). The precipitates (AS-CE) were each resuspended in Tris-NaCl buffer, dialyzed against the same, assayed for biological activity then applied to a column of Sephadex G100 and eluted with Tris-NaCl buffer. Eluted fractions were combined, lyophilized, dialyzed against PBS, and assayed for biological activity.

The biological activity of the extracts was determined by their ability to support neuron growth and process development in primary cultures. One to three day old rat cerebellums were collected, minced in PBS, and dissociated with trypsin. Cell viability ranged between 85-95% (trypan blue exclusion) and normally 1 x 10^6 cells/ml were recovered. The cells remaining in suspension following a 30-60 min. preattachment were distributed into culture flasks or micro-culture plates containing complete medium (DMEM, 10% FBS) w/ or w/o the AS-CE and G100 combined fractions. Neurons were distinguished from non-neuronal cells based on their phase-contrast morphology and staining reaction with cresyl violet.

The 55% and 75% AS-CE yielded cultures containing approximately 60-80% bipolar and multipolar neurons with extensive process development. Only non-neuronal cells were recovered in the abscence of the gland extracts. The G100 combined fractions corresponding to a mol. wgt. range of 50K-75K supported the growth of a mixed neuronal and non-neuronal culture. The G100 combined fractions corresponding to a molecular wgt. range of 25K-30K yielded nearly pure cultures of multipolar neurons. SDS and Analytical PAGE of the G100 combined fractions indicated the presence of a wide range of mol. wgt. polypeptides, including a 25K-30K dalton band which may correspond to the low mol. wgt. form of NGF (2.5 S). Additional results will be presented concerning the metabolic effects of the parotid extracts on neuronal and nonneuronal cells as well as the effects of sublethal concentrations of lead on neuron survival and process development in culture.

72.13 GROWTH AND DEVELOPMENT OF REASSOCIATED VASOPRESSIN NEURONS IN VITRO. S.L. Scharoun, D.M. Gash and M.F.D. Notter*. Dept. of Anatomy, Univ. of Rochester, Rochester, N.Y. 14642.

Results from our laboratory using a transplant model involving the grafting of vasopressin neurons into vasopressin-deficient Brattleboro rats for study of hypothalamic neurosecretory neuron development and function in vivo has been quite successful. To complement and extend these findings, we have recently developed both a reaggregate culture system and a monolayer culture system to study the effects of growth factors and hormones on the growth, development and innervation patterns of vasopressin neurons. The results of the experiments on the in vitro model system used in this present abstract will be used to select hormones and factors for test in vivo. By comparing the effects of these substances, both in cultured vasopressin neurons and neurons growing in the brain, our knowledge of factors important in regeneration and development should increase.

The anterior hypothalamus, including the supraoptic nucleus containing vasopressin neurons, was dissected out from normal embryonic and neonatal Long-Evans rats 12, 15, 17, 19 and 21 days post coitus (dpc) as well as from neonatal ages, day 1 and day 5. The cells were dispersed and resuspended in an MEM dispersion media. Various wells were co-cultured with their known target tissue, the posterior pituitary, to further analyze the influence of the target tissue on hormone production. At a designated end point, cultured cells were fixed and stained immunocytochemically with a rat neurophysin common to both vasopressin and oxytocin neurons. Radioimmunoassay of the media samples was performed for vasopressin quantification. Hypothalamic cells from all ages produced vasopressin as indicated by the RIA results of the media. Vasopressin (VP) levels in tissue after four days were 336.5 pg/10^6 cells while 507.7 pg VP/10^6 cells could be measured after eight days in culture. Hypothalamic cells co-cultured with dispersed pituitary tissue were shown to produce a significant increase in VP production. By varying serum factors, for example, replacement of fetal calf serum with maternal rat serum, an increased VP production of at least doubled levels was seen in reaggregate cultures. With the present study, we have shown that both the reaggregate culture system and the monolayer culture system can be adapted to a microassay of neurosecretory activity of selected regions of hypothalamus. Further, this microsystem can be employed quite readily to examine the effects of co-cultivation with target tissues as well as manipulation of various hormones and trophic factors on brain development at critical times.

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72.14 ORGANOTYPIC CULTURES IN DEFINED MEDIUM CONDITIONS. W.J. Hendelman, N. de Savigny* and K.C. Marxhall, Departments of Anatomy and Physiology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5.

The experimental use of organotypic cultures of the central nervous system has been limited to a certain extent by the complex medium required for their growth and maintenance. A completely defined medium would facilitate the use of these cultures in studies involving drugs and toxic agents. We have found a defined medium that results in satisfactory organotypic cultures of the cerebellum and the locus ceruleus.

Parasagittal fragments of newborn mouse cerebellum were explanted onto collagen-coated coverslips and cultured in the Maximow chamber. The adjacent peduncular region of the brain stem containing locus ceruleus neurons was explanted and co-cultured with some of these fragments. The standard medium used in such cultures consists of 50% Eagle's minimal essential medium (MEM), 25% chick embryo extract, 25% human serum, and a glucose level of 1100mg%. The completely defined medium has a basal component consisting of a 1:1 ratio of Dulbecco's MEM with either Ham's F12 or Ham's MCDB104, with the following additions: glutamine, insulin, progesterone, triiodothyronine, corticosterone, selenous acid, human transferrin, ascorbic acid, putrescine, and bovine serum albumin. Glucose levels were adjusted to 500-600mg% to produce a medium of approximately 330 mosm. HEPES was added to stabilize the pH.

It has been found that the development of the cultures requires an outgrowth of glia and processes, and "spreading" of the explant. In order for this to occur, serum-containing medium is used for the first 48 hours in vitro, and fibronectin is included in the defined medium. The main differences between the serum-fed (SF) and defined-medium (DM) cultures are: (i) myelin appears in abundance in DM cultures earlier than in SF cultures; (ii) at 3-4 weeks, there is better development of Purkinje neurons in SF cultures and more myelin; (iii) SF cultures show more spreading and more outgrowth. In cultures with locus ceruleus neurons, the catecholamine histofluorescence seemed comparable in both media conditions. The DM cultures are currently being investigated with electron microscopy.

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72.15 SLICE CULTURES OF NERVOUS TISSUE, B.H. Gähwiler, Preclinical Research, Sandoz Ltd., CH 4002 Basel, Switzerland

An attempt was made to combine the advantages of the slice and the culture techniques by culturing slices prepared from young animals. Cultured cells are organized in an organotypic monolayer and individual living neurons may be directly visualized and identified.

400 µm thick slices were prepared from 7-day-old rats and cultured for 1 to 3 months by means of the roller-tube technique. In cerebellar cultures, Purkinje cells and cells derived from the deep nuclei were recognized on the basis of their size, location within the culture and dendritic arborization as visualized following intrasomatic injection of lucifer yellow or horseradish peroxidase. The overall structure was preserved in cultures of the hippocampal formation. Pyramidal cells retained their bipolar orientation, with basal as well as optical dendrites, whereas the dendritic arbor of granule cells was always monopolar. Timms sulfide staining demonstrated the projection of granule cells to the dentate hilus and to the CA3 pyramidal cells, where the mossy fibers predominantly terminated within the supra-pyramidal layer. Like their in situ counterparts, the mossy fibers respected the CA3/CA1 borderline. In cultures prepared from the anterior hypothalamus, Golgi-like immunoperoxidase staining allowed visualization of groups of neurophysin-neurons bilaterally bordering on the third ventricle.

Co-cultivation of slices derived from different brain regions may be instrumental in elucidating mechanisms involved in synaptogenesis. As an example, AChE-positive fibers originating from a co-cultured septal explant were shown to provide a massive innervation of hippocampal neurons.

72.16 A GLIAL GROWTH FACTOR PRODUCED BY CEREBRAL ENDOTHELIAL CELLS IN CULTURE. D. N. Krause, I. E. Goetz*, J. N. A. Van Balgooy* and E. Roberts. Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

We recently reported that conditioned medium from cultures of cerebral capillary endothelial cells (CCE) contain a factor(s) which stimulates the proliferation of cultured glia cells (J. Cereb. Blood Flow Metab., 1983, in press). Cultured glial cell lines derived from bovine cerebral cortex all responded to CCE conditioned medium with a significantly greater increase in cell number as compared to controls. Preliminary observations with one line suggest that the conditioned medium also may promote differentiation of glial processes. Glial growth factor (GGF) was found in the medium of all bovine CCE lines, regardless of passage number (primary culture up to 18 passages). Serum was not required for GGF production. Soluble extracts of CCE cells also stimulated glial proliferation. It is not known whether the same factor(s) is responsible for the growth-promoting activity seen in the CCE conditioned medium and the CCE cell extract. In both cases, the activity was stable to freezing and heat labile (56°C). Cell extract activity was trypsin-sensitive. GGF in CCE conditioned medium is precipitated by 35% ammonium sulfate and is retained by an Amicon XM50 filter and a Sephadex G150 column. Preliminary results suggest that the GGF is a protein with a molecular weight between 50,000 and 100,000. This factor is much larger than a previously described smooth muscle/fibroblast growth factor derived from aortic endothelial cultures (Gajdusek, C. et al., J. Cell. Biol. 85:467). Production of GGF may be specific to endothelial cells because glial growth also was stimulated by media of cultured endothelia derived from either bovine middle cerebral artery or aorta. However, no growth-stimulating activity was seen with media from bovine aortic smooth muscle cells, human skin fibroblasts, or glial cells themselves. In situ, cerebral capillaries are seen in intimate association with astrocytic endfeet; thus an endothelial-derived glial growth factor may be important in the development and/or maintenance of glial-vascular relationships in brain. [Supported in part by USPHS grant NS18586 and the Hereditary Disease Foundation.]

72.17 OLIGODENDROCYTES DIFFERENTIATE EARLY IN SERUM-FREE MEDIUM. P.A. Eccleston* and D.H. Silberberg. Department of Neurology, Univ. of Penna. School of Medicine, Philadelphia, PA 19104.

Galactocerebroside (GalC) is a cell surface marker for oligodendrocytes. It is not expressed on immature precursors of oligodendrocytes, but is first detected by the use of anti-GalC antibodies at approximately the time of birth, in cultured rat CNS tissue. In order to study the effect of hormones and growth factors which control this stage of oligodendrocyte differentiation, we have formulated a serum-free medium.

The medium (SF-2) consists of a base (SF base) of Dulbecco's Modified Eagles Medium and Ham's F12 (1:1) with glucose (2 mg/ml) Hepes buffer, penicillin and streptomycin, supplemented with insulin, transferrin, selenium, and triiodothyronine (T3). Hydrocortisone and putrescine were found to have no effect on oligodendrocyte differentiation when included in this medium.

Dissociated cells from fetal (15-17 day embryonal) spinal cord or cerebrum or neonatal cerebrum were seeded in a medium containing 2.5% fetal calf serum on poly-l-lysine coated 12 mm glass coverslips. The next day after washing, the serum containing medium was replaced by serum-free medium or control medium.

To compare the number of oligodendrocytes present in various media, rabbit anti-GalC antisera was used followed by a rhodamine conjugated goat anti-rabbit IgG second antibody, for indirect immunofluorescence. For each experiment, the relative number of GalC+ cells was enumerated by counting 20 or occasionally 100 high power fields.

Deletions of single supplements from SF-2 resulted in a decreased number of GalC+ oligodendrocytes as compared to the complete medium. When each supplement was added singly to the SF base for six days of culture, no improvement was noted over the base alone, indicating that insulin, selenium, transferrin, and T3 have a synergystic effect when used in combination.

In serum-free medium (SF-2) GalC+ oligodendrocytes appeared earlier than in serum containing controls. This effect was most pronounced for cultured fetal tissue. In all cases tested, GalC+ cells were detected up to three days earlier than expected. Further studies revealed that this early differentiation occurred in both SF-2 and SF base, but not when 2.5% serum was added to either medium. In SF base, oligodendrocytes were demonstrated only at early time periods; after a week most cells were dead. From this we can conclude that the major function of the four supplements in SF-2 is one of promoting survival of GalC+ oligodendrocytes, rather than one of inducing differentiation, and that the removal of a factor present in fetal calf serum allows differentiation to occur.

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72.18 HORMONAL CONTROL OF ASTROCYTE PROLIFERATION IN CELL CULTURE Douglas A. Kniss and Richard W. Burry, Department of Anatomy, The Ohio State University, College of Medicine, Columbus, Ohio 43210.

Proliferation of glial cells is a common response to CNS trauma. This proliferation is often responsible for the formation of scar tissue which may impede regeneration attempts by neurons. Thus we used a cell culture model to investigate a way of controlling glial cell proliferation.

A dissociated cell culture system of CNS tissue was used to examine the ability of glucocorticoid hormones to control proliferation of glial cells. Dispersed cell cultures of 2-day rat cerebellum were prepared with standard methods and plated into Ham's F12 medium containing 3 mM potassium and 10% FCS. This low potassium concentration selects against neurons thus enriching the cultures in astrocytes and other non-neural cells. Immunocytochemistry with anti-GFAP (a gift from L. Eng) and anti-fibronectin (Cappell Laboratories) confirmed that the vast majority of cells in the culture were astrocytes (GFAP-positive), while, only a minority of cells per culture were immunoreactive for fibronectin.

On 3, 5, and 12 days in culture the cells were rinsed with buffer and switched to a chemically defined, serum-free medium containing: putrescine (100 µM), selenium (30 µM), insulin (5 µg/ml), transferrin (100 µg/ml), and BSA V (50 µg/ml). One group of cultures remained in this medium, while another group received 0.5 µM corticosterone in this same medium. Cells remained in these media for 48 hours. Twenty-four hours prior to harvesting, cells were exposed to 2 µCi/ml of ^{3}H-Thymidine (^{3}H-TdR) (20 Ci/ mmole). On the day of harvest, cells were rinsed, scraped from culture dishes, and precipitated with 10% TCA. Cell pellets were resuspended and aliquots were counted by liquid scintillation for acid-insoluble radioactivity (^{3}H-TdR incorporation). Four cultures were pooled per sample and triplicate cultures were run in all cases.

At days 5, 7, and 14 in vitro cultures receiving corticosterone incorporated significantly less ^{3}H-TdR into the acid-insoluble fraction than untreated cultures. Statistical significance was confirmed by a one-way analysis of variance ($p < .01$). Dexamethasone was also tested for its ability to inhibit proliferation and was found to be slightly more effective than corticosterone.

These results suggest that glucocorticoids may be effective in controlling astrocyte proliferation in vitro. Because of their action at the genomic level these hormones may provide a useful probe for the study of the control mechanisms of astrocyte proliferation. (Supported by NIH Grant NS-15894 to RWB and funds from the Spinal Cord Injury Research Center at The Ohio State University, NIH Grant NS-10165).

72.19 CYTOLOGICAL CHARACTERISTICS OF TRANSFORMATION IN TWO DISTINCTIVE CELL TYPES CULTURED FROM A GLIOMA. P.E. McKeever. Surgical Neurology Branch, Natl. Inst. of Neurol. and Commun. Dis. and Stroke, Bethesda, MD 20205.

Cells which retain a marker present in the parenchyma of glial tumors, glial fibrillary acidic protein (GFAP), and other cells which instead have fibronectin (FN) grow from a number of gliomas in early passage. The cells in explants of one of these gliomas were evaluated by phase contrast, by a nuclear fluorochrome and by the Giemsa method of predicting neoplastic transformation, correlated with double immunofluorescence for GFAP and FN.

Cells with GFAP and lacking FN had the following cytological features: cytoplasmic basophilia, reduced cytoplasmic spreading, hyperchromatic nuclei and high nuclear/cytoplasmic ratio. The average nuclear/cytoplasmic ratio was 0.30. These cells were smaller and less abundant than cells with FN and not GFAP.

Features of cells with FN and not GFAP varied. Some abnormal groups of cells had hyperchromatic nuclei and high nuclear/cytoplasmic ratio, while others resembled non-neoplastic fibroblast controls. The average nuclear/cytoplasmic ratio of the abnormal group was 0.32, more than triple the ratio of fibroblasts. Cells in this group had variation in nuclear and nucleolar size, shape and number, and abnormal mitoses. Cytologic features shared by transformed cells were present among each of the two distinctive types of cells which grew from this glioma. Not all features were recognized in each cell type.

72.20 NEUROTROPIC RETROVIRUS SPECIFIC VIRAL PROTEIN SYNTHESIS IN MURINE ASTROCYTES IN VITRO B.R.Brooks and E. Priester*. Department of Neurology, University of Wisconsin School of Medicine and William S. Middleton Veterans Administration Hospital, Madison, WI 53705

Non-cytopathic virus infection of cells by murine neurotropic retrovirus can be shown by co-cultivation of cells with XC cells which are transformed by Rous Sarcoma Virus and react with the viral glycoprotein on the surface of infected cells to result in fusion from without. We have prepared glial fibrillary acidic protein positive cells from the brains of mice at day 17 gestation and newborn day 1 as well as postnatal day 21 by trituration and enzyme dissociation. Such cells can be infected by murine neurotropic retrovirus without evidence of cell lysis and fuse with XC cells indicating the presence of viral glycoprotein. We have labelled cells with ^{3}H-leucine, prepared cell lysates, and formed immunoprecipitates with homologous heteroantibodies to the murine neurotopic retrovirus(CasBrM)or heterologous heteroantibodies to murine non-neurotropic retrovirus(AKR,RLV). Immunoprecipitates were also formed with homologous heteroantibodies to glial fibrillary acidic protein. The immunoprecipitates were electrophoresed in 10%, 3-12% and 3-27% polyacrylamide-SDS gels under reducing conditions. Fibroblasts were prepared from the muscles of mice at the above ages. Compared with fibroblasts, astrocyte cultures at all three ages showed a 50 kdalton protein which was precipitated with anti-glial fibrillary acidic protein. These astrocytes infected with virus showed the presence of virus specific p10, p12, p15, p30, Pr60, Pr80, and gp69/70 which were precipitated with heterologous antibody to intact virus or monospecific heterologous antibody to each of the above proteins. Synthesis of the precursor proteins Pr60 and Pr80 is similar in astrocytes and fibroblasts. The gag polyproteins and their cleaved subunits are identical in astrocytes and fibroblasts. In mouse embryo astrocytes Pr80 and gp69/70 are present but in mouse fibroblasts there is evidence for a new species of intermediate molecular weight which is demonstrable. In fibroblasts from older animals similar proteins are present. In newborn astrocytes Pr80 and gp69/70 are present but decreased gp69/70 is present in older astrocytes. These data suggest, but do not confirm, that virus specific protein synthesis is similar in embryo and newborn astrocytes and that cleavage of Pr80 to gp69/70 is decreased in astrocytes from older animals. In fibroblasts of all ages there may be a buildup of an intermediate cleavage product which is not present in astrocytes from younger animals. Therefore, virus specific protein synthesis is different for glycosylated proteins in astrocytes following infection than for non-glycosylated proteins compared to virus specific protein synthesis in fibroblasts.
(Supported in part by ALSSOA and VA Board of Medicine and Surgery)

72.21 A SPECIFIC GLIOTOXIC EFFECT OF DL-ALPHA-AMINOADIPIC ACID IN PRIMARY CELL CULTURES, S. Huck. Institute of Neuropharmacology, University of Vienna, A-1090 Vienna, Austria.

According to previous reports, DL-alpha-aminoadipic acid (aAA) is a specific gliotoxic agent not only in vivo (Olney et al., Neurosci.Lett.19,277,1980) but also in short-term incubated chick retinas (Casper et al., J.comp.Neurol.209,79,1982). We now report on DL-aAA effects in a recently characterized (Huck, Brain Res. Bull., in press) culture system with dissociated postnatal mouse cerebellum. The dissociated cerebellar cells essentially grow in a monolayer and can therefore be traced individually without sectioning or staining.

In addition to previously reported transient changes of glial cell morphology, DL-aAA induced karyopyknosis in astrocytes (identified by indirect immunoflurescence labelling of GFA) when applied in concentrations higher than 0.085mM. The number of astrocytes with pyknotic nuclei depended on the concentration of the substance and, in addition, on the duration of drug action. The presence of 0.21mM DL-aAA for 40h caused karyopyknosis in 50% of the GFA-positive cells. In a small number of astrocytes, even high concentrations of DL-aAA did not induce karyopyknosis. The majority of these resistant cells were of flat appearance and therefore differed morphologically from the affected cells which had distinctive processes. In accordance with previous studies, cells of neuronal morphology remained unaffected.

While D-aAA was found to be almost completely ineffective, a half-maximal effect could be induced by 0.097mM L-aAA in the same culture system. Since the purity of the preparations was established by gaschromatography (kindly conducted by Prof.Dr.E.Bayer, Univ. Tübingen, FRG), these data suggest that the active component of the racemate is L-aAA.

Interestingly, astrocytes in primary cultures of dissociated newborn mouse and rat cerebral cortex, but not C_6 glioma cells were found to respond to aAA.

Our data suggest that DL-aAA may be of great value to significantly reduce the number of astrocytes in primary cultures of the central nervous system. On the other hand, tissue culture experiments may take a key position in future studies on the mechanism of the gliotoxic lesion caused by aAA.
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73.1 A MONOSYNAPTIC SENSORY PROJECTION FROM THE KIDNEY TO THE BRAINSTEM. M. K. Donovan, N. Aboukarsh, S. R. Winternitz and J. M. Wyss. Depts. of Anatomy and CVRTC, Univ. of Alabama in Birmingham, Birmingham, AL 35294.

Recent evidence has suggested that renal afferent nerves participate in the pathogenesis of experimentally induced hypertension in the rat (Winternitz and Oparil, Hypertension 4, 1982). Although previous morphological and physiological studies have identified these afferents, only within the past two years have the central connections of these sensory neurons been traced. Most authors considered that these central processes would be made up of small unmyelinated fibers which had only short central projections; however, the experiments considered below indicate that at least a portion of the primary sensory afferents reach the level of the lower brainstem. Twenty adult, male, albino rats were anesthetized with nembutal and the left kidneys were surgically exposed through a dorsal incision. An injection of 2.0µl of fast blue (2% in H_2O) was placed into the kidney using a 10µl Hamilton syringe. Four days later, the animals were reanesthetized and a separate injection of 0.25µl of nuclear yellow (2% in H_2O) was placed bilaterally into the posterior medulla. Twenty-four hours later, the animals were transcardially perfused with saline followed by 10% buffered formalin. The kidney, brainstem and dorsal root ganglia (DRG) were removed and the dorsal root ganglia were placed directly on clean uncoated slides. The kidney and brainstem were cut at 30µm on a freezing microtome and placed on clean uncoated slides. All tissues were viewed under fluorescence microscopy using a Leitz filter A cube system. Inspection of the sections of the kidney indicated that the fast blue dye was restricted in most cases to a 1.5mm diameter area of the cortex and did not diffuse into the medulla of the kidney or into the renal nerve. The nuclear yellow injections were confined to the caudal one third of the medulla oblongata. Three populations of labeled DRG neurons were detected. One category included neurons labeled only by the posterior medullary injections. The nuclei of these cells were yellow and the cytoplasm was unlabeled. These cells were observed bilaterally at all DRG levels. Second, kidney injections resulted in the blue cytoplasmic labeling of ipsilateral DRG cell bodies at the T_8-L_2 levels with the greatest concentration at T_{13}. Of these labeled renal sensory neurons approximately 8% were also labeled by the medullary injection. When nuclear yellow was injected into the rostral third of the medulla oblongata no such double labeling was present. These results demonstrate that the central processes of these DRG neurons do reach caudal medullary levels, thus providing a monosynaptic renal sensory input to this region.

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73.2 ASCENDING SPINAL PATHWAYS IN THE TELEOST FISH, PRIONOTUS CAROLINUS. Thomas E. Finger. M.B.L., Woods Hole, MA 02543 and Dept. Anatomy, Univ. Colorado Sch. Med., Denver, CO 80262.

Searobins, Prionotus carolinus, possess modified pectoral fin rays which are moved actively to explore the substratum. Although the fin rays possess no taste buds and are innervated only by spinal nerves, the fin rays are chemosensitive and are used to locate food. The fin ray nerves terminate centrally in enlarged dorsal horns (spinal accessory lobes) located at the rostral end of the spinal cord (Finger, '82). Few dorsal root fibers ascend directly to the caudal medulla. HRP was injected into the spinal cord and elsewhere in the CNS to trace ascending spinal pathways.

The accessory spinal lobes project ipsilaterally to three main targets: the underlying spinal motor pool, the cerebellum, and a lateral funicular nucleus at the spinomedullary junction. Injections into the lateral funicular nucleus reveal two major ascending systems: 1) to the ipsilateral cerebellum, and 2) to a number of contralateral targets including the torus semicircularis, optic tectum and most importantly, nuc. preglomerulosus of the diencephalon. Both the direct and indirect spinocerebellar systems enter the cerebellum in the inferior cerebellar peduncle and terminate as mossy fibers in a limited paravermal portion of the cerebellar cortex.

The major fiber system emanating from the lateral funicular nucleus crosses the midline in the caudal medulla and ascends through the rostral medulla and pons along the ventrolateral margin of the tegmentum. In the caudal midbrain, the fiber bundle turns dorsally to end in a lateral subnucleus of the torus semicircularis and in a restricted ventrolateral portion of the mid optic tectum. The fibers which reach the optic tectum terminate predominantly in that portion of the optic tectum whose receptive field corresponds to that area of visual space in which the fin rays are present. The bulk of the fibers continue rostrally past these mesencephalic areas to terminate in a specific diencephalic target nucleus, the nuc. preglomerulosus. This pathway from spinal cord to ipsilateral funicular nucleus to contralateral specific thalamic target nucleus seems analagous to the dorsal column-medial lemniscus system reported in amniote vertebrates. The major difference is that in mammals, the dorsal column system consists mostly of collaterals of primary dorsal root fibers with an admixture of second-order fibers; whereas in Prionotus, the equivalent system consists almost entirely of second-order fibers. If these systems are homologous, this would lend further weight to the concept that the preglomerular nuclear complex in teleosts is homologous to the ventrobasal complex of mammals, a portion of the dorsal thalamus.

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73.3 CONNECTIONAL ANATOMY OF THE SOMATOSENSORY SYSTEM OF THE PIGEON. J. M. Wild. Dept. of Behavioural Biology, R.S.B.S., Australian National University, Canberra, A.C.T. Australia.

In order to investigate the connectional anatomy of the dorsal column-medial lemniscal system in the pigeon, 2 groups of experiments were performed. In the first, horseradish peroxidase-wheatgerm agglutinin (HRP-WGA) was (1) injected into primary feather follicles of the wing or tail; (2) applied to severed mixed, muscle or cutaneous nerves of the wing or leg; (3) injected into muscles of the wing. Frozen sections collected from the brainstem and cervical and lumbosacral spinal cord were processed with TMB and H_2O_2. Extraperikaryal terminal labeling was observed in the spinal grey following all HRP treatments: That from feather follicles was located at the dorsal periphery of the dorsal horn; that from cutaneous nerves was concentrated in the upper three dorsal horn laminae; and that from muscles and muscle nerves was located near neurons of Clarke's column and in more ventral laminae. In the medulla terminal labeling was observed following all HRP treatments except that involving tail feather follicle injections. At caudal levels the mixed leg nerves were represented in the gracile nucleus adjacent to the midline, and the mixed wing nerves in the cuneate nucleus slightly more laterally, although there was considerable overlap in their mediolateral representations. At successively more rostral medullary levels both these representations spread further and further laterally to occupy not only the dorsal column nuclei (DCN) but also the external cuneate nucleus (CuE) and parts of the descending trigeminal column (TTD). Terminal labeling was observed as far rostral as the eighth nerve. The large majority of the labeling appeared to be of cutaneous origin. In a second series of experiments ^{3}H-proline was injected into DCN and/or CuE and frozen sections were processed for autoradiography. No clear evidence for a cerebellar projection was found, but a dense accumulation of silver grains was distributed over the contralateral (and to a lesser extent the ipsilateral) dorsolateral posterior and anterior thalamic nuclei (DLP and DLA-DLM), particularly their ventral regions. Injections of HRP into these regions consistently retrogradely labeled neurons throughout the contralateral (and to a lesser extent the ipsilateral) DCN and CuE. Large injections of HRP into the cerebellum labeled only a few scattered neurons in DCN. These data demonstrate a substantial projection from the integument onto the DCN and CuE, and further show that these nuclei provide a major projection to the thalamus. The spinothalamic system was also investigated in these experiments, but very few retrogradely labeled neurons were found anywhere in the spinal cord following large injections of HRP covering the entire thalamus.

73.4 MORPHOLOGY AND TOPOGRAPHIC ORGANIZATION OF FUNCTIONALLY IDENTIFIED TRIGEMINAL (V) PRIMARY AFFERENT FIBERS IN RAT. M.F. Jacquin, R.D. Mooney and R.W. Rhoades. Dept. of Anatomy, UMDNJ-NJSOM & RMS, Piscataway, NJ 08854.

Intra-axonal injections of HRP were used to define the morphology of functionally characterized V primary afferents in rats. Thus far we have recovered 21 fibers (identified as primary afferents on the basis of high frequency following and invarient latency--range 0.4-0.9 ms--in response to V ganglion shocks). All labelled axons were visible in the V spinal tract for at least the length of subnucleus caudalis (Vc) and our analysis is restricted to this subnucleus. The topography of the primary afferent innervation of Vc was consistent with that shown in previous experiments. Fibers with rostral facial receptive fields (RF's) gave off collaterals in anterior Vc while those with caudal RF's innervated the posterior part of the subnucleus. Mandibular fibers innervated dorsomedial Vc while ophthalmic-maxillary afferents terminated ventrolaterally. There were also differences in the laminae innervated by ophthalmic and maxillary fibers. Low threshold maxillary afferents provided bouton-like swellings primarily in layers III and IV of central Vc. Ophthalmic fibers innervated laminae III and IV in ventral Vc and layer V in central Vc. Afferents innervating different vibrissae also had segregated arborizations. Dorsal and caudal vibrissae were represented in caudal and ventral Vc, primarily in lamina IV while terminals of fibers innervating rostral and ventral whiskers were located anteriorly and dorsally, largely in lamina III.

All of the collaterals from a given parent axon (an average of 4.4 were observed in Vc; no differences with respect to submodality or V division were noted) resembled each other as did those of functionally equivalent primary afferents. Differences between functional types were reflected in the shapes, laminar distributions and densities of terminal arbors. Vibration sensitive afferents had widely distributed arbors in laminae II-V and an extremely high (117) number of boutons/collateral. Rapidly adapting guard hair afferents also had widely distributed collaterals in layers III-V, but a somewhat lower number (77) of boutons/collateral. Rapidly adapting vibrissae afferents had circumscribed arborizations in lamina III or IV and an average of 51 boutons/collateral. Slowly adapting vibrissae afferents had a similar laminar distribution, but a slightly higher number (60) of boutons/collateral. Whisker afferents characterized as having a "wide dynamic range" had restricted terminal arborizations along the layer III-IV border and the fewest (40) boutons/ collateral.

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73.5 ARRANGEMENT OF TERMINAL ARBORS OF PHYSIOLOGICALLY IDENTIFIED VIBRISSA AFFERENTS IN THE RAT TRIGEMINAL SYSTEM. H. Hayashi* (SPON: S. Gobel). Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Bunkyo-ku, Tokyo 113, Japan.

Cutaneous primary afferent axons carrying sensory information from the facial skin enter the brain stem, ascend and/or descend within the spinal trigeminal tract, and give off collaterals into the underlying spinal trigeminal nucleus. The terminal arbors of these collaterals are thought to form a continuous sagittal sheet. The HRP-intracellular staining method was used to determine whether collateral arbors of vibrissa afferents conform to this description.

Adult rats were anesthetized and their brain stems exposed. Intra-axonal recordings were made in the spinal trigeminal tract with HRP-filled glass pipettes from 5 different kinds of vibrissa afferents classified according to Zucker and Welker (Brain Res., 1969) and then HRP was injected with current pulses. After the animals were perfused, frozen sections were made and processed with $CoCl_2$ and 3,3'-diaminobenzidine.

Forty-eight axons innervating vibrissa follicles on the muzzle were stained for distances of 2-11 mm. Collaterals issued from the parent axon entered the nucleus and arborized inside the nucleus. The parent axons were located in the middle of the tract.

Within the subnuclei interpolaris (Vi), oralis (Vo) and main sensory nucleus (i.e., rostral to the obex) the terminal arbors were restricted to the outer half of the nucleus with only a small gap separating their outermost extent from the overlying tract. Near the obex (i.e., the beginning of subnucleus caudalis, Vc), however, the arbors suddenly shifted deeper into Vc's lamina V. Within Vc, the terminal arbors of successively more caudal collaterals shifted superficially from lamina V into laminae III and IV.

These data indicate that the sheet-like terminal arbors of single vibrissa afferents are discontinuous at the level near the obex and consist of 2 sheets. The rostral sheet is located superficially while the caudal sheet begins deeper and then gradually shifts to a superficial location in the caudal part of Vc. Since sudden shifts of the sheets take place at the level between 0.5 mm rostral and 1 mm caudal to the obex, the rostral and caudal sheets of multiple axons overlap at this level.

73.6 PATTERN OF TRIGEMINAL AFFERENTS IN THE BRAINSTEM TRIGEMINAL COMPLEX OF THE RAT. C. A. Bates and H. P. Killackey*. Dept. of Psychobiol., Univ. of Calif. Irvine, Irvine, CA 92717

In the brainstem trigeminal complex, clusters of dense succinic dehydrogenase (SDH) staining are related in a one-to-one fashion to the mystacial vibrissae on the face. It has been suggested that the clusters of SDH activity are related to the terminals of trigeminal afferents (Belford and Killackey, JCN 183: 285, 1979). This study attempts to bridge the gap between the pattern of SDH activity and the pattern of trigeminal afferents in the brainstem.

Injections of 3-5 µl of WGA-HRP (Sigma) were made into the rostral portion of the trigeminal ganglion of six day old (PND 6) rat pups. The animals were sacrificed on PND 8 and processed according to the method of Itoh et al (Br. Res. 175:341, 1979).

Fiber labeling was observed at all levels of the brainstem trigeminal complex. In horizontal sections, four distinct areas can be distinguished by the pattern of labeled trigeminal afferents. We tentatively identify these areas as the principal sensory nucleus (PSN), and the three subnuclei of the spinal trigeminal nucleus (oralis, interpolaris, caudalis).

Rostrally, at the level of the trigeminal motor nucleus, dense terminal labeling is present in the principal sensory nucleus. No obvious pattern is apparent at this level, although there are variations in terminal density.

Posterior to PSN, in the spinal trigeminal nucleus, the most rostral portion has the lowest density of terminal arborizations and corresponds to subnucleus oralis. Trigeminal fibers leave the tract at right angles and extend across the nucleus in large bundles. Further caudally, in interpolaris, fibers are more dispersed, extending in small groups rather than large bundles. Fibers do not branch until they form terminal arborizations. These arborizations are aligned, forming a narrow rostrocaudal band extending the length of interpolaris. This pattern of terminal arborizations corresponds to the bands of SDH activity which extend rostrocaudally through interpolaris. This finding supports the idea of a one-to-one relationship between the vibrissae and trigeminal afferents as demonstrated by SDH studies. In the area related to caudalis, the arrangement of trigeminal afferents resembles that of interpolaris, however the band of dense terminals is located slightly lateral to the band in interpolaris.

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73.7 ULTRASTRUCTURE OF TRANSGANGLIONIC HRP TRANSPORT IN CAT TRIGEMINAL SYSTEM. M.A. Henry*, L.J. Johnson* and L.E. Westrum (Spon: J.D. Loeser). Depts. of Neurological Surgery and Biological Structure and Center for Research in Oral Biology, Univ. of Washington, Seattle, WA 98195.

Recent interest in dental projections has resulted in light microscopic (LM) reports of transganglionic HRP transport from teeth to brain stem. Since some controversy persists in this area ultrastructural (EM) correlates of these transport studies are essential. Since EM protocols for transganglionic transport of HRP have proven difficult the present study attempts to standardize these procedures so that the complete distribution pattern may be reproducibly seen. This would allow consistent identification of each type of ganglion cell, axon, terminal and synapse. An inferior alveolar nerve soak procedure which demonstrates consistent and dense labeling in the brain stem is used to manipulate variables in order to select the protocol that results in the maximum retention of transported HRP and optimum tissue preservation. The TMB method was used throughout. Variables to increase the amount of transported HRP to the brain stem include: survival time, use of WGA-HRP, and additives such as DMSO and poly-L-ornithine. Technical modifications to improve tissue preservation and the retention of transported HRP include: pH and H_2O_2 concentration of the incubation solutions, pH and storage time in the postincubation solution, pH and temperature of the osmication solution, and time and concentration of dehydration solutions. The standardized procedure was then used in experiments with HRP implants into single canines. Adjacent sections are processed for LM and reveal a periobex distribution of the TMB-HRP reaction product in partes interpolaris and caudalis similar to that seen in our earlier experiments. EM of these areas demonstrate a reaction product, within terminals, that is dense to pale with a lamellated or crystalline appearance. Terminals may contain either a single or multiple crystals. The terminals containing the crystals are from 1.5 to 3.0 microns in diameter, having round synaptic vesicles and aggregates of mitochondria. Sites of synaptic specialization are sometimes identifiable and are suggestive of Gray type 1 contacts. The size of some of the crystals of TMB-HRP reaction product is below the resolution for the light microscope. This further indicates that ultrastructural studies are necessary to demonstrate the submicroscopic but complete distribution of transported HRP for this or any given pathway.

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73.8 FINE STRUCTURAL ORGANIZATION OF THE BRAINSTEM SENSORY TRIGEMINAL COMPLEX IN THE RAT. L. S. Ide and H. P. Killackey*. Dept. of Psychobiology, University of California, Irvine, CA 92717.

The fine structural organization of the brainstem sensory trigeminal complex was investigated in normal tissue from adult albino rats. The main sensory nucleus as well as each of the subnuclei of the spinal trigeminal nucleus was examined. Observations on subnucleus caudalis were restricted to the deep layers.

Direct comparisons indicate that a similar synaptic organization characterizes the main sensory nucleus, the spinal subnucleus oralis, subnucleus interpolaris, and subnucleus caudalis (deep layers). Our observations on the synaptic organization of the sensory trigeminal complex generally agree with the description that Gobel and Dubner ('69) provided for the main sensory nucleus.

Throughout the sensory trigeminal complex dendrites and typically one or more synaptic terminals containing flat synaptic vesicles (F terminals) are grouped together with a large terminal containing round vesicles (R terminal) in characteristic "synaptic glomeruli." Within these glomerular arrays the large R terminal makes multiple asymmetric synaptic contacts onto dendritic elements. The F terminals make symmetric synaptic contacts onto the same elements. In addition, axo-axonic contacts occur between F and R terminals in which the R terminal is the postsynaptic element. F and R terminals in non-glomerular regions are heterogeneous in appearance. Occasional non-glomerular F terminals make distinctively long synaptic contacts onto dendrites in a manner not seen within glomeruli. Both large R and relatively large F terminals are occasionally seen to emerge from myelinated axons. Further, both of these types of synaptic terminal commonly occur as boutons en passant. In addition to contacting smaller-diameter dendrites (and spines) such terminals also make multiple synaptic contacts onto single large-diameter dendrites or onto the surface of perikarya.

While the main sensory and spinal trigeminal nuclei share many features of fine structural organization, some differences are evident. One of these is that cells receiving large numbers of axosomatic contacts are seen more commonly in the spinal nucleus. These contacts are made by both F and R synaptic terminals. Large numbers of axosomatic contacts are particularly characteristic of the larger neurons that distinguish much of the spinal trigeminal nucleus. Comparable neurons are rarely seen in the main sensory nucleus.

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73.9 A GOLGI ANALYSIS OF TRIGEMINAL NUCLEUS INTERPOLARIS IN THE ADULT RAT. K. D. Phelan and W. M. Falls, Department of Anatomy, Michigan State University, East Lansing, MI 48824

Neurons in trigeminal nucleus interpolaris (TNI) were examined in the adult rat using the Golgi method. On the basis of their overall morphology and the distribution of their axons and dendrites, at least seven distinct populations of TNI neurons were recognized. Three types of SMALL cells with rounded to fusiform somata (10-15 μm in diameter) were identified throughout TNI. The dendritic arbors of one type extended 700 μm or more in the rostrocaudal axis and were characterized by long (up to 400 μm) primary dendrites. The other two types of small cells displayed spherical to elliptical-shaped dendritic fields up to 300 μm in diameter. They differed from each other by the presence of either beaded or spine-laden dendrites. Many spiny cells were located near the spinal V tract and some exhibited extensive unmyelinated axonal arborizations confined within the dendritic trees of the parent cells. On the basis of this axonal morphology these spiny neurons are thought to function as Golgi Type II interneurons involved in the intrinsic circuitry of TNI. Three types of MEDIUM cells (15-30 μm cell bodies) were distributed throughout TNI. The first type had fusiform to triangular-shaped somata which gave rise to 3 to 4 widely spaced primary dendrites. The dendrites branched infrequently and formed spherical to elliptical-shaped dendritic arbors up to 500 μm in diameter. The second type of medium neuron was distinguished by a pyramidal-shaped cell body which generated apical and basal cone-shaped dendritic fields which together spanned nearly 350 μm of TNI. The remaining type of medium cell possessed a polygonal-shaped cell body which emitted 4 to 6 primary dendrites. Its highly branched dendritic arbor occupied an elliptical domain up to 400 μm in diameter. The latter two types of medium cells were observed in both spiny and aspiny varieties. The LARGEST cells in TNI were concentrated in the rostral half of the nucleus. They had 25-40 μm polygonal-shaped somata which emitted 3 to 4 primary dendrites. These primary dendrites gave rise to extensive dendritic fields extending over 700 μm in the rostrocaudal axis. The dendrites of these cells emitted widely scattered spines and some higher order branches traveled up to 300 μm without branching. Both medium and large TNI cells are considered to function as Golgi Type I projection neurons on the basis of the morphology of their initial axonal segment. This study provides for the first time detailed information concerning the dendritic and axonal patterns of TNI neurons and demonstrates that the populations of small and medium neurons observed in Nissl preparations are each composed of at least three morphologically distinct types. Supported by NIH/BRSG to the College of Osteopathic Medicine, M.S.U.

73.10 TRIGEMINAL AND SPINAL PROJECTIONS TO THE PARABRACHIAL AREA IN THE CAT. W.M. Panneton and H. Burton. Dept. Anat., St. Louis Univ., St. Louis, MO 63104 and Dept. Anat. and Neurobiol., Washington Univ., St. Louis, MO 63110.

In recent investigations on intratrigeminal pathways in the cat (Brain Res. 236:463, 1982) we noted numerous neurons retrogradely-labeled with horseradish peroxidase (HRP) in lamina III and lamina IV of the medullary dorsal horn (MDH) while only a few labeled cells were found in lamina I and lamina V after injections centered in the principal trigeminal nucleus. However, when the injection spread into more dorsal and lateral pontine areas, including the parabrachial and Kolliker-Fuse (KF) nuclei, numerous labeled neurons also were found in laminae I and V. The projection to the dorsolateral pons now has been investigated more fully.

In five adult cats 20-100 nl of 1% WGA-HRP was injected into the parabrachial area. After a 48-hr. survival, the brainstem was sectioned serially through the levels of the trigeminal sensory complex. In three other cases with similar injections, either transverse or horizontal sections through the spinal cord also were cut. In those cases where the injection was confined to the parabrachial complex retrogradely-labeled neurons were found in lamina I, outer parts of lamina II and in lamina V in the MDH. These were especially prominent in dorsal and medial areas of the rostral MDH. In one case where the injection did not include the Kolliker-Fuse nucleus, neurons in lamina V were not labeled. Most labeled neurons were ipsilateral to the injection but some were seen in laminae I and V on the contralateral side. Neurons in lamina I in the spinal dorsal horn (SDH) at both the cervical and lumbar levels also were labeled bilaterally, and were best seen at the apex of the SDH. In the subnucleus interpolaris a few labeled neurons were scattered throughout the dorsal half of the nucleus after such injections. Most prominent, however, was the labeling of neurons interstitial to the spinal trigeminal tract especially dorsally in the paratrigeminal nucleus on the ipsilateral side.

Injections of HRP into the MDH (2 cases) and the cervical spinal cord (1 case) were done to define the peribrachial targets of these medullary and spinal neurons. Diffuse, granular reaction product was evident in the lateral parabrachial nucleus in all cases. Such label was evenly distributed bilaterally after the injection into the spinal cord but was mostly ipsilateral after the injections into the MDH. The injections into the MDH, especially that placed closer to the obex, also produced dense label in the KF area and the caudal part of the parabrachial nucleus along the medial edge of the brachium conjunctivum. (Supported by grants PHS 5 S07 RR05388-20 and PHS NS09809.)

73.11 INTRACELLULAR LABELLING WITH HRP OF IDENTIFIED NEURONS, PRIMARY AFFERENTS AND CORTICAL AFFERENTS IN THE CUNEATE NUCLEUS OF THE CAT. S. Cheema, R. Fyffe,* A. Light and A. Rustioni. Departments of Anatomy and Physiology, University of North Carolina, Chapel Hill, NC 27514

Intracellular or intraaxonal injection of horseradish peroxidase (HRP) enables the investigator to tackle questions related to structural correlates of neuronal function. In the present study thalamic projection neurons, primary and cortical afferents in the feline cuneate nucleus were investigated.

Neurons were identified by antidromic activation from the ventrobasal nuclei of the thalamus, their receptive fields determined, and then, if successfully impaled, were iontophoretically injected with HRP. After HRP histochemistry, sections were embedded in plastic wafers and viewed by LM prior to EM examination of HRP-labelled profiles. Soma diameters of thalamic projection neurons ranged from 10 to 25 μm. Dendritic ramifications were often extensive and carried many appendages and complex axon-like profiles which had "en-passant" and terminal enlargements. The axons of cuneo-thalamic cells coursed to the medial lemniscus and gave rise to collateral arborizations in the ventral parts of the cuneate nucleus, and occasionally in dorsal regions which did not overlap with the cell's dendritic domain.

Single primary afferent axons in the cuneate fasciculus were identified following natural stimulation. Axons activated by cutaneous stimulation of the forepaw terminated in the dorsal part of the middle cuneate nucleus; the terminal arborization of each axon appeared to be focussed upon a small group of neurons with occasional side branches that terminated outside this focal point. A cutaneous primary afferent sometimes gave rise to more than one bouquet of terminal boutons. Ia fibers from forelimb muscles terminated both in a marginal zone and in the ventral parts of the cuneate. Electronmicroscopical examination of primary afferent boutons showed these to contact mainly dendritic profiles.

Corticofugal fibers were recorded ventral to the cuneate nucleus and were identified by cortical stimulation. Corticonuclear axons coursed rostrally from the pyramidal decussation and issued collateral branches which gave rise to bouton-bearing arborizations which extended up to 2.5 mm in the rostrocaudal axis of the ventral cuneate nucleus. Other corticocuneate projections arose as collateral branches of corticospinal axons; their arborizations were restricted rostro-caudally (0.1 - 0.5 mm), and bore fewer boutons than corticonuclear axons. Synapses of corticocuneate fibers were located on small and medium dendrites.

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73.12 POST-SYNAPTIC DORSAL COLUMN FIBER TERMINAL PATTERNS IN THE NUCLEUS CUNEATUS OF THE CAT. J. Pierce, A. Rustioni, S. Cheema, and R. Fyffe*. Departments of Anatomy and Physiology, University of North Carolina, Chapel Hill, NC 27514.

The dorsal column system of the spinal cord has traditionally been viewed as an ascending pathway for primary afferent collaterals projecting to the dorsal column nuclei. However, the existence of a distinguishable post-synaptic dorsal column pathway, with distinct anatomical and electrophysiological characteristics, has been known for several years. Earlier studies using the successive degeneration technique (Rustioni, Brain Res., 1974) indicated that these fibers ascended in both the cuneate fasciculus and the dorsolateral fasciculus (DLF), terminating primarily in the ventral and rostral parts of the cuneate nucleus.

We are now reexamining this projection using degeneration and anterograde wheat germ agglutinin-horseradish peroxidase (WGA-HRP) transport, in series. Initially, dorsal root ganglionectomics are performed unilaterally from C2 to T1, and the ipsilateral dorsal columns are transected at T1. Two weeks later, at a time when most or all of the involved primary afferent fibers are not expected to transport WGA-HRP, a series of ten 0.1 μl WGA-HRP injections are made ipsilaterally, from C2 to T1. After a further 48 hours the animals are sacrificed, and the tissue is prepared for light or electron microscopy, after HRP histochemistry. This approach allows for both a more sensitive mapping of the terminal field, and an examination of individual synapses at the ultrastructural level.

Post-synaptic fibers, as shown by this approach, distribute within the cuneate nucleus in a pattern similar to that described in the earlier successive degeneration studies, although their termination in the dorsal cell cluster region appears more prominent than previously observed. The electron microscopic demonstration of synapses of post-synaptic fibers is made possible by a cobalt chloride intensification reaction using pyrocatechol and p-phenylenediamine as chromogens, with remarkably good preservation of the tissue. Current experiments are also underway to investigate the spatial relationship between the projections of primary and post-synaptic afferent fibers by combining the above approach with intraaxonal iontophoresis of HRP.

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73.13 SOMATOSENSORY RESPONSES OF DORSAL COLUMN NUCLEI NEURONS PROJECTING TO THE DORSAL MESENCEPHALON. Linda L. Cooper* and Jonathan O. Dostrovsky, Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Anatomical studies have identified a projection from the dorsal column nuclei (DCN) to the dorsal mesencephalon (DM) and have shown that only a very small proportion of the neurons which project to the DM also project to the thalamus. The aim of this investigation was to verify the projection from the DCN to the DM using electrophysiological techniques and to determine the functional characteristics and receptive field properties of both DCN neurons with projections only to the DM and DCN neurons with projections to both the DM and the thalamus.

Extracellular single unit recordings from DCN neurons were obtained in 20 chloralose anesthetized cats. Arrays of bi-polar stimulating electrodes were introduced into the contralateral DM and thalamus and were used to antidromically excite DCN neurons (0.1ms, 600uA maximum). Antidromic stimulation was used to identify the axonal projections of the DCN neurons. DCN neurons projecting to the DM were characterized with regard to their functional inputs and the location and size of their receptive fields.

Fifty-two DCN neurons were antidromically excited from the DM. 59% of these neurons could also be antidromically excited from the thalamus. Stimulating sites in the DM effective in antidromically exciting DCN neurons were within the external nucleus of the inferior colliculus, the intercollicular area and the caudal region of the ventrolateral superior colliculus. 77% of the DCN neurons with projections to the DM responded only to cutaneous stimulation, 18% of these neurons responded only to deep inputs and the remainder responded to both cutaneous and deep inputs. Responses to cutaneous stimulation resulted primarily from light mechanical stimulation of hairy skin and were rapidly adapting in nature. The receptive field sizes were similar to those previously reported by others and those examined by us for DCN neurons which projected only to the thalamus. However there was a clear trend for receptive fields of neurons projecting to the DM to be located on the proximal parts of the limbs, the torso or the neck. No clear differences were found between the receptive field properties of neurons projecting only to the DM and neurons projecting to both the thalamus and the DM.

These findings reveal that the DCN relay primarily information from rapidly adapting cutaneous receptors to the DM and are consistent with the proposed role of the DM in orientation toward external stimuli.

Supported by the Canadian Medical Research Council

73.14 MODULATION OF THE SENSORY RESPONSES OF CAT TRIGEMINAL AND CUNEATE NEURONS BY ELECTRICAL STIMULATION OF THE RED NUCLEUS. Bruce G. Gray and Jonathan O. Dostrovsky, Department of Physiology, University of Toronto, Toronto, Canada, M5S 1A8.

Anatomical studies have shown that the red nucleus of the cat projects to both the trigeminal subnucleus caudalis and the dorsal column nuclei in addition to its well known projection to the spinal cord. These facts suggest that the red nucleus may play a role in modulating the transmission of somatosensory information at various relay points. The aim of this study was to determine whether electrical stimulation of the red nucleus modulates the sensory responses of either trigeminal subnucleus caudalis or cuneate neurons.

Experiments were performed on chloralose anesthetized adult cats. Extracellular single unit recordings, using glass-coated platinum-plated tungsten microelectrodes, were obtained from both trigeminal subnucleus caudalis and cuneate neurons in each cat. Bi-polar stimulating electrodes were stereotaxically placed in the contralateral thalamus and red nucleus. All stimulation sites were subsequently verified histologically. A total of 23 trigeminal neurons (14 low threshold mechanoreceptive, 6 wide dynamic range and 3 nociceptive specific) and 20 cuneate neurons were studied in 5 cats. Neurons were excited to just suprathreshold levels with either electrical or mechanical stimuli applied to their receptive fields. Conditioning stimuli delivered to the red nucleus preceded the peripheral test stimulus by 130 ms and consisted of a 100 ms, 500 Hz train of 0.1 ms pulses. A current intensity of 150 uA was used as the cutoff point for establishing if inhibition was present. Over 90% of the cells in each region were found to be inhibited, including 2/2 trigeminothalamic and 8/8 cuneothalamic neurons. The mean current intensity needed to inhibit the trigeminal neurons was 55 uA while that needed to inhibit the cuneate neurons was 39 uA. Stimulation of the red nucleus caused excitation of 4 cells, 2 in each region. None of these latter cells were found to project to thalamus. These results, together with those previously reported for the spinal cord dorsal horn, indicate that the red nucleus can exert a powerful modulatory effect on neurons receiving somatosensory inputs. Thus one function of the red nucleus may be to inhibit the rostral flow of sensory information which is not relevant to the motor task being performed.

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73.15 THE TOPOGRAPHY OF DORSAL COLUMN NUCLEAR PROJECTIONS TO SENSORIMOTOR CORTEX THROUGH THE THALAMIC POSTERIOR NUCLEAR COMPLEX IN RATS. E.Luke Bold*, R.J. Kosinski and E.J. Neafsey. (SPON: M.A. Collins). Dept. of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153.

The present study was undertaken to compare the distribution of dorsal column nuclear projections to the thalamic posterior nucleus (PO) with the location of PO neurons projecting to forelimb sensory and motor as well as hindlimb sensorimotor cortical areas in the rat.

Prior to surgery, animals were anesthetized with Ketamine HCl (100mg/kg,IM) and placed in a stereotaxic apparatus. Within the same animal, 1% WGA-HRP was injected (0.02-0.05µl) into nucleus cuneatus or nucleus gracilis while True Blue or Nuclear Yellow (2%) was injected (0.3-0.5µl) into the corresponding electrophysiologically defined forelimb sensory, forelimb motor, or the hindlimb sensorimotor cortical area. After 1-4 day survival periods animals were overdosed with sodium pentobarbital and perfused transcardially with physiological saline followed by a 4% buffered paraformaldehyde solution and then a 10% buffered sucrose solution. Brains were removed and frozen sections serially cut at 40µm. Every other section was processed for HRP histochemistry according to the method of Mesulam (1978) and then examined under epifluorescence and polarized light for retrogradely labeled thalamic neurons and anterograde HRP labeling.

Retrogradely labeled PO neurons seen following sensorimotor hindlimb cortical injections closely overlapped with nucleus gracilis terminations within PO as demonstrated with anterograde HRP. Furthermore, retrogradely labeled PO neurons observed following sensory forelimb cortical injections showed overlap with the nucleus cuneatus terminations within PO. However, retrogradely labeled PO neurons seen following motor forelimb injections showed virtually no overlap with the terminations of the DCN.

Additionally, there appeared to be a somatotopy within PO in that sensorimotor hindlimb cortical injections resulted in the labeling of cells located dorsolateral and caudally within PO while sensory forelimb injections resulted in labeled cells more rostral and more medial to the motor hindlimb labeled cells. The motor forelimb labeled cells were found dorsal to the sensory forelimb labeled cells within PO.

The lack of overlap between DCN projections and PO neurons projecting to motor cortex suggests PO is not a relay for transmission of sensory input to the motor cortex.

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74.1 RESPONSES OF RACCOON VENTROBASAL NEURONS TO RAMP DISPLACEMENTS OF GLABROUS SKIN. Andrew M. Kelahan, Susan Warren*, and Benjamin H. Pubols, Jr. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209, and Department of Physiology, New York University, New York, NY 10016.

Single neurons of the raccoon thalamic ventrobasal complex were examined for their response to controlled ramp displacements of the glabrous skin of the hand, and the results were compared with findings in primary mechanoreceptive afferent fibers. Subjects were anesthetized with either pentobarbital sodium or methoxyflurane. Displacement velocities were varied between 0.1 and 100 µm/msec, with final displacements up to 1000 µm.

As displacement velocity increases, depending upon the unit, the number of spikes per ramp may (1) first increase to a peak at velocities between 2 and 10 µm/msec, and then decline with further increases in velocity, (2) remain fairly constant, or (3) decrease continuously. However, we have not observed a monotonic increase in spikes per ramp with increasing ramp velocity, as has been reported for some primary afferent units (e.g., Pubols, B., J. Neurophysiol., 1980).

For a given displacement velocity (e.g., 10 µm/msec), the discharge pattern of both rapidly and slowly adapting units typically is one of deceleration or constant discharge rate, but rarely the acceleration characteristic of slowly adapting primary afferent fibers (Pubols, B., and Pubols, L., Fed Proc., 1983).

For most ventrobasal neurons, instantaneous discharge frequency during ramp stimulation is adequately described as a power function of ramp velocity (Warren and Pubols, Neurosci. Abstr., 1981). However, there is a smaller population of units whose behavior is perhaps best described as a discontinuous function of ramp velocity: As ramp velocity increases, discharge rate remains relatively constant until velocities of 10-20 µm/msec are reached, at which point the discharge rate suddenly increases by a factor of 5-20 X, and remains at this higher level throughout further increases in ramp velocity. We have also observed this discontinuous response function in ventrobasal neurons of barbiturate-anesthetized cats.

We conclude that the discharge properties of ventrobasal neurons in response to ramp displacements of glabrous skin are highly complex and variable and cannot necessarily be predicted from knowledge of the discharge properties of individual primary afferent neurons. (Supported by research grants NS-13418 and NS-19486, USPHS.)

74.2 RESPONSES TO DIFFERENT RETRACTION VELOCITIES AND INTERRAMP INTERVALS OF RAPIDLY ADAPTING NEURONS IN CAT SI CORTEX. J.P. Guillemot, R. Proulx*, A. Samson* and L. Richer*. Dept. of Kinesiology and Psychology, Université du Québec, Montréal, Canada.

Cutaneous stimulus identation and retraction seem psychophysically difficult to discriminate when high velocity and short temporal interramp interval (IRI) are used. These ON and OFF somatic sensations may have their substrate in the rapidly adapting (RA) cutaneous neurons. In ordre to study the nature of OFF discharges of RA neurons at higher levels of information treatment, we recorded the unit responses in cat's forepaw representation in SI area.

Acute recordings were carried out in the conventional manner with tungsten microelectrodes using curarized and N_2O: O_2 anaesthetized cats. The recording site was area 3b. Suprathreshold skin displacement were used (2000 µm). The velocity of the stimulus for both indentation and retraction was equal. Stimuli were applied with a servo-controlled vibrator on cutaneous receptive fields previously determined with a von Frey calibrated hair. Various velocities of stimulus application and withdrawal were used with either an IRI of 10 sec. or different short IRI.

Analysis of 113 RA neurons showed 3 classes of units: class A units (70%) had ON responses higher than OFF, class B units (12%) showed the inverse relationship between the ON and OFF responses and, class C units (18%) responded only with ON discharges. When indentation and retraction stimulus velocity was increased, discharge rate increased in all neurons. Different OFF responses were obtained for class A and B units with various IRI: both increases or decreases in discharge rate were observed with increasing IRI's. In addition, higher velocity was positively correlated with discharge rate. Moreover, few units showed an interaction between IRI and velocity.

These results suggest that the stimulus retraction is more difficult to discriminate such as demonstrated in the majority of neurons showing lower OFF discharges. The visco-elastic properties of skin acting on RA neurons may explain the relation between velocity and discharge rate. In addition various behaviors observed with different IRI's indicate that temporal factors may also affect discriminative properties of skin receptors.

74.3 MOTION- AND DIRECTION-SENSITIVE NEURONS IN S-I CORTEX OF ALERT MONKEYS: TEXTURE AND VELOCITY CODING. S. Warren*, H. Hamalainen*, D.R. Kenshalo, Jr. and E.P. Gardner. Dept. of Physiology and Biophysics, NYU School of Medicine, New York, NY 10016.

Direction sensitivity in SI cortex has been attributed to an asymmetric distribution of excitation and inhibition within the receptive field (Gardner and Costanzo, J. Neurophysiol. 43: 1342, 1980). Movements in the least preferred direction are thought to feed forward inhibition to adjacent skin areas which have not yet been stimulated. Therefore, if the spacing between stimulated points is increased, the directional preference should lessen. To test this hypothesis, we have stimulated receptive fields of 66 motion- or direction-sensitive SI neurons (MSNs and DSNs) with a graded series of toothed wheels of equal diameters and tooth spacing of 0.8-9.0 mm. The wheels are mounted on low torque potentiometers, and rolled across the hand and arm with only the tips of the teeth contacting the skin surface. Despite the punctate nature of the stimulus, on both glabrous and hairy skin, the wheel response qualitatively resembled that observed when stroking the skin with brushes, edges or blunt probes. DSNs also differentiated the textures of the wheels. The clearest distinction between on- and off-directions of motion was seen with the finest textures. With 0.8 mm gaps, DSNs responded weakly to off-direction stimuli, while the on-direction response was vigorous. Increasing the gap between teeth did not significantly modify the on-direction response. However, movements in the off-direction elicited higher firing rates as the gap spacing increased. Nevertheless, even with gaps as large as 9 mm, DSNs still showed a preference for movements in the on-direction. These findings suggest that DSNs are poorly suited for quantitative texture discrimination, since their firing patterns in the preferred direction of motion are unrelated to spatial frequency.

MSNs, which showed no direction preference, did not respond differentially to textured wheels. We found no difference in responses to the finest and coarsest wheels, nor was there a preference for intermediate textures. MSNs responded in a graded fashion to velocity of wheel rotation, over the range 5-40 cm/sec, showing the most vigorous activity to rapid motion.

These data suggest that both DSNs and MSNs can integrate information from widely spaced points in their receptive fields, and that rapid temporal sequencing of stimulation is more important than spatial contiguity in determining responses. The data also support the hypothesis that direction sensitivity is conferred by lateral inhibition of input from immediately adjacent skin areas, as differentiation of direction of motion is weakened by increasing the spatial separation of stimulation sites. (Supported by NIH Grant NS 11862)

74.4 RESPONSES OF VIBRISSA UNITS IN RAT SMI BARREL CORTEX TO MULTI-WHISKER STIMULATION. D.J. Simons, Dept. of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Responses of single cortical units in the SmI barrel cortex of unanesthetized rats were observed following combined deflections of contralateral vibrissae. Compact multi-angular stimulators were used to deflect whiskers in controlled temporal and spatial patterns. Following deflection of a vibrissal hair, unit discharges to subsequent deflections of the same or a different whisker were substantially reduced or abolished altogether. This suppression was strongest at short interdeflection intervals, i.e. 10 ms, and decreased progressively during the 50-100 ms following the first deflection. In many cases this time period also corresponded with a reduction in unit discharges following an excitatory response to deflection of a single whisker. Several spatial factors determined the presence and degree of a cell's response to the second of two whisker deflections: a) the angular direction(s) in which the individual hairs were moved, b) the sequence in which two whiskers were deflected, i.e. which one was deflected first, and c) the particular combination of whiskers stimulated.

Response suppression could be elicited by prior deflection of whiskers within the normally-defined excitatory RF as well as by whiskers which when deflected alone failed to elicit unit discharges; in a number of important respects this latter phenomenon is similar to surrond inhibition. Although the effects of a given whisker generally decreased with its distance from the maximally exciting hair, i.e. usually the principal whisker of a cortical column, inhibitory regions of a RF could be distributed asymmetrically with respect to the principal whisker. Moreover, in multi-whisker RF's vibrissae eliciting the most vigorous excitatory responses when deflected alone were not necessarily the same as those that caused the strongest inhibition when whiskers were deflected in combination.

In some cases a combination of temporal and spatial factors appeared to interact. These data suggest that cortical vibrissa units are differentially responsive to patterns of stimuli produced by objects moving in particular directions and/or velocities across the mystacial pad. The findings are consistent with previous observations of temporal responsivity and spatial organization of RF's in the somatosensory cortex in other species. At least some of these effects appear to be due to active inhibitory mechanisms within the cortex itself.

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74.5 THE REPRESENTATIONS OF THE HAND IN AREAS 3b AND 1 OF THE MACAQUE MONKEY, MACACA MULATTA. T. P. Pons, P. E. Garraghty, C. G. Cusick, J. T. Wall, and J. H. Kaas. Department of Psychology, Vanderbilt University, Nashville, TN 37240.

Currently there are different interpretations of the somatotopic organization of cytoarchitectonic Areas 3b and 1 in macaque monkeys. Since some investigators used Macaca mulatta while others used Macaca fascicularis, the possibility of major species differences has been raised. In the present experiments, the hand representations in Areas 3b and 1 of Macaca mulatta (rhesus monkeys) were mapped in detail with microelectrodes, and results were compared with our previous findings in Macaca fascicularis (cynomolgus monkeys) (Nelson et al., J. Comp. Neurol., '80). Results were obtained from nine rhesus monkeys anesthetized with Ketamine hydrochloride and as many as 580 recording sites were obtained in a single animal. Minimal cutaneous receptive fields were determined at each recording site from a multiunit response.

The hand representations in rhesus monkeys were devoted largely to the glabrous surface. The representations of the glabrous digits were joined at the proximal bases at the 3b/1 border and progressively more distal parts of the digits were represented progressively more caudal in Area 1 and deeper in the central sulcus in Area 3b. Due to the considerable cortical magnification of the distal phalanges of the digits, the representations of the distal phalanges approached the 3b/1 border, especially in Area 1. For both areas, representations of the glabrous pads of the palm were displaced medially and laterally, digits 1-5 were represented in order in a lateromedial sequence, and much more tissue was devoted to digits 1 and 2 than to digits 4 and 5. Representations of the dorsal hand and digits were located medially and laterally in the respective hand representations. In Area 1, islands of tissue representing the dorsal surface of the digits varied in location from animal to animal, and commonly disrupted and displaced the representations of the glabrous digits. In Area 3b, dorsal surfaces of the digits were often found represented in the depths of the central sulcus near the representations of the glabrous digit tips. The hand representations were bordered laterally by the face and medially by the wrist. The results indicate that the hand representations in both Macaca fascicularis and Macaca mulatta are quite similar.

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74.6 SOMATOTOPIC ORGANIZATION OF THE SECOND SOMATIC SENSORY AREA, SII, IN THE CEREBRAL CORTEX OF THE MOUSE (SPON: T.M. Plant). G.E. Carvell* and D.J. Simons. Depts. of Anatomy and Physical Therapy, and Physiology, Univ. of Pittsburgh, Pgh., PA 15261.

The parietal cortex of barbiturate-anesthetized, adult mice was studied using tungsten microelectrodes to record single- and multiple-unit activity in response to gentle tactile stimulation of the face and body. A complete representation of the contralateral body surface occupying approximately 3.5 sq. mm, was found immediately posterior and lateral to the representation of the face in SI. Within SII: A) Substantial areas of tissue were devoted to representations of the forepaw and face, especially the sinus hairs associated with the nares, anterior upper lip and mystacial vibrissae. Receptive fields on these body regions were small and contralateral, though larger than RF's of similar regions in SI. In particular, vibrissa RF's always included at least several adjacent whiskers. B) In regions representing proximal body parts, RF's were considerably larger, were often bilateral and sometimes included the entire body surface. C) The responsiveness of many cells was labile, waxing and waning over relatively short periods, i.e. 1-2 minutes. When present, responses could be reliably elicited by light tapping or brushing of clearly defineable body regions. Loci responding to the face were the most reliably driven. D) In some but not all animals, responses could also be elicited by auditory stimuli; overlap of auditory and somatosensory regions was largely restricted to the representation of the limbs and proximal body and was rarely found in the face region. E) The face was represented most anteriorly and the hindlimb most posteriorly. Forepaw digits and anterior aspects of the face, e.g. nares and upper incisor, were represented laterally while the back and neck were represented medially. Within SII a "musculus" can be viewed as being upright in the medial-lateral plane with the midline representation of the face and nares lying immediately adjacent to the representation of these areas in SI. F) By comparison with SI, SII is characterized by a less pronounced layer IV, which is of irregular thickness and packing density, and by less uniformity in the layering of pyramidal cells in layer V.

These results are in general accord with previous findings from evoked potential studies of mouse cortex but are at variance with some reports that the mystacial vibrissae are not represented in SII in mice. Of particular interest is confirmation of the finding that, compared to SI, SII appears to be proportionally larger in the mouse than in the closely-related rat. Supported by NSF grant BNS 80-21854.

74.7 THE SECOND SOMATIC SENSORY AREA (SII) IN A PROSIMIAN PRIMATE, GALAGO CRASSICAUDATUS. M. Carlson and H. Burton. Depts. of Anatomy & Neurobiology and Psychiatry, Washington Univ. Sch. Med., St. Louis, MO 63110.

SII in the Galago was located in a highly granular type cortex of approximately 12 mm^2 along the upper bank of the lateral sulcus (LS). In anesthetized animals, closely spaced microelectrode penetrations that were parallel to the LS were used to map the distribution of multi- and single neuron responses to low threshold, cutaneous stimuli at 50-100 μm intervals; the survey approached 30 sites/mm^2. The recordings activated from well-defined receptive fields (rfs) were distributed in a single, precise, somatotopic representation of the body in which contralateral input was dominant under these recording conditions (Fig. 1). Within this general pattern, several features were determined: (1) glabrous surfaces of the fore- and hindlimbs were separately represented; (2) hairy dorsal surfaces of the distal limbs surrounded the glabrous regions superficially and posteriorly; (3) small areas that received exclusively ipsilateral input from the hand or foot were located within the distal limb zones; and (4) an area receiving bilateral input from large, poorly defined rfs was located posterior to SII.

The representation of the distal limb zones in anterior to posterior, elongated strips in the Galago SII resembled the rf distribution described previously in Macaca SII whereas the relative segregation of fore- and hindlimb zones anteriorly and posteriorly, respectively, was reminiscent of the erect body plan of SII seen in nonprimates. The exceptional segregation of glabrous skin areas and the relatively easier accessibility of SI and SII in this species may indicate that the Galago is an ideal primate for the study of the interactions between these cortical somatic sensory areas in manual tactile discrimination. (Supported by NSF 81-15044 and NS0 9809.)

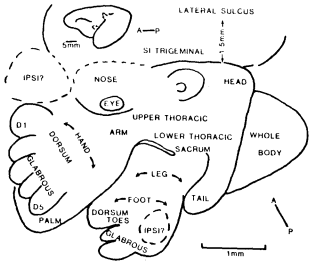

Figure 1

74.8 Somatosensory Corticotectal and Corticothalamic Projections from Anterior Ectosylvian Sulcus in Newborn Cats. Barry E. Stein and Lawrence Kruger. Dept. of Physiol. and Biophys., Med. Coll. of VA Richmond, VA 23298 and Dept. of Anat., UCLA Center for the Health Sciences, Los Angeles, CA 90024.

The fourth topographic somatosensory cortex (SIV) is located in the superior bank of the anterior ectosylvian sulcus (AES) (Clemo, H.R. & Stein, B.E. Brain Res., 235, 162-168, 1982). Somatosensory cells are also found in the fundus and inferior bank of the sulcus but are not discretely organized. Yet, both areas project heavily to the superior colliculus and thalamic posterior group. The projection from the AES represents the principal source of descending somatosensory information to the superior colliculus (Stein, B.E., Spencer, R.F., & Edwards, S.B., Neurosci. Abst., 8, 853, 1982; Clemo, H.R. & Stein, B.E., Neurosci. Abst., 7, 758, 1981). Since somatosensory cells are present in the superior colliculi of newborn cats (Stein B.E., Labos E., & Kruger, L. J. Neurophysiol. 36, 667-679, 1973) we sought to determine if a somatosensory corticotectal projection was already present at this time.

Injections of ^{3}H-leucine (.1-.3 ul) were centered in the superior bank of the rostral AES in five kittens. Three animals were 12-12 hrs., one was 3 days and one was 5 days of age, at the time of injection. All animals were allowed to survive for 20-24 hrs. The injections labeled most of the rostral two-thirds of the sulcus and spread to the inferior as well as to the superior bank.

Dense corticotectal and corticothalamic projections were seen even in the youngest animals studied. The projections appeared to be as dense or denser than that previously seen in adult cats and showed the same terminal distributions. The corticotectal projection was bilateral, much heavier ipsilaterally than contralaterally and paralleled the distribution of somatosensory cells. The distribution of terminal labeling appeared as two bands; the upper band was centered in the stratum griseum intermediale and appeared to have a puff-like organization, the lower band was centered in the stratum griseum profundum, and extended medially to invade the periaqueductal gray. Laterally the bands were fused at the border of the superior colliculus. The corticothalamic projection appeared to penetrate the ventral group in discrete fascicles terminating in a portion of the intralaminar wing and in the posterior group of thalamic nuclei. Axons running in the internal medullary lamina and running rostrocaudally in the ventral group did not reveal clear terminal labeling in the ventrobasal complex comparable to that seen following SI or SII injections in adults. These experiments indicate that the organization of the major somatosensory efferents at this cortical region occur prenatally.

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74.9 SOMATOTOPIC ORGANIZATION OF CORTICOTECTAL PROJECTIONS FROM THE ANTERIOR ECTOSYLVIAN SULCUS IN CAT. H.R. Clemo & B.E. Stein. Dept. of Physiology and Biophysics, Medical College of Virginia, Richmond, VA 23298. (SPON: L. Bruce).

A dense cortical projection to the superior colliculus (SC) arises from two somatosensory zones of the anterior ectosylvian sulcus (AES): SIV, and an area deeper in the sulcus for which no somatotopic organization has been observed. We previously showed that projections from both regions contact somatosensory cells in the SC (Clemo and Stein, Neurosci. Abst., 7:758, 1981). The present study was initiated to determine the spatial (topographic) features of this projection.

Tungsten microelectrodes were used to record and stimulate; standard orthodromic and antidromic techniques were used in 30 adult cats. Many somatosensory SC cells could be activated with low current intensities (30-100μA) from several sites in SIV and a few from the somatosensory area of the AES deep to SIV. Regardless of the source of corticotectal influence, a simple rule prevailed: a cortical site would influence only SC cells whose receptive fields overlapped that of the cortical site. Yet within this framework there was considerable corticotectal convergence and divergence. Thus, SC cells with receptive fields covering the entire forelimb could be influenced by much of the forelimb region of the AES. Similarly, a single cell in cortex representing the forepaw could project to a wide 'territory' in the SC in which cells had receptive fields that included the forepaw. The corticotectal projection from the AES was modality specific; somatosensory SC cells were activated only from somatosensory regions, auditory SC cells could be activated only from regions of the AES and somatosensory-auditory (multimodal) SC cells could be activated from both areas of the AES.

We also tested SI-SIII for corticotectal influences, but only cells in the AES gave rise to a direct, monosynaptic corticotectal projection. No responses to stimulation of SIII were observed and stimulation in SI-SII was often ineffective. If effective, these sites were usually near the borders of other corticotectal zones, required high current intensities (200-800 μA) and cells in these regions could not be antidromically activated from the SC. Thus, their influences on SC cells appear to be indirect. The only other cortical area that influenced somatosensory SC cells was in the rostral part of the suprasylvian sulcus, where an expanded representation of the forelimb has been described and corticotectal projections have been shown anatomically. The scheme of corticotectal topographic register described above was found between the suprasylvian sulcus and the SC.

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74.10 CONTRIBUTION OF THALAMO-CORTICAL AND CORTICO-CORTICAL CONNECTIONS TO RECEPTIVE FIELD PROPERTIES IN RAT SI CORTEX. J.L. Uhr and J.K. Chapin. Dept. of Cell Biology, U. Tex. Hlth. Sci. Ctr., Dallas, TX 75235.

The aim of this study was to investigate interactions between thalamo-cortical (T-C) and cortico-cortical inputs to the vibrissae area ("barrelfield") of the primary somatosensory (SI) cortex of the rat. In neuroanatomical studies we have shown that granular aggregates (barrels) in layer IV of this region receive the strongest T-C inputs, while layer V receives the strongest cortico-cortical inputs. Furthermore, receptive fields (RF's) of single neurons recorded in layer IV barrels include only one or a few whiskers, while RF's in layer V are much larger.

To quantitatively define these RF's, post-stimulus histograms were generated of responses of single units to standardized vibratory stimulation of each whisker in the RF. Both layer IV and V neurons typically responded to stimulation of the center whisker in their RF's with 6-10ms latencies. However, response latencies of layer V units increased up to 24ms post-stimulus as whiskers progressively more distant from the RF centers were stimulated.

To determine whether cortico-cortical (C-C) connections may subserve the layer V neuronal responses to these distant whiskers, adjacent regions within the barrelfield (2-3mm away from recording site) were microstimulated with a bipolar concentric stimulating electrode. Layer V neurons responded orthodromically with 3-5ms latency to stimuli between 12 and 20uA. Very few layer IV neurons responded to stimulation under 400uA. Furthermore, when such sites were lesioned (10uA;5s), the recorded layer V neuron no longer responded to stimulation of the whisker corresponding to the lesioned barrel.

Next, we investigated spatio-temporal interactions between short-latency responses to peripheral stimulation (ie.T-C input) and stimulation of nearby cortex (ie.C-C input) presented at different conditioning (C) and test (T) intervals relative to each other. Histograms were generated to measure the modulation of the T by the C stimulus. Irrespective of whether the C stimulus was peripheral or cortical, C-T intervals less than 5ms produced facilitation of the response to T and also reduced its latency by 1-2ms. With C-T intervals exceeding 5ms, a deep long-lasting inhibition of the response to T was seen. Layer IV cells exhibited a shorter and weaker inhibition than layer V cells. This pattern of interaction between peripherally and cortically driven inputs may reflect processes involved in the detection of moving objects in the spatio-temporal domain.

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74.11 RESPONSES OF CORTICOTHALAMIC CELLS TO CALLOSAL AND THALAMIC STIMULATION IN CAT SOMATOSENSORY CORTEX. P. Diadori, P. Landry and R.W. Dykes. (Dept. of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 1A1.)

To examine the effects of input from the corpus callosum onto somatosensory neurons we have recorded intracellularly and extracellularly from cells in areas 3b and 1 of the cat while driving them with peripheral, thalamic and callosal stimuli. In 19 barbiturate- anesthetized mongrel cats the cisterna was opened and a craniotomy performed. Thalamic stimulating electrodes were inserted stereotaxically into the ventroposterolateral nucleus and the contralateral somatosensory cortex was exposed and aspirated to allow stimulating electrodes to be placed in the anterior part of the corpus callosum at the midline. The depth of both sets of electrodes were adjusted to maximize the surface evoked potentials recorded in the forelimb region. Following these preparations, a 3M sodium acetate-filled pipette (20-40M) was positioned at the cortical surface and the craniotomy was sealed with 3% agar. Responses of 109 neurons were recorded along vertical trajectories through the forelimb region using conventional stimulating and recording equipment. A subset of the neurons activated from the callosum were also activated antidromically from the thalamus (latencies ranged from 1.8 to 7.6ms). Excitation from the corpus callosum ranged from 7 to 17 ms. Increasing the stimulus intensity of the corpus callosum generated IPSP's with latencies between 8 and 20ms. In individual neurons,callosal and thalamic stimulus intensities were adjusted until the IPSP's were the same amplitudes. Under this condition the duration, the time to peak and the reversal potential of the IPSP's were the same suggesting that both thalamic and corpus callosum inputs share a common inhibitory interneuron. However unlike other cortical neurons which were synaptically driven by thalamic stimulation with latencies between 2 and 5 ms, no EPSP's were detected in these corticothalamic cells. Instead of the EPSP-IPSP sequence encountered in other cortical neurons, corticothalamic cells responded with a short latency IPSP (3-4ms) lasting approximately 80ms followed by a rebound excitation at 200ms. Their receptive fields were difficult to define or were absent. We suggest that perhaps corticothalamic neurons act as relay cells, receiving information from the contralateral cortex and sending it to the thalamus to modulate thalamocortical signals.

74.12 INTERHEMISPHERIC TRANSFER OF SOMESTHETIC INFORMATION IN THE CORPUS CALLOSUM. F. Lepore, L. Prévost*, L. Richer*, J.P. Guillemot. Dept. of Psychology, Univ. of Montréal and Dept. of Kinesiology, Univ. of Québec, Montréal, Canada.

Corpus callosum (C.C.) is involved in the interhemispheric transfer of sensory information. Previous electrophysiological and anatomical experiments have shown that neurons belonging the cortical somatosensory areas (SI and SII) sent somesthetic information to the other hemisphere through axons crossing at the rostral portion of the C.C.. In the present study callosal somesthetic receptive fields (R.F.) were identified and they were characterized in terms of localization and specific sensory modalities.

Acute recordings were carried out in curarized cats using tungsten microelectrodes and N_2O: O_2 anaesthesia. The recording site in the rostral C.C. was identified visually using an operating microscope and fiber activity amplified in the conventional manner. R.F.'s were determined using von Frey calibrated hairs, puffs of air, light touch or pressure, and passive movement of the limbs.

Results indicated that various functional sub-modalities were represented originating from cutaneous, deep and paciniform receptors. Some neurons also responded to hair and whisker displacements. Moreover, both slowly and rapidly adapting axonic responses were found. R.F.'s were situated in pelvic limb (10%), trunk (15%), head (35%) or thoracic limb (25%) regions. Of the latter 75% concerned the proximal limb areas whereas 25% represented the distal limb or forepaw. These neurons all represented unilateral body parts. R.F.'s touching the midline of the body including the head and proximal limb structures were over represented in regards to distal body parts. In addition, a small number of bilateral R.F.'s were found in the face, trunk, thoracic or pelvic limbs.

These results suggest that callosal connections may be associated to different sensory functions such as midline fusion of the two halves of the body or processing of complex sensory discrimination.

74.13 THE MORPHOLOGY OF SPECIFIC THALAMOCORTICAL AXONS PROJECTING TO THE BARREL FIELD OF THE RAT. K. F. Jensen and H. P. Killackey*. Department of Psychobiology, Univ. of Calif. Irvine, Irvine, CA. 92717

The organization of thalamocortical projections to a portion of the parietal cortex of the rat (the barrel field) is very discrete and appears to preserve the spatial arrangement of receptors on the body surface, specifically the sinus hairs and vibrissae of the face. We have examined the morphology of individual thalamocortical axons which project to the barrel field.

Axons were labeled by injection of horseradish peroxidase (5% WGA-HRP, Sigma) either subcortically into fasicles of the internal capsule coursing through the caudate-putamen or into the lower portions of layer VI. The labeled axons were visualized in coronal sections with the DAB-GOD histochemical method of Itoh et al. (1979).

Individual parent axons, about one micron in diameter, ascend from the internal capsule in a step-like fashion with an occasional branch which bears terminals in layer VI. (Terminals were identified as one micron swellings, generally located on small diameter fibers.) The parent fiber courses through layer Vb and begins to branch extensively in layer Va. The vast majority of branching occurs in layer IV where the distance between branch points is short. This results in a highly branched but relatively restricted bushy terminal field that spans the height of layer IV. Within layer IV the larger diameter fibers are relatively terminal-free while the finer branches bear the majority of terminals. A few of the small caliber terminal branches extend into layer III.

The terminal field of a parent fiber appears to be associated with a single barrel. The terminal field may fill a large extent of a barrel. A number of parent fibers may have overlapping terminal fields within a single barrel.

There are variations in the size of the most extensively filled arborizations. The terminal fields of the layer IV arborizations in the posteromedial apsects of the barrel field appear wider than those in the anterolateral aspects of the barrel field. The present observations confirm and extend previous descriptions of the specific thalamocortical afferents in this system based on Golgi (Lorente de No' 1922) and Fink-Heimer (Killackey 1973) methods.

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74.14 A VIBRISSAE RELATED PATTERN OF CALLOSAL CONNECTIONS IN THE PRIMARY SOMATOSENSORY CORTEX OF THE RAT. H. Killackey*, J. Olavarria* and R. C. Van Sluyters (Spon: R. Josephson). Department of Psychobiology, University of California, Irvine, CA. 92717, and School of Optometry and Neurobiology Group, University of California, Berkeley, CA. 94720.

Previous light microscopic investigations of the callosal connections of the rat somatosensory cortex have concluded that the face region is largely acallosal. However, at least one physiological study (Pidoux and Verley, 1979) and one electron microscopic study (Perentes, 1979) have suggested the existence of such connections. Hence, we decided to reinvestigate this question using a more sensitive neuroanatomical tracing method.

Adult pigmented rats were given multiple injections of about 0.1 ul of horseradish peroxidase (HRP) across the surface of one hemisphere. After 24 hr the rats were sacrificed and the brains removed. The cortex contralateral to the injection sites was separated from the rest of the brain, flattened, sectioned parallel to its surface and reacted for HRP histochemistry using tetramethyl benzidine as the chromogen.

Following this procedure, labelled neurons and terminations are distributed in a honeycomb-like fashion within the portions of primary somatosensory cortex in which the mystacial vibrissae and facial sinus hairs are represented. This pattern seems clearest in the portion of somatosensory cortex where the sinus hairs of the snout, bucal pad and lower lip are represented. It is also detectable, although in a somewhat modified form, in the region of the vibrissae representation. In this region the callosal projections occur in broad bands that run between the representations of vibrissae rows, and narrow strands of less dense projections that cross from band to band between represenations of individual vibrissae within a row. Thus, the pattern surrounds the individual "barrels" and is largely confined to what has been termed the "septa".

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74.15 SOMATOTOPIC ORGANIZATION OF THALAMO-CORTICAL PROJECTIONS IN THE NEWBORN RAT. D. R. Dawson* and H. P. Killackey* (SPON: J. Leon). Department of Psychobiology, Univ. of Calif. Irvine, Irvine, CA. 92717

At the time of birth, the trigemino-thalamic afferents have begun but not completed their invasion of the ventrobasal complex (VB), and the vibrissae-related pattern of segmentation is not yet discernable. Further, the ventrobasal afferent end processes are located in the white matter beneath somatosensory cortex (Wise and Jones 1978). The present study examines the degree of topography in the thalamo-cortical afferents at this early time.

Subjects received discrete cortical injections (0.1 ul) of a 5% lectin-bound HRP solution on the day of birth, and were allowed to survive for 12 to 18 hours. The brains were cut in the coronal plane and were processed according to the technique of Mesulam (1976). The pattern and distribution of retrograde labelling in VB was determined, and lateral reconstructions of the cortical injection sites were generated.

The ventrobasal complex can be subdivided into two regions; a wedge-shaped main body and a narrow ventro-lateral crescent. Discrete cortical injections result in discrete groups of labelled cells within these areas, and there is a topographic order in this labelling. Cortical injections near the midline produced labelled cells in the medial tip of the ventral crescent, whereas slightly more lateral injections labelled the lateral portion of the crescent. Injections made in the most lateral portions of the parietal cortex (the presumptive barrel field) labelled the cells in the lateral and central portions of the main body of VB, whereas more ventro-anterior injections labelled the most medial area.

The topography of the thalamo-cortical projections at birth is the same as that reported in physiological studies of the adult (Welker 1971, Waite 1973). Cortical representation of torso and limbs is dorso-medial, whereas the head is more lateral. In the representation of the face, the vibrissae are represented posteriorly and the buccal pad more anteriorly. In VB, the torso and limbs are represented in the ventral crescent, with the tail most medial and the forelimbs more lateral; the main body of VB contains a representation of vibrissae and buccal pad, with the nasal-most region represented most medially.

In summary, the thalamo-cortical projections of the newborn rat appear to be as specific as that of the adult. This orderly projection forms before trigeminal input to VB is complete or the fibers have invaded their cortical target. This implies that the thalamo-cortical fibers rely at least partially on intrinsic guiding factors to align themselves and are not fully dependent on either input or target information.

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74.16 DIFFERENTIAL EFFECTS OF COMPLETE AND SUBTOTAL SENSORIMOTOR CORTEX LESIONS ON TACTILE PERFORMANCE IN INFANT- AND ADULT-OPERATED RATS. S. Finger and D. J. Simons. Psychol. Dept., Washington Univ., St. Louis, MO 63130 and Physiol. Dept., Univ. of Pitt. Sch. Med., Pittsburgh, PA 15261.

In an earlier study (Finger, Simons and Posner, Exp. Neurol., 60:347, 1978), it was found that enucleated adult rats with very large lesions of the sensorimotor cortex performed extremely poorly on a battery of five tactile discriminations, regardless of whether the lesions were made in rat pups 1-2 days old or in sexually mature adults. On the other hand infant-operated rats with 13-28% of SmI spared (typically in the anterolateral aspects of SmI) performed significantly better, although still more poorly than control animals. Because adult-operated animals with comparable spared fragments of SmI were not tested at the time, it was not known whether they would have learned the discriminations as quickly as the infant-operated rats with incomplete lesions. Nevertheless, it was hypothesized that while infant- and adult-operated animals may perform equally poorly after virtually complete lesions of SmI and SmII, the infant-operated rats with incomplete lesions would be somewhat less impaired than adult-operated rats with closely comparable lesions.

In the present study adult-operated rats with lesions sparing 15-30% of the same regions of SmI as the infant-operated rats examined previously were tested for their ability to learn the battery of tactile discriminations used before. The infant- and adult-operated rats were carefully matched on the basis of lesion size and locus by reference to remaining aggregations of cells in layer IV, i.e., "barrels", which are known to correspond to particular regions of the contralateral body. The new data showed that like the infant-operated animals with sparing of SmI tissue, adult-operated rats exhibited some learning deficits but performed better than animals with complete lesions. Infant- and adult-operated rats then were compared as two groups and as individually matched pairs of subjects. In both types of analyses support emerged for the hypothesis that infant-operated animals may be better able to use spared fragments of target tissue to guide tactile behaviors than adult-operated rats although the cells in the spared areas of infant SmI cortex seemed to show normal response properties.

These data confirm the general finding that developmental status at the time of brain damage can be an important determinant of post-traumatic performance. The role of the age variable cannot be considered alone, however, but must be viewed in the context of other experimental variables such as the presence of functionally intact tissue spared by the lesions. This observation may account for why some infant lesion studies find recovery of behavioral function while others do not.

74.17 THE S-I HINDPAW REPRESENTATION AFTER HINDPAW DEAFFERENTATION IN NEONATAL AND ADULT RATS. J. T. Wall, C. G. Cusick, and J. H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

When the hindpaw skin of an adult rat is partially deafferented by sciatic nerve transection, regions of S-I cortex usually representing sciatic inputs become responsive to hindpaw zones which remain innervated by the saphenous nerve (Wall et al., Soc. Neurosci. Abstr., 1981, 7, 758). Due to this change the cortical area representing saphenous inputs is about three times larger than that of normal adults. Even with this expansion, however, some regions of the original hindpaw representation remain unresponsive to cutaneous stimuli many months after injury. This result suggests there are limitations in the capacity of cortical neurons to recover cutaneous responsiveness if normal inputs are removed during adulthood. The present experiments tested whether similar limitations are seen if sciatic deafferentation occurs neonatally.

Hindpaws of 1 day old rats were deafferented by transecting and ligating the sciatic nerve and the S-I hindpaw representation was subsequently mapped when rats reached adulthood. During mapping, rats were anesthetized with ketamine hydrochloride and receptive fields to light tactile stimuli were defined from multiunit responses recorded in the middle cortical layers at 60-100 locations across the hindpaw region. Areas representing hindpaw inputs were then measured with a planimeter and compared to similar data from normal rats and from rats denervated as adults.

The main result is that the cortical area (mean = 0.18 mm^2,N=6) representing saphenous inputs from the hindpaw in neonatal denervates is: (1) significantly smaller [$t(14) = -10.75$, $p<.001$] than the area of the hindpaw representation (saphenous and sciatic inputs) in normal adults (mean = 0.94 mm^2, N=10), and (2) significantly smaller [$t(11) = -3.51$, $p<.01$] than the area representing saphenous inputs from the hindpaw in adult denervates (mean = 0.47 mm^2, N=7). Thus, sciatic section results in a decrease in the size of the hindpaw representation regardless of age of the animal at the time of injury. Age at injury is important, however, in determining the extent of this decrease. Neonatal denervates suffer larger losses of cortical space than adult denervates. One interpretation of these findings is that primary sensory cells in neonatal rats may be more severely affected by nerve injury than cells of adult rats. This peripheral effect may secondarily diminish responsiveness of central neurons receiving convergent saphenous and sciatic inputs, and thus result in differences in the responsiveness of parts of the saphenous representation which depend on these neurons for input. Cortical neurons may be more limited in their capacity to recover cutaneous responsiveness after neonatal deafferentation because skin to cortex connections are more vulnerable to disruption during development.

74.18 TIME-DEPENDENT EFFECTS OF DIGIT REMOVAL ON THE FUNCTIONAL ORGANIZATION OF SOMATOSENSORY (SmI) CORTEX OF RACCOONS. G. S. Doetsch and A. M. Kelahan. Depts. of Surg. (Neurosurg.) and Physiol., Med. Coll. Ga., Augusta, GA 30912, and Neurol. Sci. Inst., Good Samaritan Hosp. Med. Cent., Portland, OR 97209.

Surgical removal of the third forepaw digit in raccoons produces striking long-term and short-term changes in the functional organization of SmI cortex. At 9-12 months following digit amputation in neonate and adult raccoons, electrophysiological study of these animals under barbiturate anesthesia showed that neurons in the digit 3 cortical zone had become responsive to low-intensity stimulation of "new" forepaw skin regions, primarily on digits 2 and 4. Comparable shifts in neuronal responsiveness were found within one hour following digit removal in adult animals anesthetized with nitrous oxide or with barbiturate. The "novel" receptive fields (RFs) of neurons within the digit 3 cortical sector were larger and included the hairy skin and/or claws more frequently than in normal animals; RF location varied considerably as a function of the site of recording within cortical tissue; i.e., no orderly somatotopic representation of the "new" skin fields was found.

To determine if time-dependent variations occur in the pattern of cortical reactivation, the effects of digit 3 removal were examined at one, two and four weeks post-amputation in adult animals anesthetized with nitrous oxide or with barbiturate. At these intermediate times, the digit 3 cortical zone differed greatly from that of animals studied immediately or long after amputation. The vast majority of neurons were excited only by high-intensity stimulation of small RFs on the digit 3 stump; relatively few neurons were responsive to low-intensity stimulation of adjacent intact skin fields. Again, there was no orderly relationship between RF location and recording site.

Statistical analysis of the RF data showed that cortical reactivation at all post-amputation times more closely resembled a random distribution than a strictly topographic pattern.

The combined results indicate that, over a period of several months, the digit 3 cortical territory undergoes a dynamic sequence of changes in functional organization: after amputation, neurons normally responsive to stimulation of digit 3 first become sensitive primarily to stimulation of digits 2 and 4, then to the digit 3 stump, and finally again to digits 2 and 4. Mechanisms which might be responsible for this time-dependent reactivation are: 1) the "unmasking" of normally-present but relatively ineffective synapses, by the shifting of inhibitory and/or facilitatory influences; 2) physiological changes in peripheral nerve fibers terminating in amputation neuromas and 3) anatomical changes, including the growth of new synaptic connections within the central somatosensory system.

SPINAL CORD: CYTOCHEMISTRY AND PHARMACOLOGY

75.1 BRAIN STEM STIMULATION EFFECTS ON THE DEAFFERENTED SPINAL CORD. A. V. Apkarian, C. J. Hodge, Jr., M. P. Owen and B. S. Hansen Dept. of Neurosurgery, Upstate Medical Center, Syracuse, NY 13210.

Some central pain states that occur after nerve or spinal cord injury may be due to chronic deafferentation. Stimulation of locus coeruleus (LC) or nucleus raphe magnus (NRM) causes potent preferential inhibition of the dorsal horn interneurons responding to noxious afferent input in the intact spinal cord. Since deafferentation pain is difficult to control with either opiates or brain stem stimulation, the experiments described here were undertaken to determine if there is a change in the bulbospinal modulation of dorsal horn units consequent to deafferentation.

Seven cats underwent unilateral section of lumbar dorsal roots L1-L6 under barbiturate anesthesia. Two weeks after rhizotomy the animals were anesthetized with chloralose and the lumbar spinal cord exposed. After occipital craniectomy, stimulating electrodes were placed stereotaxically in NRM and LC. Extracellular recording were made from 110 dorsal horn units from both the intact and denervated sides of the spinal cord. The effects of brain stem stimulation were determined on the ongoing activity of the units. On the intact (control) side the effect of LC stimulation upon noxious cutaneous stimulation was determined in 15 cells. Of these, 11 were inhibited and 4 showed no effect. The effect of NRM stimulation was determined on 13 of these cells, 10 of which were inhibited and 3 showed no effect. These results are similar to earlier reports. Ninety-five cells were studied on the deafferentated side. Of these, 38 were located in lamina 4 or 5 and are reported here. In 19 cells the effects of LC or NRM stimulation were either inhibitory or absent, similar to the intact side. The other 19 cells showed facilitatory effects either from LC or from NRM stimulation. In 11 of the 19 cells the effects of LC stimulation were excitatory and the effects of NRM stimulation on the same cells were inhibitory. The remaining 8 cells were facilitated by NRM stimulation but inhibited by LC stimulation. Despite rhizotomy, 11 of the 38 dorsal horn cells had receptive fields. The effects of brain stem stimulation was not related to the presence or absence of receptive fields on the denervated side.

These results show that the effects of descending brain stem-spinal pathways are altered in the chronically deafferented cat spinal cord, and that, at least in this preparation, LC and NRM effects can be antagonistic and independent of each other.

75.2 EXPANSION OF CUTANEOUS RECEPTIVE FIELDS OF DORSAL HORN CELLS INDUCED BY 4-AMINOPYRIDINE IN THE CAT. Kazue Semba, Herbert M. Geller and M. David Egger. Depts. of Anatomy and Pharmacology, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

The receptive field (RF) organization of dorsal horn cells has been demonstrated to be altered following lesions of the spinal cord and peripheral nerves. To explain these alterations, it has been hypothesized that dorsal horn cells receive, in addition to the normally effective afferent input, some sensory input that is usually ineffective in driving the cells postsynaptically. These 'silent' synapses, when unmasked or potentiated, may give rise to altered RF of dorsal horn cells. Aminopyridines have been shown to potentiate synaptic transmission in general by enhancing transmitter release. To obtain insight into the plasticity of the RF organization of dorsal horn cells, we used 4-aminopyridine (4AP) to examine its effects on dorsal horn cell RF in the cat.

Single dorsal horn units were recorded extracellularly in laminae III-V of the L7 segment in adult cats anesthetized (α-chloralose), immobilized, and functionally spinalized at the T12-13 level. All the units tested had cutaneous RF on the hindlimb. These cells were activated by various low-threshold mechanical stimuli, including brush, touch, and pressure; some cells, in addition, responded to high-threshold, noxious stimuli. Following the administration of 4AP (0.3, 0.6, 0.9 mg/kg, i.v.), the RF of almost all the cells expanded in a dose-related manner, in some cases up to approximately twice the original area. The new RF contained the original RF, usually at the center. We did not observe the appearance of new RF remote from the original RF. We typically observed that mechanical stimuli that were previously effective activated the cells more vigorously following 4AP. In addition, in some cells, noxious mechanical stimuli which previously induced only phasic responses became effective in inducing tonic responses. Also, the level of spontaneous activity generally increased following 4AP administration. In some cats, recordings were also made from an S1 or L7 ventral root. The amplitude of compound action potentials in response to the electrical stimulation of the sural nerve increased up to 8 times following systemic injections of 4AP in parallel with the RF expansions Similar, but less pronounced dose-related increases were observed in ventral root reflex potentials elicited by electrical stimulation of the plantar cushion. This facilitation of spinal reflex pathways activated by the stimulation of the sural nerve and plantar cushion is consistent with previously reported general facilitatory effects of 4AP.

In summary, our principal findings suggest that 4AP is effective in revealing at least a portion of a subliminal fringe of RF of dorsal horn cells.

75.3 EFFECTS OF DORSAL ROOT SECTION ON UPTAKE AND RELEASE OF D-ASPARTATE IN THE GUINEA PIG SPINAL CORD S.J. Potashner and P.L. Tran*. Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032

This study attempts to determine if L-glutamate and/or L-aspartate (L-GLU/L-ASP) are transmitters of primary sensory fiber endings in the cervical spinal cord. The uptake and the release of D-[^{3}H]aspartate (D-ASP), a putative marker for L-GLU/L-ASP, was measured in the spinal cord in vitro before and 2-4 days after section of several dorsal roots.

The cervical enlargement (segments C4-T1) was dissected into left and right halves. Each half was divided into dorsal and ventral pieces. Pieces of spinal cord from unlesioned animals took up D-ASP, achieving concentrations in the tissues which were approximately 14 times that in the medium. Subsequently, electrical stimulation of the tissues evoked a Ca^{++}-dependent release of D-ASP. Section of dorsal roots C5-T1 on the right side depressed both the uptake (by 22-29%) and the evoked release (by 50%) of D-ASP in tissue dissected from the right side of the cervical enlargement. Tissue dissected from the left side had uptake and release activities similar to those in control tissue taken from intact animals.

Since the dorsal horn of the spinal cord contains a dense population of GABA'rgic neurons, the uptake and release of [^{14}C]GABA was measured to determine if these activities also are lost 2-4 days after dorsal root lesions. Section of dorsal roots C5-T1 on the right side had no effect on the uptake or on the release of GABA, unless dorsal radicular or other blood vessels were damaged. When blood vessels were damaged, there was a decrement on the right side in the uptake and release of GABA as well as a larger than normal depression of the uptake and release of D-ASP.

These findings suggest that, in the absence of vascular damage, degeneration of primary sensory fibers produces decrements in the uptake and release of L-GLU/L-ASP. Therefore, these findings support the hypothesis that primary sensory fibers use L-GLU/L-ASP as a transmitter. However, when blood vessels are damaged and presumably some spinal neurons are destroyed, there is an additional decrement in the uptake and release of L-GLU/L-ASP and of GABA. These findings imply that, in addition to primary sensory neurons, other cells which provide synaptic endings in the spinal cord may use L-GLU/L-ASP as a transmitter.
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75.4 PAROXYSMAL VENTRAL ROOT ACTIVITY AND EXCITATORY AMINO ACIDS. R.A. Davidoff, J.C. Hackman & G.P. Ryan*. Neurophysiology Laboratory, VA Medical Center & Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

There is controversy about the role of excitatory synaptic transmission in the genesis of epileptiform activity. In particular, there is disagreement about whether the prolonged depolarizations which characterize paroxysmal activity consist of synchronized ("giant") EPSPs or are the result of endogenous regenerative membrane currents. Our present experiments were designed to determine the role of excitatory synaptic transmission mediated by amino acids in the generation of convulsive activity produced in spinal motoneurons by exposure to penicillin.

We used the isolated, hemisected frog spinal cord continuously superfused with HCO_3^--buffered Ringers at 15°C. Reflex and spontaneous responses were recorded from ventral roots (VR's) using sucrose gap techniques.

Exposure of the spinal cord to the antibiotic (4.0 mM, 5 min) caused the occurrence in the VR of spontaneous, slow (5-30 sec), quasi-rhythmic (0.01-0.04 Hz) depolarizations (amplitude, 6-8 mV) with superimposed action potentials (paroxysmal VR potentials, pVRP's). It is assumed that pVRP's represent electrotonically conducted activity from motoneurons since they were eliminated by severing the VR at its entrance to the cord. Application of penicillin also caused considerable augmentation of polysynaptic reflexes caused by dorsal root stimulation (DR-VRP's).

pVRP's are generated by a synaptic process since they were eliminated by exposure of the cord to Mn^{++} ions applied in a concentration (1.5 mM) sufficient to block chemical synaptic transmission in the frog cord.

Dicarboxylic amino acids (e.g. L-glutamate, L-aspartate) released by afferent terminals and by interneurons are thought to mediate excitatory synaptic transmission in the cord. (-)β-(4-chlorophenyl)-γ-aminobutyric acid (0.1 mM), the active stereoisomer of baclofen, a drug which is thought to prevent the presynaptic release of excitatory amino acids, eradicated pVRP's and reduced DR-VRP's. Similar effects were produced by application of D,L-α-aminoadipic acid, a specific postsynaptic antagonist of excitatory amino acid actions. These effects were readily reversible. (+)Baclofen was without these actions on pVRP's and DR-VRP's and glutamic acid diethylester (1.0 mM), a weak antagonist of synaptic activity in the frog spinal cord, had minimal and inconsistent effects.

In sum, these results indicate that epileptiform activity and increased reflex transmission produced in motoneurons by the action of penicillin require the synaptic release and subsequent postsynaptic action of an excitatory amino acid such as L-glutamate or L-aspartate.

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75.5 SPONTANEOUS DORSAL ROOT POTENTIALS IN THE IN VITRO FROG SPINAL CORD. J.C. Hackman, G.P. Ryan*, C.J. Wolhberg & R.A. Davidoff. Neurology Service VA Medical Center and Departments of Neurology and Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Electrical activity in the dorsal root (DR) in the absence of stimulation has been previously observed in both frog and cat. However, the mechanisms underlying the generation and regulation of spontaneous DR activity are largely unexplored. Our present experiments were designed to investigate the nature of spontaneous slow wave activity in the frog spinal cord.

Sucrose gap recordings were made from the DR of the isolated, hemisected frog spinal cord superfused with HCO_3^--buffered Ringers solution (15°C).

Spontaneous dorsal root potentials (sDRP's) were present in all preparations. They had the same polarity as evoked DRP's (i.e., negative potentials indicating depolarization of intraspinal portions of DR's) and ranged in amplitude from just discernible to 6 mV. The largest were 25-40% of the amplitude of DRP's evoked by supramaximal stimulation of adjacent DR's. Hypoxia did not appear to be the cause of these potentials since omitting O_2 resulted in reduction of the frequency of sDRP's. Recording with K^+-sensitive microelectrodes revealed that only during the largest sDRP's did a measurable change in extracellular K^+ occur (circa 0.15 mM). This change was not large enough to account for the amplitude of the potentials.

Enhancement of sDRP's by the GABA antagonists bicuculline methiodide (10 μM) and picrotoxin (0.1 mM), the glutamate decarboxylase inhibitor, semicarbazide (2.5 mM) and low Cl^--containing Ringer's solution indicate that GABA is not responsible for the generation of sDRP's.

Intact, synaptic transmission is required to generate sDRP's since addition of tetrodotoxin (1.25 μM) or Mn^{++} (1.5 mM) to the Ringer's solution or lowering of external Na^+ (57 mM Li^+) blocked, and addition of 4-aminopyridine enhanced sDRP's.

The excitatory amino acids antagonists (-)baclofen, D,L-α-aminoadipic acid (1.0 mM), 2-amino-4-phosphonobutyric acid (1.0 mM) and D,L-α,ϵ-diaminopimelic acid (1.0 mM) reversibly blocked the generation of sDRP's. In addition the substance P antagonist, [D-Pro^2-D-Phe^7-D-Trp^9]-substance P (0.5 μM) markedly and reversibly depressed sDRP's.

In summary, it appears that intact synaptic transmission mediated by excitatory amino acids, L-glutamate and L-aspartate, or by substance P is necessary for the production of spontaneous dorsal root potentials in the isolated frog spinal cord.

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75.6 EXPRESSION OF SPECIFIC Fcγ2b BINDING SITES BY SUBPOPULATIONS OF RAT SENSORY NEURONS. J. Dodd*, W.D. Matthew and T.M. Jessell Department of Neurobiology, Harvard Medical School, Boston, MA

Small-diameter dorsal root ganglion (DRG) neurons involved in the central processing of cutaneous sensory information can be identified by their differential expression of neuropeptides and acid phosphatase isoenzymes. To examine the biochemical properties of these neurons in more detail, we have generated several monoclonal antibodies that identify antigens present in discrete subpopulations of sensory neurons. In addition, we have found that some sensory neurons express a highly selective binding site for the Fc domain of mouse IgG2b molecules.

Sections of rat DRG and spinal cord were incubated with culture supernatant or ascites fluid obtained from IgG2b-secreting murine hybridomas and then screened for specific binding, using indirect immunofluorescence techniques. Intense labelling was observed in approximately 40% of DRG neurons independent of the specificity of the variable region of each antibody and indicated the presence of an Fcγ2b binding site in these neurons. Immune staining was present throughout the cytoplasm of small-diameter neurons but was absent from large-diameter neurons. Stained fibres were visible within the DRG and in bundles in the dorsal roots. The axons of afferent fibres in the dorsal root entry zone and the tract of Lissauer were brightly labelled. In the dorsal horn, labelled fibres were present in laminae I, IIo and III, whereas fibres projecting to lamina IIi and to other regions of the spinal cord were not stained. No immunoreactivity was observed in neurons originating in the CNS. Subpopulations of neurons in other sensory ganglia and neuronal cell bodies in the superior cervical ganglion also displayed Fcγ2b binding, however, parasympathetic neurons were not labelled. Incubation of sections, under identical conditions, with IgG1, IgG2a, IgG3, IgM or IgA murine antibodies produced no detectable Fc domain-related binding. Most DRG neurons exhibiting Fcγ2b binding sites also contained the sensory neuron-specific acid phosphatase, and some overlap with substance P-immunoreactive neurons was also observed.

Selective Fcγ2b receptors are known to be expressed on the surface of lymphocytes and macrophages. The interaction of IgG2b antibodies with these receptors leads to the activation of phospholipase A_2, the release of arachidonic acid metabolites and membrane depolarization. We are currently examining the possibility that the Fcγ2b binding site in small sensory neurons is associated with similar membrane events.

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75.7 ATP EXCITES RAT DORSAL HORN AND DORSAL ROOT GANGLION NEURONS GROWN IN DISSOCIATED CELL CULTURE. C.E. Jahr and T.M. Jessell. Dept. Neurobiology, Harvard Medical School, Boston, MA 02115.

Holton provided evidence that ATP is released from peripheral terminals of primary sensory afferents and may be responsible for producing antidromic vasodilation (J. Physiol. 145, 494-504, 1959). To investigate the possibility that ATP may act as a sensory transmitter in the spinal cord we have examined the actions of ATP and related nucleotides on neurons removed from the dorsal horn of embryonic rat spinal cords and grown in dissociated cell culture. Intracellular recordings were obtained from neurons from 4 to 12 weeks in culture. Nucleotides and other drugs were applied by pressure ejection or iontophoresis. ATP (10^{-6} to 10^{-5} M) produced a rapid depolarization associated with a conductance increase in 27% of neurons tested. The response was unchanged after synaptic transmission was blocked by 200 μM CdCl. Similar depolarizations were evoked regardless of the ATP salt used (disodium, magnesium, calcium, Tris). EDTA (10^{-5} M) and pyrophosphate (10^{-4} M) were without effect. It therefore seems unlikely that the depolarization results from calcium chelation. The responsive neurons were remarkably selective for ATP. Adenosine, AMP, UTP and GTP were inactive at 10^{-4} M whereas ADP and CTP were less than one tenth as potent as ATP. Analogs of ATP that are only slowly hydrolyzed by ATPases ($\beta\gamma$methylene ATP, AMP-PNP, adenosine tetraphosphate) were effective agonists indicating that hydrolysis of the phosphate chain was not required for activity. The ATP induced depolarization was dependent on extracellular Na^+. When 90-95% of the Na^+ was replaced with either choline or sucrose, the response was blocked reversibly. This suggests that ATP opens channels permeable to Na^+. Tetrodotoxin, at concentrations that blocked the action potential, did not affect the ATP response. The ATP evoked depolarization was also unaffected by 8-phenyltheophylline, an adenosine receptor blocker, and isatogen derivatives which have been reported to antagonize ATP responses on smooth muscle. In addition to dorsal horn neurons, over 90% of dorsal root ganglion neurons in culture were also excited by ATP. The pharmacological and ionic specificities of the DRG response were essentially the same as that of dorsal horn neurons. The major difference in the responses of the two cell types was that the dorsal root ganglion neurons displayed a marked desensitization to ATP (see also Krishtal, Marchenko and Pidoplichko. Neurosci. Lett. 35, 41-45, 1983) whereas the dorsal horn neurons did not desensitize. Our results provide evidence that subpopulations of sensory and dorsal horn neurons that are likely to be involved in the processing and regulation of sensory stimuli are potently and selectively excited by ATP.

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75.8 SEROTONIN DEPOLARIZES A- AND C- TYPE PRIMARY AFFERENTS: AN INTRACELLULAR STUDY IN BULLFROG DORSAL ROOT GANGLION. G. G. Holz IV, S. A. Shefner and E. G. Anderson. Depts. Pharmacol., and Physiol. and Biophys., and Alcohol and Drug Abuse Res. and Trn. Prgm. Univ. Illinois Col. Med., Chicago, IL. 60612

We have recently reported extracellular studies showing that serotonin (5-HT) directly depolarizes primary afferent terminals and cell bodies in isolated bullfrog spinal cord and dorsal root ganglia (DRG) (Holz, G.G. and Anderson, E.G., Soc. Neurosci. Abstr., 8:791, 1982). Intracellular studies were undertaken to examine which types of primary afferents respond to 5-HT and the resistance changes which accompany 5-HT-induced depolarizations. Lumbar DRG with attached dorsal root and peripheral nerve were removed from bullfrogs, desheathed and pinned in a superfusion bath. Intracellular recordings were made at room temperature from DRG somata using 2M KCl-filled glass microelectrodes (40-80 MΩ). Neurons were classified by the latency of somatic action potentials following dorsal root or peripheral nerve stimulation. A-type cells showed conduction velocities of 5 to 18 m/sec ($\bar{x}$ = 11 $\pm$ 0.5 m/sec, S.E.M., n =40), mean input resistance of 39 $\pm$ 4 MΩ (n = 17) and mean resting potential of 65 $\pm$ 3 mV (n = 16). Since C-type neurons had conduction velocities of 0.2 to 0.8 m/sec ($\bar{x}$ = 0.5 $\pm$ 0.04 m/sec, n = 19) and a mean input resistance of 57 $\pm$ 4 MΩ (n = 8), they were clearly distinguishable from A's. The mean resting potential for C's was 59 $\pm$ 3 mV (n = 12).

Serotonin (500 μM - 1mM) was tested on 24 A-type neurons; 42% showed depolarization (4 - 12.5 mV). In all A's in which resistance measurements were obtained, the depolarization was accompanied by a decreased input resistance (15 - 38%, n = 5). The response in A's showed pronounced tachyphylaxis to subsequent 5-HT administration. Serotonin (50 μM - 1 mM) was also tested on 20 C-type neurons. Of these, 75% were depolarized. The 5-HT-induced depolarization was of two types: an initial fast, transient depolarization (9 - 33 mV, n = 4) and a more slowly developing, maintained depolarization (4 - 20 mV, n = 11). These two actions could occur independently or sequentially in the same neuron. The fast response was associated with a decreased input resistance (40 - 56%) and resembled the response seen in vagal afferents in the nodose ganglion (Higashi, H., Nature, 267: 448, 1977). The slow depolarization was associated with an increased input resistance (15 - 40%) and sometimes caused spontaneous firing. These findings support the direct depolarizing action of 5-HT on primary afferents noted with extracellular recording but also indicate that not all primary afferents respond to 5-HT. Furthermore, the action of 5-HT is qualitatively different on A- and C- type cells. This suggests a differential modulation of activity in specific subpopulations of primary afferents by 5-HT. (USPHS # DE 5390, AA 2696)

75.9 EXCITABILITY CHANGES AT INTRASPINAL TERMINALS OF AFFERENT C- AND A-FIBERS PRODUCED BY MIDBRAIN STIMULATION AND IONTOPHORETIC APPLICATION OF TRANSMITTERS IN CAT. M. Zimmermann*, E. Carstens, H. Schreiber* & H. Gilly*. II. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326 (FRG). (SPON: R.P. Scobey).

Stimulation in the midbrain periaqueductal gray (PAG) and lateral reticular formation (LRF) inhibits responses of spinal dorsal horn neurons to noxious skin stimuli. To study a possible presynaptic inhibitory action, we used the excitability testing method to determine if PAG or LRF stimulation affects the threshold for intraspinal antidromic activation of single afferent C- and A-fibers in the cat's sural nerve. We additionally tested effects of intraspinal iontophoretic application of 5-hydroxytryptamine (5-HT), noradrenaline (NA), morphine and GABA on fiber excitability, and compared these with effects of midbrain stimulation.

In cats anesthetized with sodium pentobarbital, single sural fibers were identified by their antidromic responses to intraspinal microstimulation (L6-L7 segment) using a glass-coated platinum electrode. When the threshold current to antidromically excite the fiber was stable within $\pm$5%, midbrain stimulation (100 msec pulse trains at 100 Hz, 3/s) was delivered through a bipolar electrode positioned in PAG or LRF. Drugs were iontophoretically applied from a multibarrel pipette glued to the intraspinal stimulating electrode (tip separation <50 µm). Drugs were: 5-HT HCl, morphine HCl, Na bitartrate (all 50 mM, pH 4-5), GABA (0.5 M, pH 4.5) and NaCl control (165 mM). Threshold changes during midbrain stimulation or drug application were expressed as % deviation from the control threshold.

Of 81 threshold measurements made in 42 C-fibers during PAG stimulation (100-900 µA), 60 were within $\pm$10% of control, while 18 showed increases greater than +10% and 3 decreases of more than -10%. The mean threshold increase (3.6%) was significant. LRF stimulation similarly had mixed effects on C-fiber thresholds, but the mean threshold increase (1.5%) was not significant. Midbrain stimulation had mixed effects on Aδ fiber thresholds, with PAG but not LRF stimulation producing a significant threshold increase.

Thresholds of C-fibers were predominantly raised during iontophoretic application of 5-HT (20/29 fibers), NA (10/13) and morphine (15/21), while GABA produced 3 increases and 2 reductions. Drug effects were dose-related, and control ejection of Na^+ ions did not produce threshold changes. Each drug produced threshold increases in nearly all A-fibers tested. Effects produced by each drug were plotted against the effect of PAG or LRF stimulation in the same fiber. While the majority of fiber thresholds were raised by both, the correlation coefficients were low, being highest for PAG-5-HT, PAG-NA, and LRF-NA comparisons. The observed threshold increases may correspond to a presynaptic inhibitory action. Supported by the Deutsche Forschungsgemeinschaft.

75.10 CORRELATION OF PHYSIOLOGY WITH MORPHOLOGY AND 5-HT OR SP INPUT IN CAT SPINAL DORSAL HORN LAMINA I NEURONS. V. Miletic, M.J. Hoffert, M.A. Ruda and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

Two distinct physiological types of neurons have been described in dorsal horn lamina I. Nociceptive-specific (NS) cells are activated exclusively by noxious stimuli. Wide-dynamic-range (WDR) neurons respond to innocuous stimuli but are activated maximally by noxious stimuli. We utilized the intracellular HRP technique to examine whether these distinct physiological types are associated with separate morphological neuronal classes. In addition, we combined this technique with immunocytochemistry to examine the serotonin (5-HT) or substance P (SP) input to these neurons.

Neurons in the cat lumbar dorsal horn were characterized physiologically by their responsiveness to natural stimulation of the skin, and then injected intracellularly with HRP. The spinal cord was sectioned, reacted with $CoCl_2$-intensified DAB and processed for 5-HT or SP using the PAP method. Camera lucida drawings of the HRP-filled cells and 5-HT or SP immunoreactive axons were made using a 100X objective.

We examined a total of 18 lamina I neurons, 10 of which were classified as NS and 8 as WDR. Morphologically, these 18 neurons were subdivided into four classes based on the shape of their somata and dendritic trees, number of primary dendrites, and presence or absence of spines (Gobel, S., J. Comp. Neurol., 180:375, 1978). None of these classes was associated exclusively with a distinct physiological type. In the class of aspiny pyramidal neurons (n = 9), 7 cells were NS and 2 WDR. Among spiny pyramidal neurons (n = 4) and multipolar neurons with compact dendritic trees (n = 4), 3 cells were WDR and one cell was NS in each class. In the class of multipolar neurons with loose dendritic trees only one NS cell was stained. The distribution of 5-HT or SP immunoreactive axonal contacts was similar in the dendritic trees of both NS and WDR neurons. However, somatic contacts on these cells were differentially distributed. All 10 NS neurons received 5-HT or SP axosomatic contacts. In contrast, axosomatic contacts were absent from all 8 WDR neurons.

These findings indicate that NS and WDR lamina I neurons are not exclusively associated with separate morphological classes, although there is a tendency for NS neurons to be more numerous among the aspiny pyramids, and WDR cells to appear more frequently in the spiny pyramidal and multipolar-compact neuronal classes. In addition, the absence of 5-HT or SP axosomatic contacts on the WDR cells suggests that the postsynaptic modulation of afferent input occurs on dendrites. This may explain the selective inhibition of noxious input on some WDR neurons by descending systems.

75.11 EFFECTS OF SPINALLY-ADMINISTERED MORPHINE AND NOREPINEPHRINE ON RAT DORSAL HORN INTERNEURONS AND SPINOTHALAMIC TRACT CELLS. J.L.K. Hylden and G.L. Wilcox, Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Given the spinal site of action of opioid and monoaminergic agents, the spinal cord dorsal horn is an important area to study in an effort to increase our understanding of the basic mechanisms involved in pain and analgesia. In this study, we have chosen to concentrate on the inhibitory actions of spinally-administered morphine (M) and the effects of the endogenous monoamine norepinephrine (NE) on M-induced antinociception.

Experiments were performed on rats anesthetized with chloralose (70 mg/kg, i.v.), paralyzed with gallamine triethiodide (55 mg/kg, i.v.) and maintained on a mixture of gallamine (8.3 mg/ml) and sodium pentobarbital (1 mg/ml) delivered i.v. via the jugular vein at 1.6 ml/hr. After laminectomy, a small agar reservoir was formed around the exposed lumbar spinal cord and filled with artificial c.s.f. solution (approximately 0.2 ml). Mineral oil was then poured over the c.s.f. and other exposed tissues in an effort to prevent excessive loss of body heat due to evaporation. Extracellular recordings were made from dorsal horn neurons with glass microelectrodes filled with 2% Pontamine Sky Blue in 0.5M sodium acetate. Spinothalamic tract (STT) cells were loctated by stimulation in the ventral basal complex of the thalamus with a single stainless steel electrode (800 µA or less, rectangular pulses of 0.1 msec duration at 10/sec). Neurons were identified with respect to natural stimulation of the skin (touch, pressure, noxious pinch and/or heat). Drugs were delivered directly to the spinal cord by addition to the agar reserovir or systemically through the jugular vein.

We have studied the effects of M on 20 dorsal horn cells (9 STT cells, 11 interneurons). Spinally-administered M (0.1 mg/ml) inhibited the response of wide dynamic range (WDR, 7 cells) and nociceptive specific (4 cells) interneurons and STT cells to noxious stimuli by 25-100%. A lower dose of M (0.05 mg/ml) inhibited the response of 3 out of 5 cells. The peak inhibitory effect of spinally-administered M occured within 10-15 min. Naloxone (1-5 mg/kg, i.v.) totally or partially reversed the action of M in 7 out of 10 cells. M (0.1 mg/ml) had no effect on low threshold cells (6/6 cells). NE (0.001 mg/ml) had no effect alone on the nociceptive responses of WDR neurons (3/3 cells), but was able to potentiate the inhibitory action of M (4/4 cells). NE, at this dose, had no observable effect on spinal cord blood flow. The data indicate that M can selectively inhibit nociceptive responses of STT cells and interneurons at the spinal level. This inhibitory action appears to be potentiated by the endogenous monamine NE. (Supported by USPHS Grants DA01933, RR05385, T32GM07397 and a grant from the Procter and Gamble Co.)

75.12 AN INHIBITION OF SPINAL TRIGEMINAL NUCLEUS CAUDALIS NEURONS BY LOCUS COERULEUS AND ADJACENT STRUCTURES IN THE CAT. H. Hirata, K.V. Anderson, and M. Bailey*. Dept. Anatomy, Univ. Mississippi Med. Ctr., Jackson, MS 39216

Previous studies (e.g., Sasa et al., 1974) showed that electrical stimulation of locus coeruleus (LC) inhibited the responses of neurons in the spinal trigeminal nucleus of the cat and rat. The present study differed from theirs in the following respects: (1) we recorded exclusively from neurons in subnucleus caudalis, while they recorded from more rostral subnuclei; and (2) we classified the units into three major types, low threshold mechanoreceptors (LTM), wide dynamic range (WDR) neurons, and nociceptive specific (NS) neurons.

Extracellular single unit discharges were obtained from the cat subnucleus caudalis from the level of the obex to 5 mm caudal to the obex. Animals were initially anesthetized with a combination of Nembutal and urethane, i.p., and, subsequently, with urethane, i.v., when needed. Stimulating electrodes were stereotaxically lowered into LC (P 3.5; L 2.5; H -2.2 mm) at approximately a 25° angle from the vertical and were advanced in a rostral-to-caudal direction. At the end of an experiment, radiofrequency lesions were made to mark the stimulated areas.

Thus far, the responses of LTM and WDR neurons (over 70% of the units tested) were inhibited by stimulation of LC and/or adjacent structures, such as tegmental fields and nucleus cuneiformis. We have not been able to isolate a NS neuron long enough to establish an inhibitory effect. The effective stimulus parameters used were normally 100-400 µA, 200 Hz (0.1 msec pulses), and 100 msec train. Mean percent reduction in response was 83% of the control (range= 74-100%). The responses to electrical percutaneous stimulation of receptive fields were inhibited by conditioning stimulation which usually preceded the skin stimulation by 50 msec. The responses to natural stimuli such as heat and brushing, as well as spontaneous activity, were also suppressed during and following brain stem stimulation. Histology revealed that the lesioned areas where clear inhibitions were observed included not only LC proper, but also adjacent regions and that for the latter the threshold current intensities for inhibition correlated poorly with the distance from LC.

We conclude that LC exerts non-selective inhibition on the evoked and spontaneous activity of LTM as well as the neurons receiving nociceptive inputs in the spinal trigeminal nucleus caudalis of the cat. It remains to be determined, however, whether this effect is mediated by nor-adrenergic pathways or other systems (LC of the cat is known to contain serotonergic neurons).

75.13 NALOXONE ON CAT SPINAL CORD MULTIRECEPTIVE NEURONES. P. J. Soja* and J. G. Sinclair. Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of B.C., Vancouver, B.C., Canada V6T 1W5.

Naloxone has been tested on several nociceptive systems with the hope of detecting the presence of tonically active endogenous opioid peptides. Previously, Sinclair et al. (Q. J. Exp. Physiol. 65: 181, 1980) reported that naloxone was without effects on cat spinal cord multireceptive neurones i.e. those that respond to both noxious and innocuous peripheral stimuli. Henry (Neuroscience 4: 1485, 1979; Neuroscience 6: 1935, 1981), however, found that all such neurones were enhanced by naloxone. The major difference between these studies was that Henry performed his experiments in spinal cord transected cats. Therefore we have re-examined this question by testing naloxone (2 mg/kg) on cells in the presence or absence (by cold-blocking the spinal cord) of descending influences. Naloxone was administered between 14:00 and 20:00 hours. The drug failed to produce a significant change in either the spontaneous or evoked activity using noxious radiant heat in 7 cells tested in this manner. We also tested naloxone in 6 spinal cord transected animals. In only 1 case was there a marked increase in both spontaneous and evoked activity after naloxone (up to 2 mg/kg). In 4 other experiments there was a slight increase in only the spontaneous activity while in the remaining cell naloxone produced a decrease in both spontaneous and evoked activity. Although there is a suggestion that naloxone may increase the response of these cells in spinal cord transected animals, the changes were not statistically significant when the data were grouped together. These results are consistent with the work of Sinclair et al. (1980) and Duggan et al. (Brain Res. 138: 185, 1977).

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75.14 SLOW EXCITATORY TRANSMISSION IN RAT DORSAL HORN: POSSIBLE MEDIATION BY PEPTIDES. L. Urban* and M. Randic. Dept. of Vet. Physiology and Pharmacology. Iowa State University, Ames. IA 50011.

Electrophysiological experiments were performed in an attempt to demonstrate the presence of slow synaptic potentials in rat dorsal horn neurons and their possible mediation by peptides known to be present in the spinal dorsal horn.

Rats 9-16 days old were used. After lumbosacral laminectomy 300µm thick horizontal or transverse spinal cord slices were made. Intracellular recordings from dorsal horn neurons were performed with micropipettes filled with 3M potassium acetate. Substance P, substance P antagonists and capsaicin were applied by bath perfusion in known concentrations.

In about half of examined cells, high intensity repetitive stimulation of a dorsal root (1-20 Hz for 3-5 sec) elicited, in addition to the monosynaptic and polysynaptic excitatory synaptic potentials, a slow depolarization, which reached a peak in 28 ± 10 sec (mean ± S.D., n = 29) and lasted 130 ± 53 sec. The mean amplitude of 7 ± 5 mV was recorded at resting membrane potentials between -60 and -75 mV. Although in some neurons single shock stimulation evoked a detectable depolarization, repetitive stimulation usually resulted in an augmentation of the slow depolarization and generation of synaptic and action potentials. A low Ca^{2+}- high Mg^{2+} solution or tetrodotoxin (10^{-6}M) reversibly abolished the slow depolarization in all of the ten cells tested. When the membrane potential was manually clamped, the slow depolarization was associated either with an increase or a decrease in membrane input resistance. In a few tested cells, conditioning hyperpolarization depressed the slow depolarization. The slow depolarization was also depressed or abolished in the presence of substance P, substance P antagonists and capsaicin. Attempts were made to determine the origin of the fibers eliciting slow depolarization by administering capsaicin to neonatal rats (50 mg/kg., s.c. on day 2). In these animals, the slow depolarization was evoked only in 10% of cells (n = 18).

In some cells, besides the slow depolarization, a hyperpolarization of long duration was observed. The hyperpolarizing potentials were of synaptic origin, usually associated with a decrease in membrane input resistance and were blocked by naloxone.

The present findings demonstrate the presence of the slow synaptic potentials in dorsal horn neurons. In addition, they suggest that a peptide, including substance P, may be the transmitter responsible for the generation of the slow depolarization, while enkephalins may mediate slow hyperpolarizing synaptic potentials.

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75.15 EFFECTS OF SUBSTANCE P ANALOGUES ON SUBSTANCE P EXCITATION AND SLOW EXCITATORY TRANSMISSION IN THE MAMMALIAN SPINAL DORSAL HORN. C. D. Raspantini, L. Urban,* S. Jeftinija,* K. Folkers,* and M. Randić. Dept. of Vet. Physiology and Pharmacology, Iowa State University, Ames, IA 50011 and Inst. for Biomed. Research, University of Texas, Austin, TX 78712.

Although synthetic analogues of substance P (SP) with antagonist properties have been recently developed, specificity of their central actions is presently controversial. We have, therefore, examined effects of (D-Pro^2,D-Trp^{7,9})-SP and (D-Pro^2, D-Phe^7,D-Trp^9)-SP on the SP-elicited excitation of dorsal horn neurons and on slow excitatory transmission in the mammalian spinal dorsal horn.

Extracellular recordings were made from single functionally identified dorsal horn neurons in halothane-anesthetized cats using multibarrelled microelectrodes and iontophoretic application of SP (3.7 mM), the presumed SP-antagonists (3.7 mM), and L-glutamic acid (0.2 M). Concurrent application of (D-Pro^2, D-Trp^{7,9})-SP (25-100 nA for 1-6 min) reduced excitatory responses to SP in about 60% of examined cells (n=26) irrespective of their afferent inputs. However, a depression of the L-glutamate evoked excitation, and spontaneous activity was also observed in about 10% of the cells. In addition, when larger currents or prolonged application of the analogue was used, the peptide exhibited SP-agonist activity in about 25% of examined neurons. (D-Pro^2,D-Phe^7,D-Trp^9)-SP proved to possess similar SP-agonist activity as (D-Pro^2,D-Trp^{7,9})-SP, but weaker SP-antagonist properties.

Possible interaction between (D-Pro^2,D-Trp^{7,9})-SP and the SP-induced depolarization and the slow excitatory synaptic potentials of dorsal horn neurons has been further investigated by means of intracellular recordings in the immature rat in vitro spinal cord slice preparation. Bath-application of (D-Pro^2, D-Trp^{7,9})-SP (1-3 x 10^{-5} M for 2-4 min) markedly reduced both SP-induced depolarization and the slow excitatory synaptic potentials, the latter elicited by high intensity repetitive stimulation of dorsal roots, in more than half of examined cells (n=19). The depressant effect of (D-Pro^2,D-Trp^{7,9})-SP outlasted the application period but was reversible. In several cells, the analogue reduced L-glutamate-induced depolarization, although to a smaller degree. In higher concentrations, (D-Pro^2,D-Trp^{7,9})-SP exerted depolarizing action on dorsal horn cells.

The results of these experiments indicate that (D-Pro^2,D-Trp^{7,9})-SP is a partial agonist, exercising also antagonism to SP. Further improvement of SP-antagonists with respect to potency and selectivity is desirable.

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75.16 LAMINAR ORGANIZATION OF FIVE NEUROPEPTIDES AND SEROTONIN IN THE MONKEY DORSAL HORN. N.C. de Lanerolle and C.C. LaMotte. Sections of Neurosurgery and Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT. 06510

Immunohistochemical techniques were used to identify the locations of terminals and axons containing substance P(SP)-, somatostatin(SS)-, cholecystokinin(CCK)-, vasoactive intestinal polypeptide(VIP)-, met-enkephalin(ENK)-, and serotonin(5-HT)-like immunoreactivity in the macaque monkey spinal cord. A modified picric acid-formaldehyde fixative (Zamboni fluid) best preserved the antigenicity.

Direct comparisons were made of adjacent vibratome sections from the same cord segment (C6), each reacted for a different neurochemical. Each neurochemical was found to have a unique pattern of distribution, and each dorsal horn lamina received different combinations of neurochemicals. The distribution of terminal patterns is summarized in the following table.

	Lamina I	IIo	IIi	III	IV	Ret V[1]
SP	***	***	*	*	*	**
SS	-	***	*	***	*	*
CCK	*	***	-	-	-	*
VIP	***	*	-	-	-	-
ENK	**	***	*	-	-	**
5-HT	***	*	-	-	**	*

(1= reticular nu. of lamina V; IIo and IIi = outer and inner regions of lamina II)

These data indicate some species differences from those reported for the rat (Gibson et al.,1981), particularly for CCK and VIP. The distinctive patterns closely follow traditional laminar borders, and the differential distributions imply that significant interactions between different neural systems must be quite specifically localized.

We have previously determined the ultrastructural characteristics of terminal types immunoreactive for SP, ENK & 5-HT in laminae I and IIo (Brain Res., in press). Preliminary electromicroscopic analysis of VIP-, SS-, and CCK-like immunoreactive structures in the same laminae has revealed both simple terminals containing large granular vesicles (LGV) and glomerular C-type terminals with LGV's (LGV-C type). These terminal types are similar to those found to be immunoreactive for SP. The coexistence of two or more of the neurochemicals in these terminal types is under investigation. (Supported by NS13335).

75.17 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP): DISTRIBUTION THROUGHOUT THE LENGTH OF PRIMATE SPINAL CORD. C.C. LaMotte and N.C. de Lanerolle. Sections of Neuroanatomy and Neurosurgery. Yale Univ. Sch. Med., New Haven, CT 06510.

VIP immunoreactivity in the macaque spinal cord was mapped in vibratome sections using the ABC method. VIP was localized in beaded axons, terminals, and possibly in dendrites. In cervical segments C3-C6, a few terminals and thin axons were found in lamina I; lateral to the central canal, the central cervical nu. and the intermediomedial nu. (IMM) were moderately innervated. In C8 the density of terminals increased in laminae I,IIo, and IMM; thin axons also crossed in the dorsal and ventral grey commisures (DGC and VGC). In the thoracic sample (T3), laminae I and IIo were heavily supplied with axons and a few penetrated into laminae IIi, III, IV and into medial and lateral V. Label was moderate in the DGC and VGC; somata and dendrites lateral to the central canal and in IMM were outlined also. A few terminals were seen in the ventral horn. In lumbar sections, laminae I and IIo were less heavily supplied than in the thoracic region. The IMM and region lateral to the central canal were moderately innervated. However, scattered VIP axons of medium diameter in the lateral and ventrolateral white matter traversed the cord into the grey matter. A few axons were found in the dorsal columns.

In sacral sections, VIP was denser than any other cord region. Radially oriented axons were found mainly in Lissauer's tract and in the lateral white; there were fewer in the ventral white and the dorsal columns. Large and small terminals and thin axons were densely distributed in laminae I and IIo. A heavy bundle of axons distributed along the lateral border of the dorsal horn, and continued into the lateral area of lamina V and into the lateral sacral parasympathetic nu. Axons also passed in the medial border of the dorsal horn and then coursed along the midline toward and into the central canal region where many terminals and axons were seen. In addition, large diameter, very long structures resembling dendrites were found either along the lateral and medial borders of the dorsal and ventral grey, or oriented transversely, crossing the midline in the DGC. These suspected VIP containing dendrites appeared to have spines and were also accompanied by similarly oriented VIP axons.

Immunoreactivity was quite sensitive to aldehyde fixation; moderate concentrations of aldehydes eliminated labeling except in the heavily innervated sacral regions. This may account for reports that VIP is confined to visceral afferents which supply the sacral cord. The present report indicates a broader role for VIP. Its higher density in both thoracic and sacral levels suggests its presence in visceral afferents from thoracic and abdominal as well as pelvic structures. Supported by NIH Grant NS13335.

75.18 IMMUNOREACTIVE LEUCINE ENKEPHALIN (I-lEnk), METHIOININE ENKEPHALIN-ARG-GLY-LEU (I-mEnk) AND DYNORPHIN (I-Dyn) IN THE SPINAL CORD OF THE CAT. L. J. Cruz* and A.I. Basbaum. Department of Anatomy, School of Medicine, University of California, San Francisco, CA 94143

Numerous studies have reported on the distribution of the endogenous opioid peptide, Enk, in the spinal dorsal horn. Since none of the earlier studies checked whether the antisera cross-reacted with Dyn, we repeated these studies using antisera (Weber, E.) directed against the ProEnk product, mEnk-arg-gly-leu and against the C-terminal ProDyn product, Dyn B. Absorption controls and RIA established the specificity of these antisera. The staining pattern produced by these antisera was compared with a commercially available L-Enk antiserum (Immunonuclear Corp). The studies were performed on cats perfused with 4% paraformaldehyde. Fifty micron frozen sections of spinal cord were cut and immunostained with the PAP method.

The L-Enk antiserum produced a pattern of staining identical to that previously described. There is dense terminal immunoreactivity in the superficial dorsal horn, Lamina I and in the inner (i) and outer (o) parts of Lamina II. Lamina V and the region around the central canal are also heavily stained, as is the column of Onuf located in the ventral horn of the first sacral segment. Staining with the mEnk-arg-gly-leu antiserum was similar except that Lamina IIo was consistently unstained. In contrast, the Dyn B antiserum stained Laminae I, IIo and V most densely; there was little reactivity in Lamina IIi and none in the column of Onuf. Peculiar to the S1 segment was the presence of I-Dyn cell bodies in Lamina I and IIo (without colchicine) and very dense, varicose, axonal staining in the tract of Lissauer and in the attached dorsal root. This particular staining pattern is very similar to that of immunoreactive vasoactive intestinal polypeptide in the sacral dorsal horn of the cat. Sections of spinal cord rostral to S1 showed neither the cellular, nor the Lissauer tract/dorsal root I-Dyn staining.

These data indicate that the choice of antiserum can markedly affect the pattern of dorsal horn immunoreactive opioid peptide staining. The possibility is raised that different ProEnk products, L-Enk and mEnk-arg-gly-leu are differentially distributed in the superficial dorsal horn. Finally, these data demonstrate that the distribution of I-Dyn is very distinct from that of L-Enk and there is a suggestion that I-Dyn may be present in some S1 dorsal root ganglion cells.

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75.19 DIFFERENTIAL DISTRIBUTION OF SUBSTANCE P, SOMATOSTATIN, AND CHOLECYSTOKININ IN RAT DORSAL ROOT GANGLIA. Mary M. Tuchscherer and Virginia S. Seybold. Department of Anatomy, University of Minnesota Medical School. Minneapolis, Minnesota. 55455.

Substance P (SP), somatostatin (SOM), and cholecystokinin (CCK) immunoreactivities have been shown to be present in the small type-B cells of dorsal root ganglia (DRG). A quantitative study regarding the distribution of these peptides in DRG at five different spinal levels using fluorescence immunohistochemistry was undertaken.

Male Sprague-Dawley rats (280-320gms) were implanted with chronic intrathecal cannulae of varying lengths corresponding to the DRG level to be surveyed. Colchicine was administered via the cannulae in two 50ug doses over a period of two days. The animals were then perfused with Zamboni fixative, and the appropriate ganglia were removed. Serial 10um cryostat sections were cut, and adjacent sections were processed for SP-, SOM-, and CCK-like immunoreactivities (IR) using fluorescence immunohistochemistry. An ethidium bromide counterstain was used to reveal cellular detail. Immunofluorescent cells were counted on each section and the data were statistically evaluated with reference to the total number of small cells counted at each level. Only cells containing a nucleolus were counted.

The five levels surveyed (C6, T6, T10, L2,and S1) showed significant differences in the distribution of peptide immunoreactive cells. SP-like IR occurred at its highest percentage in the L2 DRG (32%), and its lowest in the C6 DRG (10%). CCK-like IR occurred at its highest percentage in the T10 and L2 DRG (24% and 25%, respectively), and at its lowest in the C6 DRG (13%). SOM-like IR occurred at its highest percentage in the L2 DRG (13%), and at its lowest in the C6 and S1 DRG (3% and 4%, respectively). Overall, the frequency of SP and CCK positive cells was similar within a level, whereas the frequency of SOM positive cells was always less than that of the others. Thus, variability in the distribution of these peptides occurred within each level as well as between levels.

We have shown there to be significantly different proportions of SP, SOM, and CCK immunoreactive cells in DRG at the levels C6, T6, T10, L2 and S1. Correlates of these findings with the peripheral distribution and modality sensitivity of these peptide-containing primary afferents remain to be determined.
Supported in part by NS 17702.

SPINAL CORD: AFFERENT INPUT AND LOCAL CIRCUITS

76.1 NEURAL PROJECTIONS FROM GENITAL TRACT IN FEMALE RATS: AXONAL COMPOSITION AND SEGMENTAL DISTRIBUTION. J.L. Steinman*, S.M. Carlton*, C.E. Hulsebosch, R.E. Coggeshall & W.D. Willis, Marine Biomedical Institute, Galveston, TX 77550 (SPON: B. Haber)

The present study analyzes the axonal composition and location of cell bodies of afferent neurons that innervate the vagina and cervix in rats. The myelinated (MY) and unmyelinated (UN) axons in hypogastric (HYP), pelvic (PEL) and pudendal (PUD) nerves were counted from electronmicrographs. On the average, the HYP nerves had 24 MY and 2263 UN axons, the PEL had 639 MY and 2575 UN axons and the PUD had 648 MY and 1711 UN axons. In these nerves there were fewer MY axons than in the same nerves of the male. The HYP nerve also had 2 times more UN axons than the male counterpart.

The segmental distribution of sensory cell bodies innervating the vagina and cervix was determined. Horseradish peroxidase (HRP: 25% in DMSO) or HRP-wheat germ agglutinin (WGA: 5% in DMSO) was injected bilaterally into the mucosal wall of the vaginal canal after distension of the vaginal os with retractors. Rats (n=7) were sacrificed 3-5 days later and dorsal root ganglia (DRG) were removed and reacted with tetramethylbenzidine. HRP labelling was seen bilaterally in T9-T11, L1-L2 and L6-S1 DRG. Vagina, cervix, uteri and bladder were reacted with diaminobenzidine and showed heavy labelling in the epithelial layer of the vagina and cervix only. Labelling was not present in the adventitia. In 3 rats, the HYP nerve was soaked in HRP-WGA for 2-3 hrs. Reaction product was seen in L1-L2 and T9-T11 DRG on the side ipsilateral to the nerve.

These preliminary findings suggest that there is a sexual dimorphism in the axonal composition of nerves innervating the reproductive organs in the rat. We also demonstrate that HRP transport from the vagina to thoracic spinal cord levels shows a similar distribution of labelled cells as transport via the HYP nerve alone. The abundance of UN axons in the HYP nerve may provide a partial explanation for the transmission of pain due to distension of the vagina and cervix as occurs during labor. Furthermore, the present data indicate the pathways and types of fibers that may be involved in inhibition of nociceptive responses during mechanical pressure applied to the vagina and cervix. Experiments are underway to assess the effects of selective nerve transection on vaginal stimulation-produced analgesia. (Supported by NIH grants NS 07022 (JLS), NS 07062 (SMC), NS 17039 (CEH,REC), NS 09743 & NS 11255 (WDW) and a grant from the Moody Foundation.)

76.2 AXONAL CONDUCTION VELOCITY AND GANGLION CELL SIZE. K.H. Lee*, K. Chung, J.M. Chung and R.E. Coggeshall. Marine Biomedical Institute and Departments of Anatomy and Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas.

Conduction velocity is generally regarded as being closely correlated with axonal and cell body size. Thus, conduction velocity and cell body size should be correlated. The use of intracellular injections of the marker enzyme horseradish peroxidase (HRP) allows this to be tested for individual dorsal root ganglion cells and axons. Dorsal root ganglia (L6-S3) and their roots were removed from adult cats, placed in an oxygenated, Kreb's solution until use and then put in a stimulating and recording chamber at 37^{o}C where the oxygenated Kreb's solution could be perfused over the ganglion. Cells were impaled with conventional microelectrodes filled with 5% W/V HRP. Conduction velocities were calculated from conduction times and distances from stimulation to recording sites and then the cell was filled iontophoretically with HRP and visualized. Our preliminary data indicate that there is a general correlation of cell size and dorsal root axon conduction velocity, but the correlation is not close because of variability. Thus it seems that all slower conducting axons originate from small cell bodies, but some small cells have fast conducting axons.

Two other features of note were found in the morphological preparations. First it is clear that the central branch of the "T" shaped first division of the stem process of our marked dorsal root ganglion cells is considerably smaller than the peripheral branch, a finding that is in accord with the findings reported in classic studies but contrasts with more recent reports. Second, in some cells there is axonal branching within the ganglion distal to the "T" branch. If confirmed, this will indicate that some dorsal root ganglion cells are multipolar neurons. Supported by NIH grants, NS18830, NS10161, NS17039 and grants from the Moody Foundation and the Pearl and Aaron Forman Research Fund.

76.3 LEVEL LOCATION AND DISTRIBUTION OF PHRENIC PRIMARY AFFERENT NERVE FIBERS IN THE SPINAL CORD OF THE ADULT RAT. P.J. Roubal* and H.G. Goshgarian. Dept. Anat., Sch. Med., Wayne State Univ., Detroit, MI 48201.

Previous studies from this laboratory have localized and morphologically characterized phrenic motorneurons in the rat spinal cord at light (J. Comp. Neur., 201: 441-456, 1981) and electron microscopic (J. Neurocytol., 1983 in press) levels. The purpose of this investigation was to determine at light microscopic levels the origin and distribution of phrenic primary afferent nerve fibers in the adult rat spinal cord. Dry horseradish peroxidase crystals were applied to the central stump of the transected phrenic nerve in the neck to label the phrenic spinal ganglion cells and their central processes by a modification of the TMB technique. The results showed that phrenic primary sensory neurons are found in the C3-C7 spinal ganglia. The greatest number of labeled cells occurred at C5 followed closely by C4. Furthermore, the cell labeling seen at C6 and C7 exceeded the minimal labeling seen at C3. Our previous light microscopic study revealed that the majority of phrenic motorneurons were located at C4 (mean of 267 cells) whereas the C5 segment contained a smaller number of cells (mean of 129 cells). The study also showed that there was a rostral projection of phrenic motorneurons to C3 (20 cells) and a very small caudal tail of the nucleus projecting to C6 (mean of 2 cells). Phrenic motorneurons were never seen at C7. Thus, from the present study, it appears that phrenic primary sensory neurons tend to be more caudally disposed along the neuraxis than phrenic motorneurons. Transganglionic labeled central processes of phrenic primary afferent axons were seen entering the spinal cord from dorsal rootlets in both transverse and longitudinal sections. The axons were loosely fasciculated in the dorsolateral aspect of fasciculus cuneatus. In this region they were arranged along the longitudinal axis of the cord. A number of them were seen entering the dorsal horn. Axons entering the dorsal horn at the same level they enter the spinal cord were rarely seen. Axon collaterals were traced from their parent axons in fasciculus cuneatus through lamina I into lamina II and III. Several other axons were traced along the medial margin of the dorsal horn to enter the medial aspects of lamina IV. A few axon collaterals traversed laminas I-III and also entered into lamina IV. Supported by U.S. Public Health Service grant NS-14705.

76.4 PATTERNS OF ENTRY TO THE SPINAL CORD OF SAPHENOUS NERVE FIBRES IN CAT AND RABBIT. S.J.W. Lisney*, C.M. Pover*, S.K. Heaney* and P.J. Kendell*. (SPON: K.W. Horch). Dept. of Physiology, The Medical School, Bristol BS8 1TD., U.K.

In both the cat and the rabbit, the saphenous nerve supplies cutaneous sensory fibres to the medial surface of the hind leg between the groin and the ankle. To a lesser extent it also supplies some cutaneous sensory fibres to the anterior and posterior parts of the lateral surface of the leg. In both animals, saphenous fibres enter the spinal cord via the dorsal rootlets of segments L5, 6 and 7. We wanted to know whether saphenous nerve fibres were distributed amongst these dorsal rootlets in an orderly, somatotopic way.

Experiments were carried out on pentobarbitone anaesthetized cats and rabbits. After routine preparative surgery the left leg was shaved and then a lumbar laminectomy was carried out so that electrophysiological recordings could be made from the L5-L7 dorsal rootlets on the left side. The left sciatic nerve was cut in the sciatic notch to eliminate most of the non-saphenous afferent input carried by the L5-L7 dorsal roots. Starting with the most caudal dorsal rootlet of L7, the individual rootlets of each root were sectioned close to the cord and then split into 4 or 5 smaller multifibre strands. Each strand was placed in turn on a pair of platinum hook electrodes and the part of the saphenous nerve field supplied by saphenous fibres in the strand determined by brushing and stroking the skin. Only light, tactile stimulation was used. The areas supplied by the strands of each individual rootlet were drawn onto a figurine of the leg. In this way a picture of the areas of skin supplied by saphenous fibres in each of the L5-L7 rootlets was built up. The identity of the rootlets was confirmed by post mortem dissection.

There were more rootlets per root in cats than in rabbits and the area of skin supplied by individual rootlets in cats was generally smaller than in rabbits. The area of skin supplied by a rootlet was not always continuous. With both animals there was extensive overlap of the areas supplied by neighbouring rootlets and even between rootlets which were several ones apart. In both species fibres supplying the distal parts of the saphenous nerve field travelled via the more caudal rootlets of all those carrying saphenous fibres and fibres innervating the proximal parts of the field travelled via the more rostral rootlets. A gradual distal to proximal shift in the areas supplied by rootlets was evident if every third or fourth rootlet, passing from caudal to rostral, was considered.

76.5 SPINAL CORD PROCESSING OF FEMORAL VENOUS AFFERENT INPUT. B. J. Yates and F. J. Thompson. Dept. Neuroscience, Univ. Florida Col. of Med., Gainesville, FL 32610.

Electrical and mechanical stimulation of the femoral-saphenous vein elicits field potentials recordable from cord segments L-3 through S-1 in cord-transected cats, suggesting that these segments contain neurons which are activated by femoral venous afferent input. These field potentials were comprised of a series of negative waves and a slow positive wave; those potentials recorded from L-6 also had an initial triphasic spike. In most preparations the evoked potentials were abolished by transection of the caudal L-6 and the rostral L-7 dorsal rootlets, regardless of stimulation site along the vein. Thus, femoral venous afferents appear to enter the spinal cord focally in the caudalmost portion of the femoral nerve input zone, which includes all of the L-5 and L-6 cord segments.

Recordings of evoked compound action potentials from dorsal rootlets or the femoral nerve following electrical stimulation of the femoral-saphenous vein have revealed that the activated afferents include both A-α and A-δ fibers; the most prominent components of the compound action potential had conduction velocities of 58.4 m/sec (S.D. = 6.6, n = 8) and 44.5 m/sec (S.D. = 3.8, n = 8).

Minimal spinal cord processing time (measured by comparing the latency of evoked action potentials recorded from the dorsal roots and ventral roots of the input segment) for femoral venous afferent input is 6.3 msec (S.D. = 2.1 msec). In addition, intraspinal recordings have suggested the presence of multiple pools of interneurons activated by femoral venous afferent input. These data suggest that a multi-interneuronal circuit is involved in processing this input.

Thus, the feline spinal cord appears to receive a focal input from afferents, including large-diameter afferents, innervating the femoral-saphenous vein; this input activates interneurons found in a large number of cord segments. A multisynaptic interneuronal network appears to lie between the primary venous afferents and α-motoneurons.

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76.6 IMMUNOCYTOCHEMICAL LOCALIZATION OF TRANSPORTED WHEAT GERM AGGLUTININ IN THE SPINAL CORD OF THE RAT. K.E. McKenna and I. Nadelhaft, VA Medical Center and Depts. of Pharmacol. and Neurosurg., Univ. of Pitt., Pittsburgh, PA 15240

Experiments were performed on female Sprague-Dawley rats weighing 300-400 gm. The L6 dorsal root ganglia were exposed and injected with 1 to 2 μl of 1% or .25% wheat germ agglutinin (WGA). After survival times of 1 to 3 days, rats were perfused and the spinal cords were processed by immunocytochemistry using the peroxidase-anti-peroxidase (PAP) technique.

Labelled primary afferent fibers were visualized in segments L3 to S2, although the number of labelled fibers diminished greatly after one segment. Rostral to L6, fibers were labelled most heavily in the lateral portions of Lissauer's tract and marginal zone. Caudal to L6, labelled fibers were concentrated in the medial portion of Lissauer's tract and marginal zone. Labelled fibers were visualized in cross section in Lissauer's tract, in a dense plexus in the marginal zone, and extending into the substantia gelatinosa and the deeper layers of the dorsal horn. Fibers were seen extending down the medial edge of the dorsal horn into the dorsal gray commissure. Few labelled fibers were seen in the ventral horn or the dorsal columns. It is possible that large afferent fibers are not labelled with WGA.

A strong pattern of transynaptic labelling was seen in neurons innervated by primary afferents, as previously observed. (Ruda and Coulter, BR 249, P. 237, 1982). Small round cells were labelled in the marginal zone and substantia gelatinosa. Medium and large cells in the N. proprius were labelled as well as the large cells of the lateral spinal nucleus. Clusters of medium sized neurons were labelled in the dorsal gray commissure. All labelled neurons were observed ipsilaterally.

Efferent neurons (both motoneurons and parasympathetic preganglionics) were retrogradely labelled by spread of WGA into the ventral root at the injection site. The reaction product in all labelled neurons was clearly within the cytoplasm and not on the cell surface. No staining was seen in the cell nucleus. The reaction product consisted of large (> 1μ) dark clumps distributed throughout the soma and proximal dendrites. It was easily distinguished from non-specific staining which was a diffuse, homogeneous or very finely granular darkening of the neuron.

The transynaptic labelling was observed even with survival times as short as 1 day. Transport distances were about 40 mm, indicating a minimum transport velocity of 1.3 mm/hr.

These experiments demonstrate that WGA can be used to study both efferent and afferent projections. In addition, WGA is transported across synapses, labelling secondary afferent neurons.

76.7 ELECTRON MICROSCOPIC ANALYSIS OF PRIMARY AFFERENT FIBER TERMINALS FROM RAPIDLY ADAPTING CUTANEOUS MECHANORECEPTORS. M. D. Egger, K. Semba, P. Masarachia*, S. Malamed*, M. Jacquin, S. Harris* and G. Yang*. Dept. of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854

The glabrous skin of the hindlimb in the cat contains three types of low-threshold mechanoreceptors: Pacinian corpuscles (PAC), slowly adapting (SA) and rapidly adapting (RA) receptors. Following our previous electron microscopic studies on the terminals in the dorsal horn of primary afferent fibers from PAC and SA receptors, we have begun to analyze the terminals of afferent fibers from RA receptors. RA afferent fibers were physiologically identified, and iniected intraaxonally with horseradish peroxidase (HRP) in the spinal cord.

At the light microscopic level, HRP-labelled swellings, corresponding to synaptic boutons, were located in laminae II-VI with heaviest concentrations around laminae III-V. Compared with the swellings of PAC or SA afferents, those of RA afferents displayed less tendency to cluster. A tendency, seen in PAC and SA afferent swellings, for rostral swellings to be located dorsally and caudal swellings ventrally was not observed in the RA afferent swellings. At the electron microscopic level, we reconstructed serial sections of 7 boutons labelled with reaction product in laminae III-IV. The analysis of the reconstructed boutons indicated the following: (1) Labelled boutons contained clear, round synaptic vesicles, about 40 nm in diameter. (2) Labelled boutons, sometimes appearing 'scalloped' in shape, ranged from 1.1 to 2.5 μm in longest dimension; in average, smaller than PAC but larger than SA afferent boutons. (3) Individual labelled boutons appeared to form synaptic contacts, mostly of the asymmetric type, with one to four dendritic shafts or spines. (4) In addition, each of these RA boutons appeared to be in apposition to one to four unlabelled structures, all containing clear, flattened or pleomorphic synaptic vesicles. Usually synaptic vesicles were concentrated on both sides of the cleft. Some of the unlabelled vesicle-containing structures also appeared to be presynaptic to some dendritic spines that were postsynaptic to labelled boutons. (5) The complexity of synaptic organization displayed by RA afferent terminals seems to be intermediate between that of SA and PAC afferent terminals, with the PAC terminals the most complex.

In conclusion, the presence of clear, round vesicles and the predominance of axodendritic synapses provide morphological support for strong excitatory input from RA afferents to dorsal horn cells. Combined with our previous studies, the present study also suggests that various cutaneous afferent fibers may be distinguishable to some extent on the basis of ultrastructural features of their terminals in the dorsal horn.

76.8 A TECHNIQUE FOR RECORDING EXTRACELLULAR SINGLE UNIT ACTIVITY FROM NEURONS IN THE LUMBAR ENLARGEMENT OF PHYSIOLOGICALLY INTACT, AWAKE, DRUG FREE, RESTRAINED CATS. J.G. Collins. Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT 06510

Introduction. Most current techniques for the study of spinal cord neurophysiology require that animals be anesthetized or have CNS lesions which will produce a pain free preparation. While humane considerations demand that the animals be pain free, the presence of either anesthesia or CNS lesions introduces an uncontrolled variable which may significantly influence our understanding of the spinal cord. This new technique was developed in order to provide a way of studying spinal cord physiology and pharmacology in pain free, physiologically intact, awake, drug free, restrained cats.

Methods. The success of this new recording technique centers on the surgical implantation, under general anesthesia, of a stainless steel recording chamber which has a central, rectangular opening (7 mm x 12 mm). The chamber is attached to the vertebral column such that the opening in the chamber is positioned over a similar sized opening in the vertebral column over the lumbar enlargement. Following several weeks of recovery from surgical implantation, the animals, which have been adapted to restraint, are placed in a plexiglas restraint box, and a microdrive assembly is attached to the recording chamber. On a daily basis, tungsten microelectrodes (Frederick Haer & Co.) are advanced through the intact dura into the dorsal horn of the spinal cord, and electrical activity from individual neurons is recorded. The microdrive assembly can be moved in the x, y and z planes, thus permitting electrode penetration anywhere within the window.

Results and Discussion. The recordings obtained using this new technique are extremely stable. It has been possible to record for long periods of time from individual neurons. There has been no evidence of change in signal to noise ratios due either to respiratory or cardiovascular movement. In addition, it is possible to record activity from single neurons during either animal or experimenter-induced movement of hindlimbs, movement of the tail, or during animal movement of the musculature of the back. This technique promises to provide us with a means of evaluating both the physiology and pharmacology of the spinal cord in pain free, physiologically intact, awake, drug free, restrained cats. Such studies should enable us to determine not only the influence of previous preparations on our understanding of the nervous system, but should also provide us with a way of evaluating questions about spinal cord function which could not be addressed in the acute preparation.

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76.9 INTERMEDIATE NUCLEUS INTERNEURONS MEDIATING SYNAPTIC ACTIONS ON PRIMARY AFFERENTS AND SPINAL MOTONEURONS. P. Rudomín, I. Jiménez*, and M. Solodkin*. Centro de Investigación del Instituto Politécnico Nacional. México 14, D.F.

An important question pertaining the functional organization of spinal reflexes is the identification of the interneurons mediating presynaptic inhibition and of their axonal projections. In other words, do these neurons belong to private pathways, or are they shared with others which produce postsynaptic excitation or inhibition of motoneurons? This can be approached by recording the dorsal (DRP) and ventral (VRP) root potentials produced by single interneurons as indicators of synaptic actions ocurring on primary afferents and on spinal motoneurons, respectively. In cats anesthetized with pentobarbital, paralized and artificially respired, extracellular recordings were made from interneurons in the intermediate nucleus. Their spontaneous discharges were used for spike triggering averaging of DRPs and VRPs simultaneously recorded from the central ends of S_1 dorsal and ventral rootlets, respectively, the latter with the sucrose gap technique. Although the available sample is still relatively small, it is already possible to identify distinct groups of interneurons mediating specific actions on primary afferents, on motoneurons, or in both of them. 11 neurons produced negative DRPs of .33-3.6 μV and of 4.3-23 ms latency, and inhibitory VRPs of .40-4.5 μV and .5-13.7 ms latency. Peak values were attained between 40-50 ms and total durations were between 100-200 ms. Two of these neurons were activated bi- or trisynaptically by gr. I volleys in the posterior biceps semitendinosus (PBSt); three, mono- or bisynaptically by gr. I gastrocnemius soleus (GS) fibers, and nine polysynaptically by cutaneous volleys, 1.3-3 xT strength. These interneurons appear to mediate FRA and Ib inhibitory actions on motoneurons and also the PAD of Ib fibers. Three other interneurons produced negative DRPs and biphasic (excitatory-inhibitory) VRPs with the inhibition lasting as long as the DRPs. They were activated by gr. I GS but not by PBSt afferents, and polysynaptically by cutaneous fibers. Only one interneuron produced a short latency negative DRP (6.5 ms) without any VRP. This neuron was only activated bisynaptically by gr. I GS fibers and may mediate PAD of gr. Ia fibers. Four neurons produced no DRPs but only inhibitory VRPs with amplitudes of 1.4-4 μV and latencies ranging from 0.6 to 16.3 ms (mean 7.6±5.9 ms) which lasted 60-140 ms. Three of them were polysynaptically activated by gr. I PBSt, two by gr. I GS volleys, an all by low threshold (1.2-2xT) cutaneous fibers. Finally, 7 cells produced no DRPs or VRPs; they were bisynaptically activated by gr. I GS but not by PBSt or sural afferents, and polysynaptically by superficial peroneus volleys (3.13±1.48 xT strength).

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76.10 FUNCTIONAL CONNECTIONS OF INTERMEDIATE NUCLEUS INTERNEURONS MEDIATING SYNAPTIC ACTIONS OF PRIMARY AFFERENTS DISCLOSED BY MEANS OF SPIKE TRIGGERED AVERAGING OF DORSAL ROOT POTENTIALS. M. Solodkin*, I. Jiménez* and P. Rudomín (SPON: G. Meza) Centro de Investigación del Instituto Politécnico Nacional, México 14, D.F.

The purpose of these studies was to identify the interneurons in the spinal cord which produce primary afferent depolarization (PAD) and to characterize their activation patterns. The experiments were done in anesthetized cats, paralyzed and artificially respired. PAD was disclosed by using the spontaneous spike activity of intermediate nucleus interneurons to trigger the averaging of dorsal root potentials (DRPs), recorded from fine S1 and L7 dorsal rootlets. Of 113 interneurons, 43 produced DRPs with a peak amplitude in the range of 0.4-4 μV (mean±S.D.=1.58±.32μV), and a time course similar to that of the DRPs produced by electrical stimulation of gr. I muscle nerves (time to peak 53.6±22.5ms, duration 100-200 ms). Type I neurons produced DRPs with a mean latency of 7.58±2.55 ms (n=23) and responded mono-, bi- or trisynaptically to gr. I volleys in the posterior biceps and semitendinosus (PBSt) and gastrocnemius soleus (GS) nerves. Ten of these interneurons were polysynaptically activated by low threshold (2.08±.08xT) sural (SU) fibers. Type II and III neurons produced DRPs with longer latencies (means 21.49±2.2 ms, n=9 and 34.1±1.84 ms, n=5 respectively) and were monosynaptically activated by gr.I PBSt and GS volleys. It thus appears that type I neurons form part of the shortest pathways producing PAD. Since Ia fibers are not depolarized by stimulation of cutaneous nerves, interneurons were further classified in types b and a depending on whether or not they responded to these inputs. Type Ia neurons (n=4) were activated by PBSt and GS gr. I volleys (below 2xT threshold) and inhibited by stimulation of the brain stem (BS) ipsilateral reticular formation (with 600 Hz trains). Type Ib interneurons (n=10) were excited by PBSt stimuli with mean strengths of 2.28±1.0xT and also by gr. I GS fibers (below 2xT threshold). 70% of these cells were inhibited and 20% excited by BS stimulation. It is suggested, as a working hypothesis, that type Ia interneurons mediate the PAD of gr. Ia fibers. The type Ib neurons, which are excited by BS stimulation, have the requirements expected for the interneurons mediating the PAD of Ib fibers.

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76.11 CORRELATION OF RESPONSES TO NATURAL STIMULATION OF THE SKIN WITH RESPONSES TO SURAL NERVE STIMULATION: THE NATURE OF LATENT SYNAPSES. Lillian M. Pubols. Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97210.

Latent projections to dorsal horn neurons have been demonstrated by electrical stimulation of skin, nerves, or dorsal roots not supplying the natural receptive fields of those neurons. Mendell et al. (J. Physiol., 285:299, 1978) have shown that a small percentage (1.4%) of L7 dorsal horn neurons, responsive to natural stimulation of hindlimb skin in the intact cat, can discharge with monosynaptic latencies to electrical stimulation of flank skin, supplied by distant dorsal roots. A larger percentage of these neurons (31%) discharge with polysynaptic latencies, the remainder being unresponsive to distant stimulation. In view of the fact that latent synapses can be demonstrated from afferents projecting via dorsal roots 4-5 segments from the recording site, one might expect there to be a much higher proportion of neurons receiving latent projections from nearer dorsal roots. To test this hypothesis the responses of L6 and L7 dorsal horn neurons to stimulation of the sural nerve were compared for neurons having receptive fields within the region of skin innervated by the sural nerve, i.e., the lateral foot and toes, versus those having receptive fields on other parts of the foot. Extracellular action potentials were recorded in response to low and high threshold cutaneous stimuli, and to electrical stimulation of A fibers in the sural nerve. Neurons with natural cutaneous receptive fields outside the sural nerve territory never gave short, fixed latency (<5 msec), presumably monosynaptic, responses to sural nerve stimulation (n = 14). Four of these gave excitatory responses to nerve stimulation with longer, variable, presumably polysynaptic latencies, and the remainder had no excitatory responses to nerve stimulation. Four out of 5 neurons with receptive fields on the lateral foot and toes gave short latency excitatory responses to sural nerve stimulation, and the fifth had longer, more variable latencies. These data are very similar to the results of Mendell et al., and demonstrate that the proportion of neurons exhibiting monosynaptic latent inputs in the intact cat is very small, regardless of the proximity of the roots supplying the effective and latent projections. (Support: NIH, NS16634, NS19523)

EXCITATORY AMINO ACIDS I

77.1 CHARACTERISATION OF THE LOW AFFINITY KAINIC ACID BINDING SITE SOLUBILIZED FROM PIGEON CEREBELLUM. A. Dilber*, H. Henke*, M. Cuénod and K. Winterhalter* Brain Res. Inst., Univ. of Zürich, Switzerlandand and Dep. of Biochem., Swiss Federal Institute of Technology, Zürich, Switzerland).

The pigeon cerebellum contains a large number of kainic acid binding sites (B_{max} = 118 pmol/mg protein). Isotopic dilution experiments indicate the presence of a high (K_d = 30 nM) and a low affinity (K_d = 330 nM) binding site. In crude membrane preparations binding to the low affinity site shows positive cooperativity (n = 2.2). The low affinity site is specifically localized in the molecular layer of the cerebellar cortex (Henke H. et al.,1981, Brain Res., 219, 95-105). At a concentration of 0.25% the non-ionic detergent Triton X-100 solubilizes preferentially the low affinity binding site, whereas 1% Triton X-100 solubilizes also the high affinity site. Both sites were in an active form after solubilization. The affinity for kainic acid (K_d) in solution was 20 nM and 440 nM respectively for the high and low affinity binding site. Again binding with positive cooperativity (n = 1.5-2) was seen for the low affinity binding site. However, in contrast to the observations with membrane bound sites no paradoxical increment of bound ^{3}H-kainic acid (hook effect) was present in solubilized material. Kainic acid and L-glutamic acid showed similar displacing potencies in the membrane bound (IC_{50} : 2 and 170 μM) and the solubilized (IC_{50}: 6 and 250 μM) low affinity binding site. Kainic acid dimethyl ester (IC_{50} = 200 μM) and gamma-methylene-L-glutamic acid (IC_{50} = 200 μM) had a more potent displacing activity than L-glutamic acid. Dihydrokainic acid, a kainic acid derivative (obtained by catalytical reduction of the double bond in the isopropylene side chain), shows very little displacing activity (IC_{50} >1 mM). These two facts confirm the importance of the unsaturated side chain in kainic acid for its binding. Gel filtration chromatography resulted in a four to six-fold enrichement of the low affinity site, indicating a molecular weight of about 440'000, in keeping with the sedimentation coefficient of 12S obtained by density gradient centrifugation. Isoelectric focusing experiments in thin layer agarose and polyacrylamide gels gave a pI of 4.8-5.5 for the low affinity kainic acid binding site.

77.2 ANTAGONISM OF N-METHYL-D-ASPARTIC ACID EXCITATION OF RAT HIPPOCAMPAL PYRAMIDAL NEURONES IN VITRO BY PHENCYCLIDINE APPLIED IN KNOWN CONCENTRATIONS. M.G. Lacey* and G. Henderson* (SPON: Paul A. Fuchs.) (Dept. of Pharmacology, University of Cambridge, U.K.)

The dissociative anaesthetic and psychotomimetic agent phencyclidine (PCP) has been reported to reduce the action of the excitatory amino acid analogue N-methyl-D-aspartic acid (NMDA) on spinal interneurones in vivo (Lodge and Anis, Eur.J.Pharmacol., 77; 203, 1982). The action of PCP on NMDA-evoked excitation of rat hippocampal neurones has been investigated by means of intracellular recording from pyramidal cells in the CA1 region of the submerged in vitro brain slice preparation.

NMDA (100 µM) or glutamic acid (10 mM) was pressure ejected from a micropipette positioned close to the site of recording for 1-5 sec at 1-10 psi, producing rapid, transient depolarising responses which frequently resulted in the firing of action potentials. Application of PCP in the bathing fluid at known concentrations (0.3 to 3 µM) resulted in a reduction or abolition of the response to NMDA after a 10 minute exposure. Complete recovery of the response to NMDA was rarely seen and when it was, a washout of at least 2 hr for PCP was required. Responses to NMDA were reversibly inhibited by the selective NMDA receptor antagonist DL-2-amino-5-phosphonovaleric acid (2APV) (3-100 µM). In contrast, responses evoked by glutamate were unaffected by both PCP and 2APV.

Responses to NMDA were also abolished by 5 mM cobalt chloride; this and other evidence (Dingledine, J.Physiol.; in press, 1983) indicates a major role for calcium entry in the ionic mechanism of NMDA. The calcium channel blockers D600 and verapamil have been shown to be potent displacers of [^{3}H]PCP binding on rat brain membranes (Quirion and Pert, Eur.J.Pharmacol., 83; 155, 1982) and it may be that a close association of the PCP binding site with calcium channels underlies its antagonism of NMDA. At present it is unclear whether PCP antagonises NMDA by an action at the NMDA receptor or by acting at a stage in the depolarizing mechanism subsequent to that of receptor activation.

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77.3 ACTIVITY OF ANALOGUES OF QUINOLINIC ACID ON RAT CORTICAL NEURONES. Trevor W. Stone, Dept. of Physiology, St. George's Hospital Medical School, University of London, London SW 17, UK.

Quinolinic acid, a rigid analogue of N-methyl-aspartate (NMA) which exists endogenously in mammals has been shown to be excitatory when applied to neurones in the mammalian CNS (Europ J. Pharmacol. 72, 411 - 412; Brain Res. 259, 172-176). That excitation appears due to the selective activation of NMA receptors. We have now examined the effects of a number of methyl derivatives of quinolinate as potential selective agonists or antagonists. Compounds were applied by microiontophoresis to neurones in the cerebral cortex of male rats anaesthetised with urethane.

The 2-methylester of quinolinic acid showed some tendency to reduce responses to NMDA, causing a mean reduction of 26% (± 9, s.e.m) on 10 of 26 neurones when applied with currents of less than 30 nA. However, this action was complicated by agonist activity especially at higher currents, with a marked increase of neuronal firing occurring on 16 of the 26 cells. The N-methyl-2, 3-dimethylester analogue also showed weak agonist activity but no antagonist properties. The higher homologue of quinolinic acid, homoquinolinic acid (2-carboxy-pyridine-3-acetic acid) which is the rigid glutamate analogue of quinolinic acid proved to be as active an excitant as NMDA on the basis of nanocoulombs required for equivalent excitation. Homoquinolinate was thus about 5 times more potent an excitant than quinolinate. Furthermore homoquinolinic excitations could be blocked completely by the 'NMDA' antagonists aminophosphonovaleric (APV) and aminophosphonoheptanoic (APH) acids, while glutamate responses were unchanged. This implies that activation of the APH sensitive receptor is more dependent on the rigidly extended conformation of the activating molecule than merely on the length of carbon chain separating the carboxyl groups.

77.4 LESIONING EFFECTS OF IBOTENATE IN THE IMMATURE RAT BRAIN AND PROTECTION BY 2-AMINO-7-PHOSPHONOHEPTANOIC ACID, P.J. Roberts[1], Gethin J. McBean*[1], H.X.Steiner*[2], C. Kohler*[3], and R.Schwarcz[2].
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[2] Maryland Psychiatric Research Center, Baltimore, MD 21228,
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Ibotenate (ibo) like kainate (KA) will cause "axon-sparing" lesions following injection into the CNS of the adult rat. In the developing striatum or cerebellum however, KA fails to exhibit this property, possibly due to immaturity of receptor numbers or an undeveloped mechanism mediating KA-toxicity. Because of a number of dissimilarities between the neurotoxic actions of ibo and KA in adult animals, we have investigated the neurochemical and morphological sequelae of ibo injection into the immature rat striatum, hippocampus and cerebellum.

7-day old rats were anaesthetised with ether and injections of ibo (10 μg) made stereotaxically into one of the brain areas defined above, utilising coordinates established from pilot dye-injection experiments. Pups were allowed to survive for 2 days, after which time they were decapitated and brains processed for light microscopic examination, or neurochemical analyses.

Injections of ibo into striatum or hippocampus resulted in the complete loss of neuronal cell bodies. Catecholamine histofluorescence in the striatum was abolished, indicating loss of afferent terminals. In contrast with KA, "distant" damage was not observed. Injections of ibo into cerebellum , produced no detectable damage.

Neurochemical analysis showed large decreases in the uptake of labelled GABA, glu and DA into a striatal P_2 preparation, although glu uptake was least affected. In hippocampus, major reductions were seen in GABA and glu uptake. CAT activity was reduced by 31%. Co-injection of 10 μg ibo with equimolar (13.6 μg) (-)-2-amino-7-phosphonoheptanoate (APH) into the striatum, resulted in protection of both striatal cell bodies and DA nerve terminals.

Thus, in contrast to the effects seen with KA, intrastriatal injection of ibo in 7-day old rats resulted in extensive loss of neurones. Furthermore, unlike the effects seen with ibo in the adult rat, there was a striking lack of axon-sparing properties.

Finally, excitotoxicity is believed to be mediated through specific membrane receptors, and, in the case of ibo, probably via those activated preferentially by NMDA. The ability of APH to provide protection of both intrinsic neurones and DA terminals, indicates the presence of neurotoxicity-mediating receptors on these cellular elements (including afferent fibres) in the immature rat striatum. Thus, it is clear from this study that the development of KA and ibo receptors, and the associated mechanisms for excitotoxicity follow different ontogenetic patterns.

This work is supported by grants from The Wellcome Trust to P.J.R. and by a NATO Research Grant to P.J.R. and R.S.

77.5 STRIATAL NEURODEGENERATION CAUSED BY LOCAL INJECTION OF DL-THREO-3-HYDROXYASPARTATE AND BY LONG TERM ADMINISTRATION OF L-GLUTAMATE, Gethin J. McBean* and P.J. Roberts, Dept. Physiology & Pharmacology Univ. of Southampton, Southampton, SO9 3TU, U.K. (SPON: A. Nistri).

The low efficacy of the endogenous excitatory amino acid L-glutamate as a neurotoxic agent, when compared with kainate and ibotenate, may be attributed to its rapid removal from the site of injection by efficient reuptake mechanisms. We have tested the ability of the potent glu uptake inhibitor, DL-threo-3hydroxyasp, to cause degeneration of striatal cells following a single, local injection. We have also tested whether continuous infusion of a high dose of glu will result in similar degenerative changes.

DL-threo-3-hydroxyasp (170nmol in 1μl PBS,pH 7.4) was injected stereotaxically into the left striatum of female Wistar rats, and assays were performed 2 weeks later. There was a significant reduction in both CAT activity (30%) and Na^+-dependent ^{3}H-GABA uptake (40%) in the lesioned,compared with the contralateral striatum. For histological analysis by light microscopy, brains were perfusion-fixed in glutaraldehyde-paraformaldehyde, embedded in wax, and 6 μm coronal sections stained with thionin. An area of pronounced neuronal degeneration surrounding the site of injection was evident.

To enable long-term administration of glutamate, rats were implanted with left striatal cannulae and, one week later, Alzet® osmotic minipumps containing 1M L-glutamate in 0.1M PBS, pH 7.4 were fitted. Control rats received 0.1M PBS only. Glutamate infusion (flow rate approx= 0.5μl/h) was continued for a period of 1 week. Na^+-dependent ^{3}H-GABA uptake and CAT activity were determined in triplicate, in both the lesioned and control striata. Both these indices of striatal function fell by 35-38% in the lesioned side, whereas infusion of 0.1M PBS alone for one week did not result in any significant changes. Examination of striata by light microscopy indicated in the glu-treated animals, a wide area of neuronal degeneration, extending on either side of the cannula tract, wherein the neurones were dark and misshapen. In the control striatum, the cannula tract was easily identifiable, yet there was no evidence of damage to the surrounding cells.

These results indicate that prolonged exposure to a sufficiently high concentration of glu, will cause degeneration of striatal neurones, as will a single dose of a potent glu uptake inhibitor.

This work highlights the important role played by the high- and low-affinity uptake systems in the prevention of striatal neurodegeneration by potentially toxic levels of glutamate.

This work was supported by a grant from the Wellcome Trust to P.J.R.

77.6 GLUTAMINASE AND ASPARTATE AMINOTRANSFERASE AS MARKERS FOR GLUTAMERGIC / ASPARTATERGIC NEURONS IN RODENT AND MONKEY CEREBRAL CORTEX R.A. Altschuler, M. Parakkal, W.G. Haser, R.J. Wenthold and J.P. Donoghue, Lab. Neuro-otolaryngology, NIH, and Lab. Neurophysiology, NIMH, Bethesda, MD, Dept. Biochem., Univ. Pittsburgh, Pittsburgh, PA, and Lab. Neurophysiol., Univ. Wisc., Madison, WI.

It has been proposed that glutaminase and/or aspartate aminotransferase, enzymes involved in the routine metabolism of glutamate and aspartate, may have an additional function in the regulation and replenishment of neurotransmitter pools of excitatory amino acids. These enzymes would then be present in increased levels in such neurons and be able to serve as markers in immunocytochemical studies. In this study antisera to glutaminase (GLNase) and antisera to aspartate aminotransferase (AATase) were used in an immunocytochemical examination of the cerebral neocortex of the rat, guinea pig, and monkey (M. fascicularis).

In the rat and guinea pig neocortex dense GLNase-like immunoreactivity (ir) is present in pyramidal cells located mainly in layers V (the origin of most descending corticofugal pathways) and VI (the origin of corticothalamic fibers), and to a lesser extent in layer III. Layers I, II and IV contain very few well-labeled cells. A similar pattern is evident in monkey neocortex in the primary visual cortex (area 17) and in area 3b of the primary somatic sensory cortex, but in areas 1,2,5 and 7 labeled cells are also common in the deeper part of layer III. In areas 2, 5 and 7 the large, deep layer III pyramids are densely labeled. The motor cortex (area 4) contains numerous labeled cells throughout layers III-VI. However, the giant layer V pyramidal (Betz) cells, are not labeled.

Antisera to AATase produces an entirely different pattern In total, fewer neurons are labeled than with antisera to GLNase. AAT-ir is present mainly in non-pyramidal neurons, but some pyramidal cells also appear to be labeled. All cortical laminae contain some AATase-ir neurons but they are more common in layers II and III throughout the neocortex and in layer IVc of the monkey primary visual cortex.

The localization of GLNase-ir neurons to layers V and VI supports the hypothesis that corticothalamic and many other corticofugal pathways use an excitatory amino acid transmitter. Some cortico-cortical neurons, especially those in layer III of non-primary sensory areas of monkey cortex may also use such a transmitter. The lack of GLNase-ir in many layer II-III pyramidal cells suggests that some cortico-cortical neurons either use a different transmitter or a different synthetic pathway.

77.7 DESENSITIZATION TO GLUTAMATE AND ASPARTATE IN RAT PREPYRIFORM CORTEX SLICES. D. J. Braitman. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, Maryland 20814.

In a previous report (Hori et al., J. Neurophysiol., 48: 1289, 1982) my colleagues and I presented electrophysiological and pharmacological evidence that neither L-glutamate (GLU) nor L-aspartate (ASP) appeared to be the excitatory neurotransmitter from lateral olfactory tract to prepyriform cortex (PPC) pyramidal neurons. However, kainic acid (KA) and N-methyl-dl-aspartate (NMA) exhibited pharmacological profiles similar to that of the endogenous transmitter. In the present study I investigated the ability of repeated applications of these amino acid receptor agonists to desensitize the endogenous synaptic receptors in the in vitro prepyriform cortex slice preparation.

Double shock orthodromic stimulation to the LOT delivered every 4 sec throughout the experiment evoked field potentials that were recorded from the pial surface of the submerged, constantly perfused, tangential slice of rat PPC (350μ-450μ thick). To quantify the action of amino acids added to the perfusate, the amplitude of the slow wave component of the field, representing the population excitatory postsynaptic potential, was measured and compared to control values. Initial application of 2×10^{-3} M GLU for 5 min resulted in a significant decrease in the amplitude of the field potential due to the depolarizing action of GLU. The amplitude of the field potential returned to control, i.e., pre-drug values, 10 to 15 min after termination of the drug application. A second perfusion of 2×10^{-3} M GLU was less effective; i.e., the amplitude of the field potential decreased less than it did after the first application of GLU, suggesting desensitization of the receptor-mediated glutamate depolarization. Successive application had even smaller effects. However, the amplitude of the field potential continued to return to control value after termination of the GLU perfusion even though the GLU receptors were still desensitized. The response to repeated application of ASP desensitized in a fashion similar to GLU while the responses to repeated applications of KA and NMA did not desensitize. The responses to D-glutamate, which is not taken up by the high-affinity uptake system, also desensitized after repeated applications. This indicates that the phenomena seen in these experiments did not involve the facilitation of an inactivation mechanism.

Thus, although exogenously applied L-glutamate and L-aspartate desensitize their postsynaptic receptor sites, they do so without affecting the receptors to the endogenous neurotransmitter. On the other hand, the responses to KA and NMA, which have a pharmacological sensitivity to the antagonist 2-amino-4-phosphonobutyric acid similar to that of the endogenous transmitter, did not desensitize. The failure to find cross-desensitization between L-glutamate and L-aspartate receptors and the receptors at the terminal synapses of the lateral olfactory tract is further evidence that neither glutamate nor aspartate is the excitatory transmitter on prepyriform pyramidal neurons.

77.8 PHENYLALANYL-L-GLUTAMATE ENHANCES SPECIFIC [^{3}H]-2-AMINO-7-PHOSPHONO HEPTANOIC ACID AND [^{3}H]-L-GLUTAMATE BINDING AND INHIBITS SPECIFIC [^{3}H]-KAINIC ACID BINDING TO RAT BRAIN MEMBRANES IN VITRO J. Ferkany, R. Zaczek*, A. Markl*, and J.T. Coyle. Depts of Psychiatry, Neuroscience, Pharmacology, Div. Child Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Electrophysiological and in vitro binding studies have identified 3 classes of excitatory amino acid receptors in brain which are preferentially activated by N-methyl-D-aspartate (NMDA), glutamate (Glu) or kainic acid (KA). In the present study, we have investigated the effects of a number of dipeptides on the specific binding of [^{3}H]-L-Glu, [^{3}H]-APH, a reputed NMDA antagonist, and [^{3}H]-KA to rat brain membranes in vitro. For assay, a previously frozen buffy-coat preparation from brain was washed 4 times by centrifugation and preincubated for 30 min at 37°C in Tris citrate buffer (0.05mM pH 7.2). One ml of the tissue (0.4 mg/ml) was added to 1 ml of buffer containing the ligand and compounds of interest; and the incubation continued for 90 min. The reaction was terminated by centrifugation; and radioactivity was measured in the solubulized pellets.

Phenylalanyl-L-glutamate (PG) (EC_{50} 4.9 uM) enhanced the specific binding of both [^{3}H]-L-Glu and [^{3}H]-APH to brain membranes in a dose dependent manner. Specific binding of [^{3}H]-strychnine and [^{3}H]-spiroperidol were unaffected by PG. The maximal increase in [^{3}H]-APH binding (+445+19%) was greater than for [^{3}H]-L-Glu (+292+25%); but in both instances, the increase in binding was due exclusively to alterations in the apparent B_{max} of the ligands. The action of PG(50 uM) on [^{3}H]-APH binding was greatest on hippocampal (+950+100%) and least on cerebellar (+320+10%) membranes. PG failed to alter the non-specific binding of [^{3}H]-APH to peripheral organs or heat treated brain sonicates. The pharmacological profile of [^{3}H]-APH binding in the presence and absence of PG was identical and consistent with binding of the ligand at a Glu-type receptor. The action of PG was mimicked by Tyr-Glu(EC_{50}=11.3uM). Binding of [^{3}H]-APH was also enhanced by Tryp-Glu, Leu-Glu, and Meth-Glu (EC_{50}=29,5,5.5) although these compounds were less efficacious than PG or Tyr-Glu. The order of potency of the compounds to inhibit specific [^{3}H]-KA binding to brain was Meth-Glu>PG>Leu-Glu >Tyr-Glu>Try-Glu (K_I=3,7,8,19,70 uM). While the action of PG (50uM) and Tyr-Glu (100uM) on [^{3}H]-APH binding were not greater than PG (50uM) alone, Meth-Glu (50uM) significantly attenuated the binding of [^{3}H]-APH in the presence of PG (50uM) suggesting that Meth-Glu may be a partial antagonist at the PG site. The results of the present study demonstrate an interaction of a class of Glu-containing peptides with both Glu and KA type receptors in brain and suggest that alterations in one receptor may influence the other.

77.9 ONTOGENESIS OF KAINIC ACID INDUCED SEIZURES AND BRAIN DAMAGE: THE CRUCIAL ROLE OF HIGH AFFINITY [^{3}H] KAINIC ACID RECEPTORS IN THE AMYGDALA. M.Berger*, E.Tremblay*, L.Nitecka* & Y.Ben-Ari. Lab.Physiol.Nerveuse, CNRS, F-91190 Gif-sur-Yvette, France.

Systemic and intracerebral injections of kainic acid (KA) into adult rats produce a typical limbic seizure - brain damage syndrome which is a useful model for human temporal lobe epilepsy (Ben-Ari et al., Neuroscience 6/7,1361-1391, 1981). The involvement of the entire limbic circuity (hippocampus, amygdala, septum, limbic cortex etc.) is clearly revealed by ^{14}C 2-desoxyglucose (2-DG) studies (ibid.). In contrast, in immature rats (<18d), KA i.p. produces tonico-clonic generalized seizures without limbic signs, with the exclusive metabolic activation of the hippocampus (Tremblay et al., Neurosci.Abstr. 287/8, 1982), and no clearcut brain damage (Nitecka et al., submitted).

We have now investigated the ontogenetic appearance of [^{3}H] KA receptors in rat brain, differentiating between low and high on-off rate receptors, and found both populations to be heterogeneous. In particular, we discovered 3 types of low on-off rate KA receptors, with low (K_D=20nM), high (K_D=6nM), and very high (K_D<1 nM) affinities for the ligand. In rats younger than 16d, we found high affinity receptors in the hippocampus, but not in the amygdala, where this receptor type appeared after the 18th day of life. This is exactly the time point when 1) the animals first display limbic motor seizures, 2) the 2-DG maps reveal an activation of most of the limbic circuity, including the amygdala, 3) irreversible brain damage occurs in vulnerable structures (in particular the hippocampus), and 4) the convulsions give rise to a greater vulnerability to limbic seizures eliciting mechanisms when the animal has become adult (Nitecka et al., submitted).

Our results add further evidence for the crucial role of the amygdala in the expression of limbic seizures and offer for the first time a correlation of one of the most prominent actions of KA with a well defined subpopulation of KA receptors. The physiologic ligand for these receptors remains to be identified.

77.10 CONTENT AND IN VITRO RELEASE OF ENDOGENOUS AMINO ACIDS IN THE AREA OF THE NUCLEUS TRACTUS SOLITARIUS (NTS). M.P. Meeley, M.D. Underwood*, W.T. Talman and D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine potential amino acid (AA) neurotransmitter candidates in the area of the NTS in rats. Twenty AAs were analyzed by reverse-phase HPLC with fluorescence detection (100 fmol limit). Rats were decapitated, brains removed and sliced (1mm), and micropunches (1mm) taken of NTS and, for comparison, Caudate Nucleus (CN) and Ventrolateral Medulla (VLM). Micropunches of NTS, extracted with 0.4N perchlorate, contained notably high amounts of Glu (446+32 nmol/mg prot., n=7), Gly (375+50) GABA (359+35) and Gln (214+14), and moderate to low levels (170-5 nmol/mg) of Tau, Asp > Ser > Arg > Ala > Thr > His > Tyr > Trp > Met > Asn, Val, Phe, Ile, Leu, but no B-Ala. Glu levels in NTS were intermediate to those measured in CN (691+56, n=6), a glutamatergic area, and VLM (249+24), which has no known Glu input. The GABA content of NTS was significantly higher than that of CN (235+18, $p < .05$) or VLM (119+13, $p < .001$). Gly content was greatest in NTS ~ VLM > CN; Asp, NTS ~ CN > VLM. To examine non-selective release, bilateral micropunches of NTS (CN or VLM) from 3 rats were pooled and superfused (200uL/min) with normal and high K+ (56mM, 4 min) media without Ca2+. Basal release values in NTS were highest for Gly > Glu > Asp > Tau > GABA. Stimulation with K+ resulted in release of all AAs measured, with a variable range of response between experiments (e.g., Glu, 1-3 fold increase over basal levels). To determine the transmitter-specific component of release, calcium was added to the media and basal and K+-stimulated release measured. Ca2+-dependent release in NTS was observed for Glu (36+7% of total releasable pool), GABA (87+5%), and Gly (24+6%) (n=5). In CN, a larger fraction (> 90%) of the total Glu released upon stimulation was Ca2+-dependent, , with only moderate release of GABA, Gly, and Asp. A slight response to K+(+calcium) was noted for Glu and GABA in VLM. We conclude: (a) the abundance of Glu and its Ca2+-dependent release in NTS is strong additional support for the transmitter role of Glu in this area; (b) high GABA pools and dramatic releasability of GABA in NTS is compelling evidence for transmitter function of this AA in the region; and, (c) Gly may also act as a transmitter in NTS, given its substantial content, though modest Ca2+-dependent release. (Supported by Grant HL 18974 and HL 07378.)

77.11 RECEPTOR-MEDIATED EXCITOTOXIN-INDUCED LYSIS OF NEUROBLASTOMA CELLS IN CULTURE. A.T. Malouf, R.L. Schnaar and J.T. Coyle. Depts of Neuroscience, Psychiatry, and Pharmacology and Experimental Therapeutics, Div. of Child Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The N18-RE-105 neuroblastoma x embryonic retina hybrid cell line was previously shown to have a high density of specific binding sites for [^{3}H]-L-glutamate (GLU) in washed membranes with a B_{max} of 15.5 pmoles/mg protein and a K_D of 654 nM (Malouf, A.T., et al,(1982) Soc. Neurosci. Abstr. 8,878). Pharmacological studies revealed a high correlation (r=0.92) of the K_I's of several drugs which bind with high affinity to the [^{3}H]-GLU receptor in rat brain membranes when compared to their K_I's of binding to the neuroblastoma hybrid cell membranes. In both cases, [^{3}H]-L-GLU appears to label a quisqualate-preferring receptor.

To determine if the binding sites labelled by [^{3}H]-GLU on N18-RE-105 neuroblastoma membranes could mediate the cytotoxic effects of acidic amino acids on intact cells, neuroblastoma were grown in the presence of various concentrations of neuroactive amino acids and their analogs. Cell viability was assessed by lysing the cells firmly attached to the culture dish in phosphate buffer containing 0.5% Triton X-100, and measuring the activity of the cytosolic enzyme lactate dehydrogenase (LDH). In addition, LDH activity released into the growth medium by cell lysis during culture was also determined. After 24 hours of cell culture in the presence of 10 mM L-GLU, the cell associated LDH activity was decreased by 25±1% ($p<0.025$) whereas LDH activity in the media was increased 415±17% ($p<0.001$). The number of viable neuroblastoma cells continues to decline for up to 3 days in the presence of GLU, resulting in a 90% reduction in cell number compared to control cultures. Microscopic inspection of the cultures grown in 10 mM GLU revealed a predominance of distended cells filled with vacuoles. As assessed by the reduction of cellular LDH, the increase in media LDH and morphological alterations, quisqualate was the most potent cytotoxin (1mM) followed by L-GLU and ibotenate (10mM); D,L-2-amino-4-phosphono butyrate (10mM), a mixed antagonist, was only partially effective. In contrast, kainate, N-methyl-D,L-aspartate and GABA, which do not compete at the [^{3}H]-GLU receptor site, exhibited no cytotoxic effects on the N18-RE-105 cells at 10mM.

The results indicate a correlation between cytotoxic potency and affinity for the [^{3}H]-GLU receptor of excitatory GLU analogs; furthermore, the cytologic alterations accompanied by cell lysis are consistent with observations on the excitotoxic action of these compounds on CNS neurons. Supported by NIH NS13584, and MOD grant #5-302.

77.12 EFFECTS OF KYNURENATE ON ROOT POTENTIALS EVOKED BY SYNAPTIC ACTIVITY AND AMINO ACIDS IN THE FROG SPINAL CORD. Keith S. Elmslie* and Doju Yoshikami. Dept. of Biology, University of Utah, Salt Lake City, Utah 84112.

Perkins & Stone (Brain Res. 247: 184, 1982) have reported that kynurenate (Kyn) inhibits the effects of certain excitatory amino acids in mouse cortical cells. We report here our findings of the effects of Kyn on dorsal and ventral root potentials evoked by synaptic activity and by bath application of amino acids in isolated, hemisected spinal cords of frog. Kyn (0.5 mM) rapidly and reversibly blocked at least 90% of the synaptically mediated ventral root potential produced by stimulation of the dorsal root. Spontaneous activity recorded from both ventral and dorsal roots was also abolished by Kyn. Kyn is not, however, a general inhibitor of synaptic transmission since Kyn concentrations as high as 2.5 mM had no effect on synaptically mediated dorsal root potentials produced by stimulation of the ventral root. In addition, Kyn had no effect on synaptic transmission in sympathetic ganglia of frog.

In spinal cords treated with TTX, ventral root potentials (VRPs) induced by N-methyl-D,L-aspartate, quisqualate, kainate, aspartate, and glutamate were all significantly reduced by 2.5 mM Kyn. In contrast, GABA-induced depolarizations measured from the dorsal root were not antagonized by Kyn. Kyn itself, when applied to a TTX-treated spinal cord, did not produce any ventral root response.

The dose-response curve to quisqualate was shifted to the right in 2.5 mM Kyn. Furthermore, when Kyn is washed out, the rate of recovery from Kyn block appears to be accelerated by the presence of quisqualate. These results suggest that quisqualate and Kyn compete for common binding sites. However, although concentrations of Kyn in range of 1 mM or greater generally reduced quisqualate-induced VRPs, lower concentrations of Kyn (e.g. 0.1 mM) potentiated the peak of the response to saturating concentrations of quisqualate by as much as 30%. Interestingly, the duration of the potentiated quisqualate responses were significantly shorter than the unpotentiated responses. Thus, Kyn does not act simply as a competitive inhibitor of quisqualate. These results, taken together, raise the possibility that there is more than a single class of binding sites for quisqualate and Kyn in the cord.

Detailed experiments on the action of Kyn on the responses to other amino acids remain to be performed.

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77.13 IONIC MECHANISMS UNDERLYING THE ACTION OF PUTATIVE TRANSMITTERS ON ENZYMATICALLY DISSOCIATED HORIZONTAL CELLS. R. Shingai* and B. N. Christensen. Dept. of Physiology & Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

Glutamate is one suggested candidate for the excitatory transmitter that is released from photoreceptors at synapses made with horizontal cells in the fish retina (Laster, E.M. & Dowling, J.E., Proc. Natl. Acad. Sci., 1982). The action of this substance is to depolarize the cell membrane of both horizontal cells in the intact retina as well as horizontal cells enzymatically isolated from intact retina. A prevalent hypothesis that explains the modulation of horizontal cell membrane potential during changes in luminosity is that the excitatory transmitter is continuously released from the photoreceptors. It would therefore be expected that transmitter activated receptors do not desensitize in the continuous presence of the transmitter. Furthermore, the reversal potential of the transmitter should be at a minimum near zero mV in order to explain the low resting potential recorded from horizontal cells in the intact dark adapted retina. We investigated the ionic basis of the physiological responses produced by the application of glutamate to horizontal cells enzymatically isolated from the catfish retina. Experiments using both current and voltage clamp were done to examine the changes in membrane potential and ionic currents during application of the agonist.

The putative neurotransmitter l-glutamate was pressure ejected onto isolated horizontal cells under both current and voltage clamp conditions. Pulses of the transmitter (500 uM in the pipette) produced a depolarizing potential and an inward going current. Inward currents of up to 1 nA could be elicited. The drug response did not inactivate over a period of time lasting up to 20 sec. This current reversed at a membrane potential of about +5 mV. Above this potential the current became outward and the amplitude of the current was linearly related to the holding potential. The reversal potential shifted to about -5 mV when the external sodium was replaced with choline suggesting a small contribution of sodium to the glutamate activated current. Under current clamp conditions, application of the transmitter evoked an action potential which was similar in every respect to one produced by direct application of current to the intracellular electrode. The action of the transmitter appeared to have little effect on the voltage sensitive sodium and calcium currents. Because glutamate receptors do not desensitize in the presence of the agonist and because of its positive reversal potential, glutamate appears to be a good candidate for the excitatory transmitter at these cells. Supported by grant EY-01897.

77.14 IN VITRO AUTORADIOGRAPHY FAILS TO DEMONSTRATE A PATTERN OF SPECIFIC NEURONAL BINDING OF ^{3}H FOLIC ACID IN RAT BRAIN. T.A. Fuller, Washington Univ. Sch. Med., Dept. Psychiatry, St. Louis, MO.

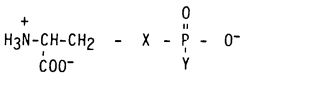
Direct injection of folic acid (pteroyl-L-glutamic acid, PGA) into rat striatum, amygdala or substantia inominata induces a syndrome of limbic convulsions and neuropathology distant from the injection site quite similar to that induced by the glutamate analog kainic acid (Olney et al., Nature 292, 165-167, 1981; McGeer et al., Brain Res 260, 107, 1983). The PGA metabolites N-5-formyltetrahydrofolate (FTHF) and methyltetrahydrofolate (MTHF) also produce this neurotoxic syndrome following intraamygdaloid injection, MTHF being 20-30 fold less potent than either PGA or FTHF. Despite an initial report that MTHF is a potent competitor for KA binding sites on rat cerebellar membranes, it now appears unlikely that kainate preferring receptors underlie the folate induced toxic syndrome because significant folate competition for kainate binding has not been found in striatal membranes (Ferkany et al., Neurobehav Toxicol Teratol 4, 573-579, 1982) or telencephalic homogenates (M. Price, unpublished). In order to determine if specific folate binding might underlie the folate toxicity, in vitro autoradiography of ^{3}H-PGA was undertaken.

In brief, thaw-mounted 15 µm frozen sections of lightly fixed brains of adult male Sprague-Dawley rats were incubated in the dark in either a 4 or 8 nm solution of ^{3}H-PGA (35 Cu/nmol, Amersham) in Tris HCl buffer (0.17 M, pH 7.4) or in an identical solution which also contained a 1000-fold excess of unlabeled PGA for 30-60 min at 4° or 25°C. After incubation, the sections were rinsed twice in cold Tris buffer for 30 sec or 5 min. Slide-mounted tissue sections were placed in cassettes and tritium-sensitive ultrafilm (LKB) was directly apposed to the tissue. The film was exposed for 3 or 4 weeks at -70° C and developed in Kodak D19. The sections were stained with thionine for microscopic viewing.

The various incubation conditions resulted in variable amounts of nonspecific binding of ^{3}H-PGA seen in those sections incubated with the solution containing excess unlabeled PGA. The only significant specific accumulation of label was localized in choroid plexus at all levels of brain, probably attributable to the folate-binding protein known to reside in the plasma membranes of that structure. There was no pattern of specific neuronal binding of ^{3}H-folate discernible in any of the sections. These preliminary studies neither support the contention that PGA significantly binds to the kainate-preferring receptors which have been demonstrated by similar in vitro autoradiography (Monaghan & Cotman, Brain Res 252, 91-100, 1982) nor demonstrate a neuronal binding site for PGA. The mechanism underlying the folates' excitatory and toxic abilities remains to be clarified. These experiments were supported by RSDA MH-00330.

77.15 ANTAGONISTIC ACTIVITY OF ω-PHOSPHORUS-CONTAINING GLUTAMATE ANALOGUES IN THE PERFORANT PATH. R.K. Freund, S.L. Crooks*, J.F. Koerner, and R.L. Johnson*. Depts. of Biochemistry and Medicinal Chemistry, Univ. of Minnesota, Minneapolis, MN 55455.

The finding that the glutamate analogue L-2-amino-4-phosphonobutanoic acid (L-APB) antagonizes excitatory responses in the perforant path of rat hippocampal slices [Koerner and Cotman, Brain Research, 216 (1981) 192] has prompted an investigation of the structural features required for antagonism at these synapses. On the basis of published extracellular criteria, [Koerner and Cotman, Brain Research, 251 (1982) 105], the pharmacology of several γ-substituted glutamate analogues was compared on dentate granule cells [Koerner et al., Brain Research, in press]. Only L-APB and O-phospho-L-serine (O-PLS) [Fagg et al., Neurosci. Lett., 31 (1982) 59] exhibited antagonism. Planar vs. tetrahedral geometry of the γ-substituent could not explain agonist vs. antagonist activity differences. The only obvious differences are: (1) that L-APB and O-PLS can bear a side chain dianion, while side chains of the known agonists are obligatory monoanions at physiological pH, and (2) that these antagonists contain a phosphorus atom at the δ-position. In order to explore the influence of side chain charge on antagonism, we have synthesized and tested the activities of two additional γ-substituted glutamate analogues, DL-2-amino-4-(methylphosphino)-butanoic acid (DL-AMPB) and O-methylphosphonyl-L-serine (O-MPLS). The compounds were classified as antagonists by their pathway selectivity and other criteria, which indicates that a dianionic side chain is not a strict requirement for antagonistic (vs. agonistic) activity. DL-AMPB and L-APB (X = CH_2) were more potent and displayed a higher degree of pathway selectivity than O-MPLS and O-PLS (X = O), respectively (Table). Also, L-APB and O-PLS (Y = OH) were more potent than their methyl-substituted (Y = CH_3) analogues DL-AMPB and O-MPLS. Although methyl substitution may have a variety of deleterious effects (e.g., steric, hydrophobic, etc.), the lower potencies of DL-AMPB and O-MPLS are also possibly due to the absence of dianionic character of the γ-moiety. (Supported by PHS NS17944.)



Cmpd	X	Y	K_D (med) μM	K_D (lat) μM	K_D (med)/K_D (lat)
L-APB	CH_2	OH	45	2.5	18
DL-AMPB	CH_2	CH_3	2200	110	20
O-PLS	O	OH	125	23	5.4
O-MPLS	O	CH_3	8100	3600	2.3

77.16 VALPROIC ACID AND HUMAN PLASMA AMINO ACIDS, G.N. Ko, E.R. Korpi, L.B. Bigelow, S.J. Zalcman, R.J. Wyatt. Adult Psychiatry Branch, NIMH, Saint Elizabeth Hospital, Washington, D.C. 20032

Introduction: Valproic acid is an anticonvulsant that has been administered to individuals with the schizophrenic syndrome. It has little or no beneficial effect. The purpose of the present study was to examine its effects on plasma amino acid concentrations.

Methods: Morning plasma from six schizophrenic subjects was obtained and stored at -50°C until assayed by an automated HPLC amino acid analyzer. Plasma was obtained while patients were valproate free and again after receiving valproate for four weeks.

Results: Plasma glutamate and aspartate were decreased by valproate (two-tailed, T=3.36; $p < 0.05$; T=2.75; $p < 0.05$, respectively). Taurine was elevated (T=4.84; $p < 0.05$). Eleven other plasma amino acid concentrations were unaltered during valproate treatment. No improvement in schizophrenic symptoms was noted during the valproic acid treatment period.

Discussion: Sodium valproate may confer its anticonvulsant properties by its action on these excitatory (glutamate and aspartate) and inhibitory (taurine) amino acid concentrations, as well as its well known ability to raise GABA concentrations.

The changes in amino acid metabolism apparently do not coincide with any improvement in the clinical state of patients with the chronic schizophrenic syndrome.

77.17 KYNURENATE ANTAGONISM OF HIPPOCAMPAL SYNAPTIC FIELD POTENTIALS. M.B. Robinson, K.D. Anderson*, and J.F. Koerner. Dept. of Biochemistry, Univ. of Minnesota, Minneapolis, MN 55455.

Kynurenate, a tryptophan metabolite, is an antagonist of excitatory amino acids in the cerebral cortex [Perkins and Stone, Brain Research, 247 (1982) 184], but the extent of the antagonist action in the CNS is unknown. We have previously shown that antagonists can be distinguished from agonists by extracellular recording of synaptic field potentials [Koerner and Cotman, Brain Research, 251 (1982) 105]. A slice of rat hippocampus, submerged in superfusing medium, was exposed to bath-applied kynurenate at threshold concentration for inhibiting the stimulus-evoked extracellular synaptic field potential. The drug concentration was doubled every four minutes until two doublings of the bath concentration no longer reduced the synaptic field potential or until the response was reduced >70%. Inputs to CA3 pyramidal cells were measured by stimulating in the hilus of the dentate and recording in the stratum lucidum of the regio inferior (containing mossy fiber inputs to CA3 pyramidal cells) or in the stratum radiatum of the regio inferior (containing commissural inputs to CA3 pyramidal cells). The mossy fiber inputs are (–)-baclofen insensitive; the commissural inputs are (–)-baclofen sensitive [Ault and Nadler, J. Pharmacol. Exp. Ther., 223 (1982) 291]. This differential sensitivity to (–)-baclofen was used to verify electrode placement. Inputs to granule cells were measured in the middle or outer one-third of the molecular layer, the medial or lateral perforant paths respectively, and electrode placement was verified by the differential sensitivity of the two pathways to DL-APB [Koerner and Cotman, Brain Research, 216 (1981) 192]. Inputs to CA1 pyramidal cells were measured by stimulating and recording in the stratum radiatum of the regio superior.

Kynurenate was identified as an antagonist by its sigmoidal concentration-response curve, the differential pathway sensitivity of different inputs to the same neuron type, and absence of the spiking induced by agonists. As an antagonist, it was found to be approximately equipotent for the medial perforant pathway, the Schaffer collateral inputs to CA1 pyramidal cells, and the baclofen-sensitive inputs to CA3 pyramidal cells (K_d = 200-300μM). It appears to be slightly more potent in the lateral perforant pathway but by no more than a factor of two. This is the most potent compound known to non-selectively antagonize these four pathways which putatively utilize acidic amino acid transmitters. In contrast, synaptic field potentials of the mossy fiber-CA3 synapses, for which no known transmitters have been implicated, were inhibited less than 20% by 8mM kynurenate. [Supported by PHS NS17944]

77.18 REGIONAL DISTRIBUTION OF L-[^{3}H]GLUTAMATE BINDING SITES AS DETERMINED BY QUANTITATIVE AUTORADIOGRAPHY. J. T. Greenamyre, J. B. Penney and A. B. Young, Neuroscience Program and Neurology Dept. University of Michigan, 1103 East Huron, Ann Arbor, MI 48109.

Electrophysiological and biochemical evidence suggest glutamate as a putative excitatory neurotransmitter in the mammalian CNS. High affinity binding of L-[^{3}H]glutamate to CNS membranes has been demonstrated by several groups. L-[^{3}H]Glutamate binding is saturable, reversible, highest in synaptic-enriched fractions, inhibited by various glutamate analogues and is apparently related to synaptic glutamate receptors. Anatomical localization of glutamate binding sites has been limited by available dissection methods and results have been contradictory. We report the use of quantitative autoradiography to study the regional distribution of [^{3}H]glutamate binding in rat brain.

Rat brains were quickly removed, blocked, cooled in ice-cold buffered 0.32 M sucrose and frozen on microtome chucks under powdered dry ice. Twenty micron brain sections were thaw-mounted onto subbed slides, washed for 30 min in ice-cold 50 mM Tris-HCl (pH7.4) containing 2.5 mM $CaCl_2$ and blown dry. Slides were incubated for 30 min at 37°C with 50-1000 nM [^{3}H]glutamate in 50 mM Tris-HCl containing 2.5 mM $CaCl_2$. Nonspecific binding was determined in the presence of 1 mM unlabelled glutamate. After incubation, sections were rinsed 3 times with cold buffer, followed by a rinse with cold 5% glutaraldehyde in acetone. Total rinse time was 10 sec. Autoradiograms were prepared and analyzed as previously described (Penney et al., Science, 214: 1036, 1981).

Glutamate bound to a single population of sites with a K_D of about 1.0 μM. The pharmacology of binding was similar to that observed in homogenate studies; quisqualate and ibotenate had IC_{50}s of 2 μM and 16 μM, respectively. There was marked regional variation in glutamate binding. Binding was greatest in stratum moleculare of the hippocampus, followed by stratum oriens and stratum radiatum. Binding was also high in cortex (layers 1 and 2), striatum, medial and lateral geniculate bodies, stratum griseum of the superior colliculus, the cerebellar molecular layer and substantia gelatinosa. Binding was intermediate in cortex (layers 5,6), other thalamic nuclei, substantia nigra, interpeduncular nucleus, periaqueductal grey matter and intermediate and ventral grey zones of spinal cord. Little binding was present in globus pallidus, hypothalamus, reticular formation or pontine or medullary tegmentum. The distribution of binding correlated well with proposed glutamatergic pathways and their pharmacology suggests that they are related to postsynaptic glutamate receptors.

Supported by NSF grant BNS-8118765, United Cerebral Palsy Foundation Grant R-305 and NIMH Individual Predoctoral National Research Service Award (MH 08922-01) to JTG.

78.1 SPECTRAL ANALYSIS OF CORTICAL EEG EFFECTS PRODUCED BY THE REVERSIBLE CHOLINESTERASE INHIBITOR PHYSOSTIGMINE IN RATS. Marsha L. Bloodworth-Barlow*, Christine U. Eccles and Gerald A. Young. Dept. of Pharmacology and Toxicology, Univ. of Maryland School of Pharmacy, Baltimore, MD 21201.

Physostigmine is a relatively simple and reversible cholinesterase inhibitor. Characterization of EEG and behavioral changes following physostigmine administration should serve as a foundation for the further study of central nervous system effects of other anticholinesterase agents. Cortical EEG power spectra associated with the behavioral states of sleep, REM sleep and wakefulness in the rat have been previously characterized (Young et.al., Pharmacol. Biochem. Behav. 8:89, 1978). In the present study, power spectral analysis was utilized to delineate changes in cortical EEG in the rat following acute administration of varying doses of physostigmine.

Adult female Sprague-Dawley rats were implanted with chronic cortical EEG and temporalis muscle EMG electrodes and intravenous (i.v.) cannulae for drug administration. EEG and EMG recordings were obtained with a Grass polygraph and were stored on tape with a Hewlett-Packard recorder. EEG samples were subjected to spectral analysis, utilizing a Nicolet MED-80 minicomputer. EEG was digitized at a sampling rate of 100/sec.; spectral power densities were estimated at 0.1 Hz intervals from zero to 50 Hz.

Following i.v. administration of higher doses of physostigmine, two predominant spectral peaks were produced in the cortical EEG power spectra. These spectral peaks were within the 1-3 Hz and 5-6 Hz bands. At these doses of physostigmine, behavioral manifestations of physostigmine (i.e., tremor, urination, defecation) were present. At lower doses of physostigmine, at which no behavioral effects of physostigmine were apparent, one predominant spectral peak occurred in the theta band (5-10 Hz). The above effects of physostigmine on EEG and behavior persisted continuously for about one hr. In contrast, control wakefulness after saline administration was associated with relatively low amplitude, high frequency cortical EEG that alternated with frequent occurrences of sleep and REM sleep.

In conclusion, the data obtained so far with physostigmine indicate that our experimental model should allow the comparative delineation of the effects of other anticholinesterase agents upon EEG and behavior.

78.2 TRIETHYLTIN-INDUCED ENCEPHALOPATHY IN PERINATALLY EXPOSED RODENTS; BIOCHEMICAL AND MORPHOLOGICAL EVIDENCE OF NEURONAL DAMAGE. B. Veronesi*, Health Effects Research Laboratory United States Environmental Protection Agency, RTP, NC 27711, (Northrop Services Inc.) and S.C. Bondy* Laboratory of Behavioral and Neurological Toxicology, NIEHS, RTP, NC 27709 (Spon: C.F. Mactutus)

In the rat, acute exposure to triethyltin (TET) on day 5 of postnatal development (D5) results in CNS hypomyelination, edema and errors in compaction in young (D20) animals. However, these myelin abnormalities are corrected by D60. Since behavioral and electrophysiological abnormalities persist in adult-tested, D5-treated rats, we elected to examine the CNS neuronal population. For this, Long-Evans hooded D5 pups (n=48) were injected (IP) with saline, 6 or 9 mg/kg TET-bromide and sacrificed at D20, and D60. Gross examination of the whole brain at both ages revealed a dose dependent weight loss and a graded anterior-posterior cortical thinning, especially prominent in the entorhinal cortex. In the D20 pups, examination of the posterior cortex revealed a range of cytological abnormalities, including swollen, vacuolated and hyperchromatic neurons in all layers and a marked microglial infiltration. In the adult (D60) brain, overt cellular necrosis was not seen, although the neuronal lamination pattern of the posterior cortex was poorly defined. An immunocytological stain, specific for glial acidic fibrillary protein revealed the neuronal population of the entorhinal cortex to be largely replaced by astrocytes. Golgi-stained material indicated that the surviving macro-neurons in this region had thinner, shorter, apical dendrites and fewer, secondary and tertiary, basilar dendrites. Biochemical analyses of DNA/RNA/protein ratios derived from the anterior and posterior cortex, the hippocampus and cerebellum of 6 mg/kg, TET-D5 exposed pups sampled at D20, indicated a reduction in the number and size of cells, most evident in the posterior cortex and hippocampus. The surviving cells appeared richer in protein, reflecting the astrocyte population. No selective depression of acetycholinesterase levels was seen in the posterior cortex. This study demonstrates that perinatal insult by TET results in the destruction of cortical neurons. This damage, which predomates in the entorhinal cortex, results in a smaller brain and an altered cortical cytoarchitecture in both D20 and D60 animals. Although these neuronal changes are most evident in the D20 pups, they are not transient and are clearly demonstrable histologically in the adult brain of perinatally exposed animals. Consequently, these data suggest neuronal damage rather than myelin abnormality underlies the persistent behavioral and electrophysiological abnormalities reported in adult tested, TET-D5 exposed rats.

78.3 EFFECTS OF TRIMETHYLTIN ON NEURAL ACTIVITY OF PYRAMIDAL AND GRANULE CELLS OF MOUSE HIPPOCAMPAL SLICES. D. L. Armstrong, M. J. Wayner and F. C. Barone. Division of Life Sciences, University of Texas at San Antonio, San Antonio, Texas 78285.

The systemic administration of trimethyltin (TMT) produces necrosis of the pyramidal and granule cells of the hippocampus. The mechanism of TMT's neurotoxic action and its effect on single cell neural activity have not been determined. To study the effects of direct application of TMT on nerve cells a perfused mouse hippocampal slcie preparation was employed. Trimethyltin chloride (0.01M) was injected into the perfusion line in a volume of 0.1cc during the extracellular recording of single unit spontaneous activity of pyramidal and granule cells. Also, the effects of TMT on responses to paired pulse stimulation of the mossy fiber system and perforant path were determined. In addition, slices were prepared from mice that had been injected with a single i.p. dose of 3.0mg/kg TMT. The spontaneous activity and evoked responses of these slices were examined 6, 12, and 24 hours following the injection in an effort to detect electrophysiological changes prior to irreversible cell loss. Direct application of TMT produced irregular bursting patterns in the spontaneous activity of granule cells in the dentate gyrus and pyramidal cells in the CA1 and CA3 region. Control injections of normal perfusate had no effect on cell activity. A repetitive application of 3 injections within a 1 hour period suppressed all spontaneous activity and responses to afferent stimulation. A single application did not affect the amplitude of CA3 and granule cell stimulation evoked population spikes. Significant changes were not observed in slices prepared 6 and 12 hours following the systemic injection. However, after 24 hours well developed synchronous population spikes of granule and CA3 cells were not observed. Individual cell spikes appeared superimposed on the population response. In conclusion, the direct application of TMT to the slice medium produces a bursting response pattern in pyramidal and granule cells that resembles the effects observed after perfusion with penicillin, which is believed to reduce inhibitory activity in the hippocampus (Schwartzkroin and Prince, Ann. Neurol. 1:463, 1977). Electrophysiological changes could be detected in slices prepared from TMT treated mice prior to significant cell loss. The results suggest that the slice preparation can be employed as a neurotoxicological screen and further tests on TMT are being carried out.

78.4 EFFECTS OF HALOTHANE ON DENDRITIC BRANCHES AND SYNAPTIC DENSITY, E. Uemura, E. D. Levin, N. K. Laughlin and R. E. Bowman. Dept. of Vet Anat., Iowa State Univ, Ames, IA 50011, and Dept. of Psych., Univ. of Wisc., Madison, WI 53715.

Halothane neurotoxicity on synaptogenesis was studied in the cerebral cortex during postnatal development. Sprague-Dawley rats were exposed to halothane in chambers during the entire gestation period. There were three exposure conditions: chamber control, 25 ± 5 ppm intermittent halothane (8 hrs/day, 5 days/week), and 25 ± 5 ppm continuous halothane (24 hrs/day, 7 days/week). Each condition consisted of 6 rats. Rats were sacrificed at day 5 and 21 postpartum. The cerebral cortex was processed for light microscopic analysis of dendritic growth as revealed by the Golgi-Cox stain and for electron microscopic analysis of synapses stained with 1% ethanolic phosphotungstic acid. A significantly fewer number of dendritic branches ($P < 0.001$) and shorter dendritic lengths ($P < 0.001$) per neuron were found in rats exposed to halothane. Continuous halothane exposure had most severe effects on rats at both day 5 and 21 postpartum ($P < 0.001$). Similarly, the synaptic density in rats exposed to halothane was lower than those found in the control ($P < 0.001$). The difference was particularly prominent in rats which had been exposed to halothane continuously and examined at day 5 postpartum ($P < 0.001$). These data suggest that the initial growth of dendritic branches was affected by halothane exposure resulting in the delay of synaptic maturation.

Supported by NIH Grant NS17107.

78.5 BEHAVIORAL AND NERVOUS-SYSTEM SPECIFIC PROTEIN CHANGES ASSOCIATED WITH EARLY POSTNATAL EXPOSURE TO TRIMETHYLTIN (TMT). D.B. Miller & J.P. O'Callaghan*, Biol. Sci. Res. Cntr., UNC and Neurotox. Div. U.S.E.P.A., MD-74B, Research Triangle Park, NC 27711

Acute administration of TMT to the adult rat is reported to produce relatively selective damage to the limbic system as indicated by behavioral, biochemical, and pathological alterations. Postnatal day 5 (PND5) exposure to TMT produces adult hyperactivity and decreases in whole-brain and hippocampal weights (Ruppert et al., Soc. Neurosci. Abs. 8:82, 1982). The present study characterizes the neurotoxicity of early postnatal exposure to TMT using tasks related to hippocampal function (e.g., memory and learning) in the neonate and juvenile rat. Because the morphological maturation of the CNS is characterized by ontogenetic changes in proteins associated with critical processes (e.g., synaptogenesis, myelinogenesis, neurogenesis), toxicant-induced effects on the developing nervous system may be reflected in alterations of proteins specific to the diverse cellular and subcellular elements composing nervous tissue (O'Callaghan & Miller, Trends in Pharm. Sci., 1983). Thus, behavioral evaluations were accompanied by measurements of nervous-system specific proteins. Long-Evans rats were cross-fostered and reduced to 8 pups/litter on PND1 (birth = 0). I.P. injections of 0.0, 5.0, or 6.0 mg/kg of TMT-OH were given on PND5. Behavioral changes in the neonate included a dose-related increase in trials to learn an alleyway task, as well as a decreased ability to retain the task over a delay period. As juveniles, TMT rats were less accurate in a radial-arm maze (RAM), and either (1) did not learn or (2) required more trials to acquire a passive avoidance task. Open-field activity during the neonatal period gradually progressed from a dose-related hypoactivity to a hyperactivity by weaning. Hyperactivity was also a concommitant of RAM behavior. PND5 TMT produced a transient retardation in body weight and dose-related decreases in wet weights of whole brain, hippocampus, forebrain, and cerebellum, the hippocampus being the most affected on a percentage basis. The synapse-specific phosphoprotein, protein 1 (P1), was extracted from hippocampus as well as forebrain and assayed by phosphorylation with exogenous cAMP-dependent protein kinase. Although the total amount of P1 decreases as a function of TMT dosage, the percentage of total protein accounted for by P1 either increased (hippocampus) or remained the same (forebrain). In addition, the percentage of total forebrain protein accounted for by proteins specific to myelin did not vary as a function of TMT dosage. The content of myelin proteins obtained from optic tracts, however, showed a dose-related decrease. The data reported here indicate that early postnatal exposure to TMT can interfere with development of the limbic system.

78.6 CAT NEUROHYPOPHYSIS FINE STRUCTURE AFTER SUB LD50 SOMAN OR DFP. K.C. Sikora-VanMeter, T. Ellenberger, J. Willetts* and W.G. VanMeter. Dept. Veterinary Physiology and Pharmacology, Coll. Vet.Med., Iowa State University, Ames, Iowa 50011.

Fine structural changes in male or spayed female adult mongrel cats have been studied after acute intravenous or chronic cutaneous exposure to diisopropylfluorophosphate (DFP) or SOMAN. Chronic administration was given in daily doses of 0.3mg/kg, or 0.5mg/kg for 14days (DFP), and 1.0mcgm/kg, 2.5mcgm/kg, or 5.0mcgm/kg until a total of 5.0mcgm/kg was attained (SOMAN). All animals were killed by barbiturate overdose 24h after the last injection, perfused with glutaraldehyde and prepared for conventional transmission electron microscopy.

The fine structure of axons and pituicytes in the hypophysial stalk and neural lobe of cats receiving acute doses of DFP (0.4mg/kg) is indistinguishable from that of untreated animals and changes in neurosecretory granulated vesicles (NGV's) remain unclear until morphometric analysis can be carried out. On the other hand, acute exposure to SOMAN (5mcgm/kg i.v.) causes a decrease in axon terminal NGV's, and increase in small electron-lucent vesicles, and the pituicytes within the neural lobe contain a greater number of lysosomal elements.

The fine structure of the cat neurohypophysis is markedly effected by chronic low dose exposure to DFP (0.3mg/kg/day or 0.5mg/kg/day for 14days. Total dose: 4.2mg/kg and 7.0mg/kg respectively) or to SOMAN (1.0, 2.5, or 5.0mcgm/kg/day until a total dose of 5.0mcgm/kg was attained). Chronic administration of DFP results in the appearance of large multilamellar structures within the hypophysial stalk and the neural lobe. In the neural lobe, these structures may have derived from individual nerve fibers while in the hypophysial stalk, they may be axons in the process of demyelination since myelinated axons are observed in the hypophysial stalk of untreated cats. The well developed endoplasmic reticulum and Golgi complexes in the pituicytes of these animals indicate a high level of activity and a large number of axons invaginate the pituicyte cytoplasm. On the other hand, chronic exposure to SOMAN increases the number of dilated axons and evokes early signs of degeneration. The myelin of the few myelinated axons in the hypophysial stalk remains intact and no multilamellar bodies are found in the neurohypophysis.

Work supported by U.S. Department of Defense Contract. Nr.DAMD17-80-C-0106

78.7 RETENTION OF MOTOR DEFICITS PRODUCED BY PERINATAL EXPOSURE TO POLYBROMINATED BIPHENYLS (PBB). J.W. Henck*, C.L. Carlson*, D.H. Rezabek* and R.H. Rech. Dept. of Pharmacology/Toxicology, Michigan State University, East Lansing, MI 48824.

Pregnant Sprague-Dawley rats received polybrominated biphenyls (0 or 6 mg/kg) daily in a peanut butter vehicle from day 6 of gestation through day 24 postpartum. Open field activity of offspring was measured from days 12-24 postpartum. Number of squares traversed, latency to move from the center square, and number of rearings were assessed during the 3-minute test period. Control male and female rats exhibited the characteristic peak in squares traversed at 16 days of age, followed by a decline and subsequent increase to adult levels by 24 days of age. Peak number of squares traversed was delayed to day 18 for rats exposed to 6 mg/kg. The PBB-treated rats also were significantly less active than controls by postpartum day 24. Latency to move from the center square decreased with time in a comparable manner for control and treated rats. A peak in number of rearings was observed on day 14 for control rats; no peak was observed for treated rats. This parameter was significantly depressed for treated rats throughout most of the 12-day observation period. No sex-related differences were observed for control or treated rats with any of the parameters tested.

At 5 months of age, all animals previously examined for open field activity were tested for d-amphetamine-induced stimulation of locomotor activity. Unaccommodated locomotor activity was examined for 1 hour, during which both control and treated rats exhibited habituation. Rats were then injected with 2 mg/kg d-amphetamine and monitored for 3 additional hours. Control and treated male rats exhibited comparable activity throughout the observation period. Peak stimulation occurred within 30 minutes of injection, while offset of action occurred at 2 hours post-injection. Peak stimulation for control and treated female rats occurred within 45 minutes of injection. Control females exhibited a second peak of stimulation at 1.5 hours post-injection, while treated females did not; activity of treated females was significantly less than that of controls for the duration of the observation period. Offset for control females occurred at 3 hours, while that for treated females occurred at 2.5 hours. Because of a large variability in the activity of female rats, testing was repeated to attempt to correlate the motor activity of control and treated females with stages of the estrous cycle. Treated female rats were found to be less active than controls during estrus and proestrus (high estrogen activity), but equally active during metestrus and diestrus.

These data indicate that perinatal exposure to 6 mg/kg PBB produces motor deficits in neonates which are retained in the female up to 5 months of age; these deficits appear to relate in part to the role of estrogens in locomotor activity. (Supported by NIEHS grant ES02783.)

78.8 OBSERVATIONS ON THE TOXICITY AND CHOLINERGIC DYSFUNCTION RESULTING FROM INTRACEREBROVENTRICULAR INJECTIONS OF CHOLINE MUSTARD AZIRIDINIUM ION (ChM Az). E. H. Colhoun*, D. J. Brajac* and R. J. Rylett (SPON: M. A. Cook). Department of Pharmacology, University of Western Ontario, London, Ont., Canada, N6A 5C1.

In the current literature the suggestion has been made that monoethylcholine mustard aziridinium ion (MEChM Az; also AF64A), first investigated as a potent and irreversible blocker of choline transport (Rylett and Colhoun, 1980), may be the drug of choice in the selective destruction of central cholinergic neurons and thus provide an animal model for dementia of the Alzheimer type. We have investigated the toxicity and actions of a number of choline mustard analogues, ChM Az, MEChM Az and ethoxycholine mustard aziridinium ion (EChM Az). The acute toxicity of the compounds in mice in order of potency is ChM Az > MEChM Az > EChM Az. Utilizing rat brain synaptosomes, the order of potency of the mustard analogues as inhibitors of sodium-dependent, high-affinity choline transport was ChM Az > MEChM Az > EChM Az. ChM Az was also the most potent inhibitor of choline acetyltransferase. ChM Az and MEChM Az showed hemicholinium-like signs of toxicity in mice; EChM Az produced acute toxicity similar to that seen with acetylcholine and at a subacute dose produced delayed toxicity (10-14 days) similar to that seen with nitrogen mustard HN2. This action was not observed with ChM Az or MEChM Az. Utilizing stereotaxic placement, ChM Az (120 nmol) was injected into the lateral ventricle of mature Sprague-Dawley rats. This dose was acutely toxic with 4 of 5 rats dying within 30 hours. ChM Az-treated rats (0-24 hr) vocalized, showed contralateral circling movements, hypokinesia, piloerection and aggression. At 24-30 hr the rats exhibited rigidity and paralysis leading to death. Synaptosomes, prepared from a rat surviving at 3 days, showed decreases in choline transport (choline=0.45 μM) of 60% and 30% in hippocampus and cortex respectively; choline transport was not significantly reduced in striatum. Injections (i.c.v.) with 40 nmol ChM Az incurred no mortality during a 7 day observation time but rats exhibited piloerection, hypokinesia and lethargy. High affinity choline transport measured in synaptosomes prepared from hippocampus, cortex and striatum showed decreases of 45%, 30% and 0% compared to controls at 3 days after ChM Az injection. Assay of choline acetyltransferase yielded similar decreases at 3 days after treatment, with somewhat greater inhibition observed after 7 days. ChM Az, which is more structurally related to choline than is MEChM Az is a highly toxic substance and has apparent central cholinergic neurotoxic actions in rat brain.
(Supported by the Medical Research Council of Canada).

78.9 NEURON-SPECIFIC PHOSPHOPROTEINS AS BIOCHEMICAL MARKERS OF NEUROTOXICITY: EFFECTS OF ACUTE ADMINISTRATION OF TRIMETHYLTIN (TMT) TO THE RAT. J.P. O'Callaghan*, D.B. Miller and H. Makkawy* (Spon: M.I. Gage). Neurotoxicology Division, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Recently, several neuron-specific phosphoproteins that serve as endogenous substrates for calcium- and cyclic nucleotide-dependent protein kinases have been identified in the central nervous system (CNS) (e.g. see Greengard, Harvey Lec. 75: 277, 1981). These phosphoproteins exhibit different patterns of distribution with respect to brain region, subcellular localization and neuron type. Because of their intimate relationship to the cellular and functional heterogeneity of the CNS, we have proposed that neuron- and other cell-type specific proteins may serve as useful biochemical markers of neurotoxicity (O'Callaghan and Miller, Trends Pharmacol. Sci., 1983). In order to evaluate this hypothesis, we are assessing the effects of known neurotoxicants on cell-type specific proteins throughout the nervous system.

In the present study, neuronal phosphoproteins, including Synapsin 1 (Protein 1), were examined following the administration of TMT, a neurotoxicant that has been reported to produce pathological changes in the rat CNS that are largely restricted to limbic system neurons. Crude synaptic membrane fractions were prepared from hippocampus and frontal cortex of rats that had received TMT or saline. These fractions were then preincubated for the purpose of converting phosphoproteins entirely to the dephosphorylated form. Membrane proteins were subsequently phosphorylated, in vitro, by the addition of Mg^{++} - (^{32}P) ATP, either by endogenous kinases or by acid extraction of the proteins followed by addition of exogenous cAMP-dependent protein kinase. The two procedures gave similar results. Following resolution by SDS-PAGE, specific protein phosphorylation was detected by autoradiography and quantified by microdensitometry or liquid scintillation spectrometry. TMT (9.0 mg/kg, i.v.) caused a time-dependent decrease in several phosphoproteins, including two bands that comigrated with the synapse-specific phosphoprotein doublet, Synapsin 1 (M_r 80-85K). Maximal loss of hippocampal phosphoproteins occurred at 3 weeks and most phosphoproteins remained significantly reduced 14 weeks after TMT administration. TMT (3.0-9.0 mg/kg) caused a dose-related decrease in Synapsin 1 in the hippocampus (to 50% of control) but not in the frontal cortex. Microdissection of slices of dorsal hippocampus revealed regional differences in the extent to which TMT affected Synapsin 1. Results will be discussed in terms of the neuropathological effects of TMT. These data indicate that nervous-system specific proteins may be useful biochemical indicators of neurotoxicity.

78.10 BEHAVIORAL AND ELECTROPHYSIOLOGICAL EFFECTS OF ASYMPTOMATIC LEAD EXPOSURE AS A FUNCTION OF DEVELOPMENTAL STAGE IN RATS. L. J. Burdette* and R. Goldstein*. (SPON: L. Grant). Dept. of Psychology, Washington University, St. Louis, MO 63130.

Chronic behavioral and electrophysiological effects of brief low level lead exposure during development were investigated to determine whether the pattern of impairment was affected differentially by the developmental stage of poisoning. Dams were exposed to a volume of lead acetate solution calculated to deliver a daily dosage of 270 mg/kg during one of three preiods: Days 16-23 of gestation (G); Days 1-8 of nursing (1N) or Days 9-16 of nursing (2N). Control dams received an equivalent volume of distilled water. Free erythrocyte protoporphyrin (FEP), hematocrit and hemoglobin levels were monitored in female pups during Days 16-32. Open field activity and spontaneous T maze alternation behavior were measured in male offspring during Days 40-59, following which bipolar and screw electrodes were implanted in hippocampal field CA3 and visual cortex, respectively, for recording electroencephalography (EEG) and visual evoked potentials (VEPs). A decrease in FEP values, and a parallel increase in hematocrit and hemoglobin levels, were observed until 28 days of age when all measures approached asymptotic limits. FEP values of poisoned G subjects were significantly elevated above control levels at 32 days of age, the only group tested after blood parameters were stable. Temporal and spatial activity patterns were sensitive to the timing of lead exposure, as evidenced by the failure of poisoned G animals to exhibit normal decrements in peripheral field activity over time and by their increased exploration of the center field. Spontaneous alternation behavior was not observed in control or experimental groups. Power spectrum analyses indicated that lead exposure selectively depressed 6-7 Hz hippocampal signal energy, independent of the timing of exposure providing additional evidence for functional differentiation of low (5-7 Hz) and high (8-12 Hz) frequency theta. All other hippocampal and cortical frequencies were unaffected. Similar to other reports of lead poisoning effects on VEPs, early component latencies of both cortical and hippocampal waveforms were decreased significantly in all poisoned groups. Component amplitudes were not influenced reliably. The data indicate that the observed effects of lead poisoning depend on both the function assessed and the timing of lead exposure.

78.11 AXOPLASMIC TRANSPORT OF NEUROTOXIC ESTERASE IN HEN SCIATIC NERVE. C.D. Carrington* and M.B. Abou-Donia. Laboratory of Neurotoxicology, Duke University Medical Center, Durham, NC 27710.

Neurotoxic esterase (NTE) is a putative enzyme which has been postulated to be the site at which organophoshorus compounds induce delayed neurotoxicity in chickens and other animals. This hypothesis has been faulted because NTE activity returns in brain, spinal cord, and sciatic nerve long before the onset of the neuropathy. However, organophosphorus induced delayed neurotoxicity (OPIDN) has been characterized as a distal neuropathy in which the distal portions of the longest axons are the most sensitive to the neurotoxicant. Consequently, we have determined the rate at which NTE activity returns to distal and proximal portions of hen sciatic nerve following inhibition by in vivo administration of either diisopropyl phosphoroflouridate (DFP) or phenyl methyl sulfonyl flouride (PMSF). DFP is neurotoxic whereas PMSF is not. We also measured anterograde and retrograde transport rates for NTE. NTE activity was measured by the method of Johnson (Arch. Tox. 37:113-5, 1977), except that the incubation period was extended to up to two hours; the reaction rate was found to be linear over this period. Anterograde and retrograde transport rates were calculated from measurements of enzyme accumulation distally and proximally to double ligations. NTE recovery rates in the proximal portions of the nerve were similar to those reported previously, with 50% recovery after about 7 days following inhibition with either DFP or PMSF. Recovery in the distal portions of the nerve differed in two respects: First, recovery of activity in the most distal segment lagged about two days behind the most proximal segment. Second, activity in the distal segments was significantly more inhibited 10 and 14 days following inhibition by DFP than at the same time points following PMSF administration. Anterograde transport was found to occur at a rate of 113±37 mm/day, with 10.5% of the total activity moving at this rate. Retrograde transport was found to occur at a rate of 48±26 mm/day, with 17.1% moving at this rate. These results suggest that although resynthesis of enzyme is the major determinate of recovery rate in sciatic nerve, NTE transport is sufficiently slow to cause a two-day lag in recovery in distal portions of the nerve. This decay may make the longer axons more susceptible to OPIDN. (Supported in part by NIEHS Grant No. ES02717).

78.12 POSTNATAL EFFECTS OF PERINATAL EXPOSURE TO LEAD ACETATE AND CARBON MONOXIDE. Z. Annau. The Johns Hopkins University, Baltimore, MD. 21205

Male and female adult Long Evans Hooded rats were exposed to 0.1% lead acetate or distilled water in their drinking water for 70 days prior to mating. Females were maintained on lead acetate (Pb) or distilled water (W) through gestation and lactation. Subgroups of pregnant females were exposed to 0.1% carbon monoxide (CO) through lactation. Litters were reduced to 8 pups and behavioral testing was started on postnatal day 4. Measures of locomotor activity indicated that by day 21 control animals were more active than animals exposed to Pb or CO or both. Homing behavior and negative geotaxis were affected by treatment in more complex ways by the experimental treatments. At weaning, half the animals were either removed from the Pb in their water or placed on it to counterbalance the post weaning lead exposures. Adult animals were tested in a two way avoidance task at ages 90,91 and 120 days. Each animal was tested for 100 trials on each day. No significant differences appeared in the first two training sessions. On the third session however, animals exposed to lead made more avoidances than controls.

Animals that had been exposed to Pb through their lives were exposed to hypoxia or hypercapnia and cerebral blood flow measurements were taken during exposure, using the radioactive microsphere technique. Both control and Pb treated animals increased their blood flow to a CO_2 challenge although the Pb treated animals had higher flow levels both in normocapnia and hypercapnia. The cerebral blood flow of controls increased as inspired oxygen concentration decreased, but the blood flow of Pb treated animals plateaued in mild hypoxia and did not increase further.

The results of these experiments demonstrate that low level Pb exposure has irreversible behavioral and physiological consequences, and that the potential health risks of these consequences may be severe.

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78.13 LOCOMOTOR ACTIVITY AND DOPAMINE METABOLISM IN MANGANESE TREATED NEONATAL RATS. P. Kontur and L.D. Fechter. Div. of Toxicology, The Johns Hopkins U. Sch. of Hyg., Baltimore, MD 21205.

Manganese (Mn) toxicity is thought to result from the disruption of dopaminergic neuronal function. We have shown that neonatal administration of Mn while increasing brain regional Mn levels does not change the levels of dopamine (DA), serotonin (5-HT) or their metabolites in the striatum and hypothalamus or alter the normal development of locomotor activity (Neurotox., 3, 143,1982). This surprising lack of toxicity especially in 2 major DA containing areas was further studied by using amphetamine administration to assess the ability of Mn treated rats to compensate for any damage not evident as disrupted normal development.

13, 17, and 21 day old control, vehicle intubated and 25 and 50 ug/g Mn intubated rats were administered saline, .5, 1, or 3 mg/kg of amphetamine and their locomotor response measured over a 1 hour session. The animals were sacrificed immediately after the session and the brains dissected for analysis of levels of DA, 5-HT and their metabolites in the striatum, nucleus accumbens, and olfactory tubercle.

Control and vehicle intubated animals showed the expected stimulation of activity by amphetamine at each age and amphetamine dose. In these groups maximal stimulation at 13 and 17 days occurred at 1 mg/kg whereas at 21 days maximal stimulation occurred at 3 mg/kg.

At all ages Mn treated animals injected with amphetamine showed enhanced levels of activity relative to that seen in vehicle intubated animals receiving amphetamine. Amphetamine dose response effects appeared to predominate over Mn effects. That is, at each age, the Mn groups did not differ from each other at any amphetamine dose but within each age there were amphetamine dose dependent effects. Thus in 13 and 21 day old Mn treated animals 1 and 3 mg/kg of amphetamine enhanced activity whereas in 17 day old animals .5 and 1 mg/kg enhanced activity above that seen in vehicle intubated rats.

Whether this differential sensitivity of 17 day old Mn treated rats to amphetamine as well as the overall enhanced locomotor response of Mn treated animals to amphetamine will be reflected in altered neurotransmitter metabolism is currently under investigation and will be presented along with the activity data.

In addition to the analysis concerning Mn toxicity the normative control data relating the development of locomotor activity to regional DA metabolism and the effects of amphetamine on these parameters will be presented and discussed. These relations have not been previously characterized.

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DISEASES OF THE CNS

79.2 AXONAL AND MYELIN LESIONS IN β-MANNOSIDOSIS: ULTRASTRUCTURAL CHARACTERISTICS. K.L. Lovell and M.Z. Jones. Pathology Dept., Michigan State Univ., E. Lansing, MI 48824.

Ultrastructural characteristics of axonal and myelin abnormalities were delineated in the nervous system of goats affected with β-mannosidosis. This autosomal recessive defect of glycoprotein catabolism is associated with a deficiency of β-mannosidase and with tissue accumulation of oligosaccharides. Severe neurological deficits present at birth include an intention tremor, pendular nystagmus, ataxia and inability to stand. Previous morphological examination revealed widespread cytoplasmic vacuolation, axonal spheroids, and severe myelin paucity in the brain, but not spinal cord or peripheral nerves. The objective of the present study was to delineate ultrastructural characteristics of axonal and myelin lesions in selected regions of the central and peripheral nervous system.

Four day old and 4 week old affected goats and age-sex-matched control goats were anesthetized and perfused intracardially with a glutaraldehyde/paraformaldehyde mixture in 0.1 M phosphate buffer. Tissue was post-fixed in osmium tetroxide, stained en bloc in uranyl acetate and embedded in Epon-Araldite. Two μm sections were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate.

Contents of axonal spheroids included dense bodies, electron lucent vesicles with a double membrane, membranous whorls, mitochondria and axonal filaments. Different proportions of each component were seen in individual spheroids. The origin of the abnormal components is not clear, but the vesicles did not originate from transported cytoplasmic vacuoles, since vacuoles consistently were characterized by single membranes. In the central nervous system, many normal appearing axons without myelin sheaths were present in regions of myelin paucity. Most of the remaining myelin sheaths appeared to be of normal thickness. The presence of an internode with myelin adjacent to an internode without myelin suggested that axonal abnormalities were not the principal reason for the myelin deficit. In the peripheral portion of cranial nerves V and VIII, myelin sheaths surrounded most axons as in control animals, but there appeared to be an increased percentage of smaller axons in the affected animals. The pathogenesis of the specific types of myelin lesions in β-mannosidosis remains to be established.

Supported by BRSG funds from the College of Osteopathic Medicine, Michigan State University to KLL and NIH grant NS-16886 to MZJ.

79.3 EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN MICE: ADOPTIVE TRANSFER OF DISEASE IS MODULATED BY THE PRESENCE OF NATURAL SUPPRESSOR CELLS. I. N. Montgomery* and H. C. Rauch. Dept. of Immunol. and Microbiol. W.S.U. Sch. of Med. Detroit, MI 48201.

Experimental allergic encephalomyelitis (EAE) is primary demyelinating disease induced in mice by an intradermal inoculation of the encephalitogen emulsified in complete Freund's adjuvant (CFA) accompanied by an intravenous injection of pertussis vaccine. Direct adoptive cell transfer in mice requires treatment of the recipient or the cells prior to transfer. Only when the donor cells are cultured with basic protein prior to injection into the recipient or when the recipient is subjected to low dose irradiation prior to the injection of the donor cells is disease transfer succcessful. To examine the nature of the restriction observed in direct adoptive cell transfer of EAE we have undertaken cell-transfer of EAE using both SJL and F_1(BALB/c x SJL) mice and using cyclophosphamide (CY) as a recipient treatment substitute for low dose irradiation. The temporal dynamics of the lymphocyte population in response to encephalitogenic challenge were then explored.

Untreated recipients of EAE-donor lymphocytes did not develop EAE and do not respond to subsequent encephalitogenic challenge which suggested the presence of suppressor-like cells in the recipient. Subsequent challenge, however, did induce clinical EAE in CY-treated cell recipients which did not display clinical signs after receiving cells from EAE donors. Direct evidence is presented for the presence of naturally occurring suppressor (N_S) cells in the following experiment. Splenic lymphocytes (2×10^8 cells) were transferred from naive mice to untreated recipients [suppressive] or to Cy-treated recipients [permissive]. The development of EAE following encephalitogenic challenge was significantly reduced in recipients of the naive spleen cells. Further data indicate that the temporal dynamics of lymphocyte induction in EAE donor mice is such that adoptive transfer is successful 4-8 days post-challenge. That a regeneration of the N_S population occurs shortly following CY-treatment was ascertained by measuring the time interval during which the CY-treated recipients remained permissive for adoptive transfer of EAE. CY treatment of donors following encephalitogenic challenge dramatically reduced disease incidence only during the early post-sensitization period. These studies indicate the presence of a naturally occurring suppressor-like cell which modulates the function of adoptively transferred T-effector lymphocytes. (Supported in part by NIH grant NS18898).

79.4 ANTI-CEREBELLAR ANTIBODY LEVELS IN THE SERA OF AUTOIMMUNE MICE. S.A. Hoffman, D.N. Arbogast*, P.M. Ford*, D.W. Shucard and R.J. Harbeck*. Brain Sciences Laboratories, Department of Pediatrics, NJH/NAC Denver, CO 80206.

The NZB strain of mice has been shown to develop brain reactive autoantibodies similar to those seen in systemic lupus erythematosus patients with CNS involvement (Hoffman, S.A., et al., Brain Res., 142:477, 1978; Harbeck, R.J. et al., Clin. exp. Immunol., 31:313, 1978). We have recently undertaken to extend these findings by examining other autoimmune strains of mice, viz., NZB/W, BXSB & MRL/l. The objective was to assess strain differences in the development of brain-reactive antibodies during the life of these animals.

Starting at two months of age, 20 mice from each of the autoimmune strains, as well as the normal MRL/mp and the non-autoimmune BDF_1 strain, were bled at monthly or bimonthly intervals. Prior to their expected deaths, based upon observation and life span estimates, the animals were anesthetized and sacrificed by exsanguination. Using an indirect immunofluorescence technique the sera were examined for brain-reactive antibody against dissociated cerebellar cells from 6-10 day old BDF_1 mice. The table shows the percentage of mice with "positive" sera. Positive sera were defined as those sera which immunofluorescently stained more cells than two standard deviations above the mean of cells stained by sera from the 2,4 and 6 month old BDF_1 mice (6.25%).

Age+	BDF_1	NZB/W	NZB	BXSB	MRL/l	MRL/mp
2	0(20)*	40(20)	18(17)	27(15)	10(20)	10(19)
3				69(13)	53(17)	
4	5(19)	21(19)	32(19)	55(11)	67(15)	21(19)
5				86(7)		
6	10(20)	33(18)	31(19)			5(19)
8	15(20)	56(9)	84(19)			37(19)
10	55(20)	100(5)	33(15)			23(13)

+Months
*Parentheses indicate the number of animals tested

All strains of mice tested showed some degree of serum reactivity against the cerebellar cells. The data indicate, however, that there were elevated percentages of mice with positive sera among the autoimmune as compared to the normal strains of mice. There was also a general, but not absolute, increase in serum reactivity with increasing age of the mice. Thus, there is not a qualitative difference between autoimmune and normal mice with respect to the development of anti-cerebellar antibodies, but the autoimmune strains do appear to develop these antibodies more rapidly.

79.5 NEUROPHARMACOLOGY OF TOURETTE'S SYNDROME. R.L. Borison and B.I. Diamond. Psychiatry Research Laboratory, Downtown V.A. Hospital and Medical College of Georgia, Augusta, Georgia 30910.

The spontaneous neurological disorder, Gilles de la Tourette's Syndrome (TS) involves multiple and migratory motor and vocal tics. This disorder first occurs before the age of 15 years, and runs a lifelong course. Although the etiology of TS is unknown, it appears that there is a strong genetic component to this disorder, with a variable penetrance. The basic pharmacology of TS suggests a striatal hyperdopaminergic-hypocholinergic state, similar to tardive dyskinesia. Historically, dopamine receptor blockers, in particular haloperidol, have been used to mask the signs of TS. In a double-blind comparison of three dopamine receptor blockers- haloperidol, fluphenazine, trifluoperazine -in 10 TS patients, we found that all three neuroleptics were capable of producing equivalent tic control, yet haloperidol produced more central side-effects, including sedation, apathy, and depression. Although TS is presumed to involve a hypocholinergic striatal balance, anticholinergic drugs are often used to treat the acute extrapyramidal side-effects produced by haloperidol in TS patients. We studied the actions of a prodopaminergic drug, amantadine (100-300mg daily) versus an anticholinergic drug, benztropine mesylate (4-6mg daily) in seven TS patients with haloperidol-induced recurrent dystonias and pseudoparkinsonism. We found that amantadine not only did not exacerbate the underlying TS, but proved to be at least as effective as benztropine in the reversal of the haloperidol-induced side-effects. More recently it has been suggested that the alpha receptor agonist/antagonist, clonidine, may be an effective agent for treating TS as shown in open label trials. We studied the efficacy of clonidine and haloperidol in TS using a double-blind placebo-controlled design. The study group consisted of 15 TS patients, and haloperidol was used in doses from 2.25 - 7.5mgs daily, whereas the dosage of clonidine was between 0.35 - 0.75mg daily. Both clonidine and haloperidol proved to be statistically significantly superior to placebo, however, no statistical significance could be found when comparing clonidine to haloperidol in efficacy. The side-effect profile of the two drugs was much different. Haloperidol produced sedation in greater than 50% of patients, and produced extrapyramidal side-effects as well as apathy and depression. With clonidine treatment, sedation only occassionally occured, otherwise the major side-effect was a relatively short-lived dry mouth in approximately 33% of patients. Hence, clonidine is equal to haloperidol in its therapeutic efficacy in treating TS, but has fewer side-effects, making it a preferable therapeutic agent. The efficacy of clonidine in TS also suggests either a major clinical role for norepinephrine in striatal functioning, or a noradrenergic neuron in series with a dopaminergic neuron as modulating the signs of TS.

79.6 L-PYROGLUTAMIC ACID: A NEUROTOXIC IMINO ACID THAT PRODUCES A DRUG INDUCED ANIMAL MODEL OF HUNTINGTON'S DISEASE AND WITH A POTENTIAL ROLE IN THE ETIOLOGY OF HUNTINGTON'S DISEASE. G.K. Rieke, A.D. Scarfe* and J.F. Hunter*. Dept. of Anat., College of Med., and Vet. Physiol. and Pharmac., College of Vet. Med., Texas A&M Univ., College Station, Tx. 77843.

Intrastriatal injections of L-Pyroglutamic acid(L-PGA) in the mouse produces an animal that presents postural asymmetries(twist- of the head and trunk), hyper- or hypoactivity, tremor, ataxia and incoordination, and possible dyskinesias(head snapping or flinging movements, body twitches). The movements of the head and body appear to be involuntary as they occur sporadically and interrupt ongoing directed behaviors. The injected animals also rotate predominantly toward the side of the injection. The turning response consists of 360° turns with a radius of turning equal to one body length of the mouse. The rates of ipsilateral rotation in the experimental group(20 nmoles L-PGA) are significantly greater(Duncan $P \leq 0.001$) compared to the saline-injected control group. L-PGA produces a neuropathological response similar to kainic acid; however, L-PGA is approximately 200 times less potent than kainate. The neuropil in the vicinity of the injection site contains dilated profiles, degenerating neurons and oligodendroglia, and active microglial-like cells many of which are active phagocytes. The swollen profiles are associated with intact presynaptic boutons. L-PGA does not destroy neurons in parts of the brain at long distances from the injection site, that is, L-PGA does not have the distance effect of kainic acid. L-PGA destroys neurons in the hippocampus, prepiriform cortex, nucleus accumbens septi and the amygdala when it is injected directly into these areas. L-PGA is a neurotoxin with a distinct dose-response effect upon the cells of the caudatoputamen(CPU). For example, a 200 nmole dose of the toxin destroys approximately 28% of the CPU, while 20 nmoles destroys approximately 1.4% of the CPU. The saline(0.9% NaCl) vehicle injected into control animals in conjunction with the mechanically-induced lesion involved less than 0.2% of the CPU. If the neurotoxic hypothesis for HD is correct and L-PGA, as a neurotoxin, plays a possible role in the etiology of HD then an analysis of fluid levels of L-PGA in HD patients and their offspring "at risk" might strengthen the correlation between the L-PGA-induced mouse model of HD and the disease in man. Presently we are conducting an analysis of the plasma of HD patients, their off-spring "at-risk" and age matched nonchoretic controls by means of microcapillary gas chromatography and mass spectrometry.

This work was funded in part by the Center for Comparative Medicine, Texas A&M University, grant 18820-3.

79.7 STUDIES ON ACETYLCHOLINESTERASE ACTIVITY IN MANIC-DEPRESSIVE ILLNESS. J. H. Thakar* and Y. D. Lapierre* (SPON: J. Metuzals)

The etiology of manic-depressive illness (MDI) is not known. The major hypotheses on etiology indicate cell membrane abnormality, cholinergic-adrenergic malfunction and biogenic amines imbalance. Acetylcholinesterase (AChE) is an important regulator of the synaptic transmission. The role of this enzyme in cell membrane function in MDI is not understood.

To study biochemical abnormality at molecular level, it is difficult to obtain the CNS tissue from patients as well as controls. However, the studies of peripheral systems such as erythrocytes, leukocytes and platelets have been well accepted as a model for CNS system (Rowland, L.P. Muscle and Nerve 3, 3-20, 1980). We have studies AChE activity in erythrocyte membranes of 28 bipolar MDI subjects, 24 well relatives and 14 normal volunteers.

Erythrocyte membranes were prepared by washing the cells with ice-cold saline and hemolyzing them in 10 mM imidazole buffer containing 1mMNaCl and $MgCl_2$ each, at pH 7.4. The membranes were washed three times and harvested at 48,000 g by centrifuging for 20 minutes. AChE activity was measured by the procedure of Ellman et al (Biochem. Pharmacol. 7, 88-95, 1961). The hydrolysis of actylthiocholine was measured by coupling thiocholine formation with dithiobisnitrobenzoate at 412 nm using a recording spectrophotometer.

Our preliminary data (MDI 0.84 ± 0.14, well 0.79 ± 0.13 and normal 0.63 ± 0.26 expressed as mean $\pm$ S.D, moles/min/ G membrane proteins) show a significant increase in the AChE activity in MDI subjects ($P \leq 0.002$) and their well relatives ($P \leq 0.02$) as compared to normal volunteers. These activities were inhibited by NaF (2mM), quinidine (0.4mM) and dibucane (1mM). These data will be presented and discussed to evaluate the role of AChE and membrane functions in MDI.

79.8 TREATMENT WITH FLUOSOL-DA OF SPINAL CORD INFARCTION IN THE RABBIT. J. F. Toole and I. J. Miller, Jr., Depts. of Neurology and Anatomy, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

An infarction was induced in the lumbosacral spinal cord of rabbits to test the effectiveness of a perfluorochemical preparation (Fluosol-DA) and oxygen treatment on this ischemic lesion. A balloon catheter was inserted through the femoral artery to the thoracic aorta and inflated for 20 minutes under light penobarbital anesthesia supplemented with ether. This procedure resulted in sensory and motor defecits in the inferior extremities which include analgesia and paresis as well as flaccid paralysis in more severe cases. Following release of the occlusion, animals received an intravenous injection of 20 cc of Fluosol, saline or no treatment. Some animals in each treatment group were placed in a 95% oxygen - 5% carbon dioxide mixture at atmospheric pressure during the 4 hours following the occlusion, while the remaining animals recovered in room air. Animals were sacrificed after 48 hrs, and the spinal cords were prepared for microscopic examination using paraffin sections 8 microns thick and cresyl violet staining. Cytological features were compared among ventral horn cells in lumbar segments 6 and 7, and cervical segments were examined as controls. Ten sections separated by 100 microns each were selected for scoring, and each section was rated with an integer from 0 - 3 as follows: 0 for normal tissue, 1 for tissue containing chromatolysis in up to 50% of ventral horn cells, 2 for chromatolysis in more than 50% of ventral horn cells, and 3 for cavitation and cellular degeneration. Tissue from animals without treatment (N=4) received a mean score of 1.8 $\pm$ 0.2 (S.E.M.), while tissue from animals receiving intravenous saline was scored 2.0$\pm$0.3 (N=4). Animals treated with intravenous Fluosol yielded tissue which was scored 2.0 $\pm$ 0.1 (N=8). Scores of the cytological lesions varied from 1 - 3 among animals in all groups, but the most severe lesions were found in animals with the most profound neurological symptoms. Treatment with oxygen produced a mean score of 2.0 + 0.1 (N=8) across groups, while animals recovering in room air were scored a mean of 1.9$\pm$0.1 (N=8). In these experiments, neither Fluosol nor oxygen proved effective in the treatment of the effects of ischemia in the rabbit spinal cord.

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79.9 DISTRIBUTIONS OF TUMOR METASTASES IN HUMAN CNS. S.M. de la Monte* G.W. Moore* and G.M. Hutchins* (SPON: A.W. Clark). Department of Pathology, The Johns Hopkins Medical Inst., Baltimore, MD. 21205.

It is not known whether the observed distributions of CNS metastases are due to trophic influences or simply a mass effect of tumor dissemination. The purpose of this study was to compare patterns of metastases from tumors of different histogenetic origin. Data were derived from review of the clinical histories, autopsy protocols, and histologic slides of 361 patients with metastases: malignant melanoma (56 cases), small cell lung carcinoma (85), breast carcinoma (188), and neuroblasoma (32), all subjected to complete autopsy at The Johns Hopkins Hospital. Data were analyzed using standard statistical tests.

CNS metastases from malignant melanoma occurred most frequently in cortical grey matter (64%) and leptomeninges (46%). While metastases in leptomeninges and dura mater were correlated with metastases in endocrine and mesoderm-derived organs including gonads (all $p<0.05$ or better), metastases in cortical grey matter occurred independent of other sites, and a strong negative correlation between cortical grey matter and hepatic metastases was observed ($p<0.001$). CNS metastases from small cell lung carcinoma occurred in 56% of the cases, and in 75% of those patients, cortical grey matter and nuclei were involved. Metastases to grey matter were positively correlated with metastases to the adrenal and kidney, while metastases to white matter were negatively correlated with metastases in bone marrow (all $p<0.001$). Metastases to dura or leptomeninges were not correlated with metastases to extra-CNS sites, and mainly occurred by extension of tumor from metastatic foci in adjacent CNS parenchyma. For breast carcinoma, progressive declines in the frequencies of CNS metastases with increasing age were observed with respect to cortical grey, grey matter nuclei, and leptomeninges, but not dura mater or white matter. Metastases to grey matter were correlated with metastases to the adrenal ($p<0.001$), metastases to dura and/or leptomeninges were correlated with metastases to endocrine organs ($p<0.005$) and extra-CNS mesoderm derived structures ($p<0.001$). Neuroblastoma metastasized primarily to dura mater and/or leptomeninges; metastases to neuroectoderm derived structures of the CNS were uncommon. CNS metastases were correlated with metastases to endocrine and mesoderm derived structures of the head and neck ($p<0.05$), the pancreas ($p<0.05$), and gonads ($p<0.05$).

The results show that for several different tumors, metastases to dura mater and/or leptomeningese are correlated with metastases to endocrine and mesoderm-derived structures outside of the CNS. In contrast, metastases to grey matter were not uniformly correlated with metastases to particular extra-CNS tissues, and these distributions varied as a function of tumor histogenesis.

79.10 RELATIVE PRESERVATION OF FRONTAL GLUCOSE METABOLISM IN EARLY ALZHEIMER'S DISEASE. N.L. Foster and T.N. Chase (SPON: H. Lansdell). Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health, Bethesda, MD 20205.

Alzheimer's disease causes gradual intellectual decline associated with neuronal loss. Despite the traditional view that frontal lobes are particularly susceptible to damage in this disorder, recent quantitative post-mortem neuronal counts and studies of cerebral atrophy have suggested that frontal cortex may be spared relative to temporal and parietal cortex. Using 18-fluorodeoxyglucose positron emission tomography, regional glucose utilization can be determined during life relatively early in the course of the disease. Analyses of PET scans performed in 17 right-handed patients (12 men, 5 women, mean ± SEM age 61 ± 1.5 years) with clinically diagnosed Alzheimer's disease were compared with results in 5 right-handed age-matched normal volunteers (2 men, 3 women, age 57 ± 1.9 years). All indicies of general intellectual function were significantly ($p < .0001$) lower in the Alzheimer's group. For example, WAIS Full Scale IQ scores were depressed by 35% (130 ± 3.1 in controls vs 84 ± 3.1 in patients). The Wechsler Memory Quotient by 45% (134 ± 5.9 vs 74 ± 3.2), and the Mattis Dementia Scale score by 30% (142 ± 1.0 vs 100 ± 4.7). Overall cerebral glucose metabolism averaged 30% lower in the Alzheimer's patients (range 3.9 to 6.7 mg/100g/min) than in control individuals (range 5.0 to 9.9 mg/100g/min). Among normal volunteers frontal glucose metabolism was approximately the same as in the temporal and parietal lobes (7.6 ± .68 mg/100 g/min vs. 7.5 ± .73 and 7.5 ± .89). By contrast, Alzheimer patients averaged a significantly higher glucose metabolism in frontal regions (5.7 ± .22) as compared to temporal (4.4 ± .21) and parietal (4.8 ± .23) areas. 16 of the 17 patients showed highest absolute glucose metabolic rates in frontal regions and 15 had the least percent reduction compared to normal values in these regions. On the average, Alzheimer's patients had a 25% frontal, 40% temporal and 36% parietal reduction of glucose metabolism compared to normals. Relative preservation of frontal brain activity may be due to less cortical neuron loss or less damage to its afferent projections. These findings may have implications relative to the critical participation of certain neurotransmitter systems in the pathogenesis of Alzheimer's disease.

79.11 DISPLACEMENT OF MYELIN SHEATHS BY THE NEURITIC PLAQUES OF ALZHEIMER'S DISEASE. W.C. Mobley, C.A. Kitt, R.G. Struble and D.L. Price. Neuropharm. Br., Dept. of Med. Neurosci., Div. of N.P., Walter Reed Army Inst. of Res., Washington, DC 20307, and Dept. of Neurol., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Myelin sheaths within the central nervous system can be immunocytochemically stained by antibodies to the myelin basic protein (Itoyama, Y., et al., Ann. Neurol., 7:157, 1980). Neuritic plaques are one of the diagnostic features of Alzheimer's disease. We have employed an antiserum to myelin basic protein in an immunocytochemical examination of myelinated fibers in cortical areas containing neuritic plaques. Serial 8 µm sections were prepared from formalin fixed, paraffin embedded brain tissue of three Alzheimer's disease patients. Sections were stained for myelin basic protein, for neurites and amyloid by the Sevier-Monger silver method, for amyloid by thioflavine T, or by H&E. Neuritic plaques were identified in silver stained sections by their characteristic accumulation of argentophilic rods and granules. Thioflavine T demonstrated plaque amyloid. Except for an occasional positive sheath, neuritic plaques failed to stain for myelin basic protein. This was true in neocortex and in hippocampus. The absence of staining produced, in effect, a negative image of the plaque, highlighting it in relation to the myelin sheaths of the adjacent neuropil. These negative images were confirmed as neuritic plaques by staining the same section for both myelin basic protein and for amyloid (thioflavine T). Myelin sheaths very near the plaque frequently followed curvilinear paths. In those instances where such myelin sheaths were visible over long distances, they appeared to be draped around the edge of the plaque and to be displaced by it. Two mechanisms can be envisioned to account for the curvilinear path followed by myelin sheaths near plaques. It may be due to regrowth of fibers to avoid plaques. Alternatively, and more likely, existing fibers may be displaced by active accretion of material within plaques. Our observations do not discriminate between these possibilities. They do demonstrate that plaques can disrupt the normal cytoarchitectural arrangement of myelinated fibers. The physiological significance of this disordered arrangement is unclear.

79.12 QUANTITATION OF VASOGENIC EDEMA IN CONTUSED SPINAL CORD
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Spinal cord contusion initiates pathophysiologic responses including tissue edema. The developing pathology is probably responsible for much of the permanent loss of function following contusion. Therefore, a better understanding is needed of the relation between tissue responses such as edema and permanent post-contusion loss of function.

This study of experimental spinal cord injury in the ferret quantitated spinal cord edema as a function of the amount of cord compression, with velocity of compression constant. A 20% cord compression had no long-term effects on conduction through the injury site, as measured by somatosensory evoked potentials 4 hours post-injury. Compression of 50%, on the other hand, resulted in reduced amplitude and increased latency of the evoked potential at 4 hours post-contusion. In comparison between 20% and 50% compression, increased amount of compression resulted in increased edema at four hours.

The time course of edema development differed for the two levels of compression, however. Edema developed gradually after a 20% compression, continuing to increase even after apparent functional recovery of the injured region. On the other hand, post-contusion edema developed within 20 min of a 50% compression, and did not increase significantly over the remainder of the 4 hour monitoring period. In the clinical situation, therefore, spinal cord edema may be well-developed prior to therapeutic intervention, and may affect the viability of spinal cord tissue.

Attempts to generate local edema without cord compression were unsuccessful. Surface application of a cryogenic probe did not produce edema significantly different from laminectomy-only controls, nor were permanent changes in neuronal conduction observed. Direct application of dry ice produced edema, but long-term changes in spinal cord conduction were variable.

79.13 PERSISTENT DEPRESSION OF CALCIUM LEVELS IN CEREBRAL CORTEX FOLLOWING TRAUMATIC BRAIN DYSFUNCTION. D.C. Nathanson* and O.R. Hubschmann. Neurosurgery Section, Veterans Administration Medical Center, East Orange, NJ 07019, and University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ 07103.

Recently the pathogenic effects of the disruption of calcium (Ca++) homeostasis and intracellular calcium accumulation have been reported in a number of tissues including the brain. The influx of Ca++ into the cell, resulting from mechanical injury or ischemia, has a number of deleterious effects upon the cell (e.g., membrane damage and loss of mitochondrial function) and may lead to irreversible cell injury. We have studied the changes in the concentration of extracellular Ca++ following injury in order to determine whether these levels could be used as an indication of the functional state of the brain. The dynamic changes in Ca++ activity in the cortical microenvironment were monitored in 20 cats in whom either intracerebral hematoma was produced by injecting 0.5cc of autologous blood, or cerebral ischemia was produced by the injection of air into the carotid artery. The cellular response was monitored using extracellular potassium (K+) and Ca++ ion-specific microelectrodes, cortical DC potential and electrocorticogram.

The cortical response was characterized by a profound cellular depolarization, extracellular accumulation of K+, extracellular Ca++ depletion, and an attenuation of the ECoG. Ca++ normally maintained at 1.12 ± .14mM fell to levels ranging between 0.26 and 0.8mM in the cortex following cerebral injury. Localized or reversible injury resulted in a transient decrease in Ca++ levels which returned to near or slightly below normal levels (0.9 - 1.1mM) within 20 minutes, accompanied by cellular repolarization and recovery of the ECoG. In those animals with terminal injuries the calcium levels remained low (0.26-0.48mM), accompanied by the loss of ECoG activity and, in 3 animals, the cessation of cardiac function. Finally, in one group of animals, the calcium levels recovered to levels below normal (0.68-0.8mM) with the return of an attenuated ECoG. It is believed that while these animals may be capable of survival, there is severe neuronal damage as a result of their injury.

These results suggest that Ca++ levels in the extracellular space may be indicative of the functional state of nervous tissue following cerebral injury and may give some indication of the chances for recovery.

79.14 NERVE TERMINAL AND NERVE FIBER DEGENERATION FOLLOWING SINGLE AND MULTIPLE CEREBRAL CONCUSSIONS. L. C. Parsons and M. D. Guthrie. University of Virginia, Charlottesville, VA 22903 and UTHSC, San Antonio, TX 78201.

Twenty-four adult laboratory rats, following implantation of a concussion disc, recieved either one, two, or three experimental cerebral concussions (ECC) separated by 7 day time intervals. The force of the impact(s) ranged between 26 and 42 lbs./PSI. Criteria used to identify ECCs included the presence of convulsive seizures, and loss of corneal and postural reflexes. A Nissl stain was used to identify cell somas and brain stem nuclei while contiguous brain sections were stained, using either a Fink-Heimer or cupric silver technique. The cupric silver technique was used to verify the presence and extent of nerve terminal degeneration (ntd) and nerve fiber degeneration (nfd), observed and previously reported (Parsons and Guthrie, Neuroscience Letters, 24:199, 1981) to occur following ECC. Both afferent and efferent fiber tracts traversing regions identified to be in the zones of coup, contrecoup and craniocervical junction lesions, demonstrated nfd. The extent of nfd as well as ntd appeared to correlate well with one and two blows. Differentiation between two and three blows was more difficult and probably could be attributed to the brain's macrophage system. Findings suggest further verification for the accumulative effects of ECCs.

79.15 NUCLEAR MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY IN EXPERIMENTAL BRAIN EDEMA. H. M. Bartkowski, L. H. Pitts, M. Nishimura*, M. Brant-Zawadzki*, M. Moseley*, G. Young*. Neurosurgical Research Laboratory, University of California, San Francisco, CA 94143.

Cerebral edema was produced by the classical cold-lesion technique in a canine model. A stainless steel cylinder, 2.0 cm in diameter, was cooled in liquid nitrogen (-70 degrees C) and applied to posterior frontal dura through a burr hole. Nuclear magnetic resonance (NMR) proton imaging of the animals was performed 3, 6, and 24 hours after production of the lesion, at a field strength of 0.35 Tesla utilizing a multiparametric spin-echo (SE) technique. The animals were euthanized immediately after imaging and brain samples were studied with NMR proton spectroscopy utilizing a Fourier-transform spectrometer at a field strength of 3.5 Tesla and a Nicolet 1180 computer to determine longitudinal (T1) and transverse (T2) relaxation values. In addition, brain samples were analyzed for water content by microgravimetric and wet/dry weighing techniques.

Data analysis of water content, NMR spectroscopy, NMR imaging, and T1 and T2 relaxation values calculated by the SE technique was performed. NMR imaging detected the cold-lesion at 3 hours. The two-second pulse interval SE technique was most sensitive in the detection of the lesion and provided optimal differentiation of the gray and white matter. Close correlation between tissue water content and T1 and T2 relaxation values was found. Representative images and data will be presented and discussed.

79.16 SEROTONIN ANTAGONISTS INCREASE RESISTANCE OF CNS TO ISCHEMIA WITHOUT ALTERING BLOOD FLOW RATE. J.A. Zivin and J.A. Venditto* Department of Neurology, University of Massachusetts Medical Center, Worcester, MA 01605

We tested the effects of 3 serotonin antagonists, lysergic acid diethylamide, 2-bromolysergic acid diethylamide (BOL), and cinanserin on the susceptibility of rabbit spinal cord to infarction. Blood flow to the spinal cord was obstructed by occlusion of the abdominal aorta, one of the drugs was administered 5 min later, and after 15 to 60 min of ischemia, blood flow in the aorta was restored. We found that, compared with controls, each drug was able to increase the average duration of ischemia required to produce permanent neurological deficits (ET_{50}). The most effective drug was BOL which was able to increase the ET_{50} by as much as 49%.

Since serotonin is known to have potent circulatory effects, we conducted a study to determine whether BOL produced its beneficial action by altering perfusion of the spinal cord. Five min after obstruction of the aorta, either saline or 3 mg/kg BOL (the dose most effective for protecting the spinal cord from infarction) was injected intravenously. Twenty min after occlusion of the aorta, spinal cord blood flow was measured using [^{14}C]-iodoantipyrine as a diffusible tracer. In control animals, blood flow in the normally perfused thoracic region of the spinal cord was 20.7 $\pm$ 2.66 ml/100g/min (mean $\pm$ s.e., n = 3) and in ischemic lumbar areas flow was 0.389 $\pm$ 0.127. In treated animals, blood flow was 22.8 + 4.99 ml/100g/min (n = 3) in the thoracic cord and 0.514 $\pm$ 0.044 at the lumbar levels. Analysis of variance revealed no significant differences between control and treated animals.

Thus, although BOL increased the resistance of spinal cord to ischemic damage, it did not significantly alter the blood flow rates in the spinal cord during ischemia.

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79.17 HIPPOCAMPAL CELL DEATH IN AREA CA1 FOLLOWING ISCHEMIA IN THE GERBIL: ELECTROPHYSIOLOGICAL RESULTS OBTAINED IN VITRO. T. S. Whittingham, W. D. Lust, Y. Yasumoto* and J. V. Passonneau. Lab. of Neurochemistry, NINCDS, NIH, Bethesda, Md. 20205.

Gerbil brains made ischemic for 5 min by bilateral carotid artery occlusion exhibit cell death of CA1, but not CA3, pyramidal neurons within 4 days following the ischemic episode (Kirino, Brain Res., 239:57, 1982). We are using the in vitro brain slice preparation to study possible electrophysiological changes, such as a hyperactivity of CA1 or CA3 neurons, which might lead to this cell death.

Horizontal brain slices of 300 μm were prepared from control (non-ischemic) and post-ischemic (1,2,3, or 4 days) gerbils. Evoked and spontaneous field potentials were recorded in areas CA1 and CA3 using 3MΩ glass microelectrodes. Orthodromic evoked responses in area CA1 of control slices were typically 20 msec in duration and contained a single population spike. Secondary population spikes occurred at stimulus frequencies of 2 pps or greater, with moderate (~25%) fatigue of the response occurring by 10 sec of 10 pps stimulation. Spontaneous burst discharges were seldom observed. Responses obtained from animals at 1 to 3 days post-ischemia were substantially the same as in control slices. Surprisingly, the electrophysiological responses were also unchanged at 4 days of recovery. These slices were stained with thionin and exhibited no neuronal loss in area CA1.

More recent work by Kirino suggests that CA1 neurons are lost only in the dorsal portion of the hippocampus. Thus, sagittal slices were prepared approximately 3 mm from the midline to include the sensitive neurons and maintain intact hippocampal circuitry. CA1 electrophysiological characteristics in control slices and through 2 days recovery were very similar to those obtained from horizontal slices. Two of the four animals allowed to recover for 3 days also maintained normal responses. However, the remaining 3 day, and all 4 day, post-ischemia animals exhibited no CA1 activity. The slices which failed to respond electrically all showed marked neuronal degeneration in area CA1. Pyramidal cell responses in area CA3 did not vary during the 4 day recovery period in both horizontal and sagittal slices.

These results indicate that if the cell death phenomenon is to be studied in vitro, sagittal slices should be used. Given the similarity of the control responses, horizontal slices from the same animal might be used as internal controls of insensitive CA1 areas. The results fail to detect any abnormal neuronal activity prior to the sudden death of CA1 neurons at 3 to 4 days of post-ischemia. It is possible that the ischemia imposed by slice preparation masks any activity changes which may take place in vivo.

79.18 UNUSUAL STRUCTURAL CHANGES OF ASTROCYTES ENCOUNTERED IN A SPONGIOFORM DEGENERATION OF THE MOUSE BRAIN. N.A. Azzam, R.N. Azzam*, J.V. Bready* and P.A. Cancilla*. Dept. of Anatomy, Fac. of Med., Kuwait Univ., Kuwait and Dept. of Pathology, UCLA Sch. of Med., Los Angeles, CA. 90024.

A spontaneous disorder of a Charles River strain of Swiss-Webster mice that is characterized by an autosomal recessive pattern of inheritance, cranial enlargement, swelling of the brain and a spongy transformation of the white matter has recently been described by our group. The spongy state is due to a remarkable swelling of the cell body and processes of astrocytes associated with fluid accumulation, dispersion of organelles and appearance or membranous arrays and myelin figures. Microvascular endothelium appears normal. Detailed ultrastructural analysis of astrocytes in relationship to blood vessels, ependyma and pia mater has been conducted to document the range and diversity of alterations present in this disorder. One of the most striking changes encountered was the presence of numerous hemidesmosomes in the foot processes of the astrocyte not only in relationship to the blood vessels but also associated with the subpial and subependymal extensions of the cells. These hemidesmosomes were regularly distributed and closely spaced along the membrane always in association with an adjacent basal lamina. Often, this change was accompanied by a retraction of the cytoplasmic membrane which led to an infolding or pinching-up of the basal lamina, with concomitent streaming and contouring of adjacent glial filaments. Since these changes were also present in astroglia in non-vascular regions including ependymal and pial surfaces they were likely a reactive change to enhance support for cells undergoing the spongioform change. The second unusual change was found in the subependymal astroglia. The alteration consisted of vacuolar dilatation of the endoplasmic reticulum with a uniform distribution within affected cells. The vacuoles, lined by a single membrane with some associated ribosomes were filled with a uniformly dispersed granular material. The cells were otherwise characterized by folded nuclei, a well defined Golgi zone, sparcely distributed glial filaments and an association with vessels or ependyma. Because the vacuoles were associated with dilatation of the cisternae of rough endoplasmic reticulum they were presumably related to a synthetic process rather than to pinocytotic uptake by the cells. Since these cells are sometimes associated with redundant basal lamina, it is possible that they are reactive and are synthesizing some components of the basal lamina. Although the changes described here are part of a genetic disorder they are regarded as secondary rather than primary features of the disease.

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79.19 ULTRASTRUCTURAL PROPERTIES OF CNS TUMOR MICROVASCULATURE. M. Miyagami*, B. H. Smith, T. Baginski*, M. A. Greenwood*, P. McKeever, and P. L. Kornblith, Surgical Neurology Branch, NINCDS, National Institutes of Health, Bethesda, Maryland 20205.

CNS tumor microvasculature is important to understanding of tumor biology as well as delivery of therapeutic agents. We have undertaken a systematic study of human tumor microvessel properties utilizing specimens obtained from selected tumor zones at surgery and examined by standard electron microscopic techniques as well as quantitative image analysis methodology. Ten cases (astrocytoma Grade IV,6; astrocytoma Grade II, 1) oligodendroglioma, 1; meningioma, 1; and malignant meningioma, 1; have been evaluated with respect to a) vessel number per tumor zone (central, intermediate and peripheral tumor as well as brain adjacent to tumor (BAT)); b) endothelial cell number and organelles; and c) blood-brain barrier related properties (endothelial cell, tight junctions, fenestrations, pinocytotic activity). Vascular numbers were calculated from examination of 3 fields from each of five blocks from each tumor zone. For ultrastructural studies 10-22 vessel profiles from each zone were evaluated.

Vessel numbers were greatest in the periphery of malignant tumors and lowest in the (often necrotic) tumor centers. Thickened vascular walls (containing increased endothelial cell numbers) as well as narrowed lumens were characteristic. Benign tumors showed no such zonal variation in vessel number. Tubular bodies were common in the marginal zones of malignant, but not benign tumors. Up to 40 percent of vessels in malignant tumors had tubular bodies, whereas benign tumors and BAT, a maximum of 10 percent. Such a finding is consistent with neovascularity. The endothelial cells themselves were increased in number and thickness most prominently in the malignant tumors. Luminal microvilli were increased in the endothelial cells of malignant tumors as were mitochondria and rough endoplasmic reticulum. Two populations of endothelial cells, one with electron-dense cytoplasm and the other with more electron-lucent cytoplasm could be distinguished. In contrast to earlier reports, endothelial cell tight junction abnormalities were not clearly different in malignant and benign tumors. In necrotic areas of malignant tumors, more open junctions could be found. Factor VIII-related antigen was increased in endothelial cells of malignant as compared to benign tumors (Miyagami et al., unpublished data).

These results support the notion of important zonal variation in the brain tumor vasculature of malignant, as opposed to benign, human brain tumors; question the extent of tight junction (and hence blood-brain-barrier) abnormality; and indicate the rapid neovascularization of malignant tumor marginal zones. The latter suggests the active release of one or more angiogenesis "factors" by glioma cells. In addition, the utility of quantitative image processing techniques is demonstrated.

79.PO NEW TREATMENT FOR MULTIPLE SCLEROSIS. A. Winter. Livingston, New Jersey 07039.

Multiple sclerosis is a demyelinating disorder of the central nervous system. The spasticity and pain prevent the patient from functioning normally. A new treatment regime is described comprising transcutaneous nerve stimulation (TNS) and D-phenylalanine (DPA). The significant improvement in signs and symptoms suggest additional factors in the etiology of multiple sclerosis and treatment.

79.PO PROGRESSIVE DETERIORATION OF THE CEREBELLAR CIRCUITRY IN ALUMINUM ENCEPHALOMYELOPATHY. B. Ghetti, M. Goheen* and O. Bugiani.* Indiana University School of Medicine, Indianapolis, In. and Istituto Neurologico "Besta" Milan, Italy.

The injection of aluminum (Al) powder into the cerebrospinal fluid of adult rabbits induces a progressive encephalomyelopathy, characterized by postural alterations, myoclonic jerks, muscle weakness, and ataxia. The neuropathological hallmarks of this toxic encephalomyelopathy are the neurofibrillary tangles, composed of 10 nm neurofilaments. Aim of the present study was to determine the degree of susceptibility of the cerebellar neurons to aluminum toxicity, and to establish whether cerebellar neurons are lost after having developed the neurofibrillary tangles. Rabbits were injected into the cysterna magna with 0.15 ml of a 1% suspension of aluminum powder and then perfused with glutaraldehyde between 1 and 85 days after injection. The cerebellum was studied by light and electron microscopy. The Purkinje cells (Pc) appeared to be the most susceptible cerebellar neurons to the aluminum toxicity and they developed neurofibrillary tangles. Occasionally interneurons (e.g. basket cells) showed the presence of the intracytoplasmic neurofilament bundles. No neurofibrillary tangles were observed in the granule cells nor in the neurons of the dentate nucleus. The accumulation of filaments in the Pc extended to the main dendrite. Prominent swellings were also noticed in the initial segment of the Pc axon: in addition to the extensive accumulation of neurofilaments, mitochondria were seen in large numbers, mostly in the subaxolemmal region. In the molecular layer, numerous Pc dendrites underwent degeneration: they appeared as electron dense osmiophilic debris, surrounded by hypertrophic astrocytic processes. In animals sacrificed between 1 and 2 months after injection, the number of degenerating dendrites became progressively larger; severe Pc losses could be also noticed, in the vermis and in particular at the crown of the folia. In the second and third month the loss of Pc dendrites was followed by progressing losses of parallel fibers and granule cells. In the molecular layer, extensive glial proliferation could be observed. Within the dentate nucleus, axons underwent Wallerian-like degeneration; numerous axon endings had degenerated and they appeared as electron dense debris. The present results indicate that there is a significant loss of Purkinje cells, secondary to aluminum toxicity. The Pc loss produces a deafferentation of the dentate neurons and a marked granule cell degeneration.

OPIATES I

80.3 PENTAZOCINE AND TRIPELENNAMINE: EFFECTS ON BRAIN-STIMULATION REWARD. E.M. Unterwald* and C. Kornetsky (SPON: L. T. Kucharski). Laboratory of Behavioral Pharmacology, Boston University School of Medicine, Boston, MA 02118.

In recent years, the abuse of the combination of pentazocine (Talwin^{R}) and tripelennamine (PBZ^{R}, Pyribenzamine^{R}) has been reported (Showalter, C.V., J. Am. Med. Assoc., 244:1224-1225, 1980). This drug combination is commonly referred to as "T's and Blues" and is administered intravenously by the abuser.

The mechanism by which tripelennamine, an antihistaminic/anticholinergic, enhances the abuse liability of pentazocine, a mixed agonist/antagonist opioid, is not well understood. In order to investigate the reinforcing properties of this combination, we determined the acute effects of tripelennamine, pentazocine, and several combinations of the two on the threshold for rewarding brain stimulation to the medial forebrain bundle - lateral hypothalamic (MFB-LH) region in the rat. Many drugs of abuse including morphine, cocaine, amphetamine and phencyclidine have been shown to lower the threshold for brain-stimulation reward suggesting that this is a useful model of abuse liability and euphoria (Kornetsky, C., et al., Arch. Gen. Psych., 36:289-292, 1979).

Male albino rats (CDF, Charles River Laboratories) were stereotaxically implanted with bipolar electrodes aimed at the MFB-LH area. Thresholds for rewarding brain stimulation were determined using a modification of the psychophysical method of limits. Tripelennamine (1.25-20.0 mg/kg) or pentazocine (1.25-10.0 mg/kg) administered intraperitoneally caused a dose-dependent lowering of reward thresholds for intracranial stimulation. Doses of tripelennamine and pentazocine that were ineffective alone in lowering the threshold for brain-stimulation reward, were then administered concomitantly. The threshold for rewarding brain stimulation was significantly lowered following this combination. The magnitude of this lowering effect was much greater than what was observed with either drug alone at any dose. These results suggest a marked synergistic increase in euphoria when these two drugs are concomitantly administered intravenously in man.

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80.4 INTRACRANIAL SELF-ADMINISTRATION OF METHIONINE ENKEPHALIN. N.E. Goeders, D.A. Ingham, J.D. Lane and J.E. Smith, Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

The abuse liability of opiates depends in part upon their reinforcing properties. Rats will self-administer morphine, an opiate with a high affinity for mu opiate receptors, into the ventral tegmental area (Bozarth and Wise, Life Sci. 28, 551, 1981), lateral hypothalamus (Olds, Brain Res. 168, 351, 1979) and nucleus accumbens (Olds, Brain Res. 237, 429, 1982) at concentrations generally corresponding to the in vitro affinity of the agonist for the major receptor subtype in that region. This investigation was initiated to identify the neuronal circuitry mediating the reinforcing properties of an endogenous delta opiate receptor agonist, methionine enkephalin (met-enkephalin), using intracranial self-administration (ICSA) methodologies. Experimentally naive male Fisher F-344 rats were stereotaxically implanted unilaterally with guide cannulae into the nucleus accumbens, a structure containing primarily delta opiate receptors. The animals were allowed to self-administer 100 nl microinfusions of met-enkephalin directly into the nucleus accumbens by depressing a lever located on one wall of the experimental chamber. The microinfusions were delivered by an adaptation of the electrolytic microinjection transducer (EMIT) system described by Bozarth and Wise (Life Sci. 28, 551, 1981). Concentrations of zero to 750 pmoles of the drug dissolved in artificial cerebral spinal fluid were tested for ICSA. The delivery of met-enkephalin into the nucleus accumbens initiated a reinforcing stimulus in a dose-dependent fashion, with maximal rates of ICSA observed with 500 pmoles of the drug. The reinforcing nature of this self-administration was demonstrated with intermittent schedules of reinforcement and a two-lever discrimination procedure. Naloxone effectively blocked the self-administration of this endogenous ligand, implying that delta opiate receptors are responsible for the initiation of the reinforcing stimulus produced by microinfusions of met-enkephalin into the nucleus accumbens. This suggests that the activation of opiate receptors outside the ventral tegmental area can result in the initiation and propagation of a reinforcing stimulus, which is inconsistent with the dopaminergic hypothesis of opiate reinforcement.

80.5 NALOXONE ENHANCEMENT OF NOCICEPTIVE RESPONDING.
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The highly specific and potent opiate antagonist, naloxone, has emerged as a useful tool for the study of endogenous opiates. However, investigations of naloxone's effect on responsivity to painful stimuli in animals and humans have yielded ambiguous and conflicting results (cf. Goldstein, in Beers and Bassett, eds., Mechanisms of Pain and Analgesic Compounds, 1979). Some variability can be attributed to differing pain tests and doses of naloxone.

The present studies were designed to systematically examine the effects of naloxone alone and in combination with other agents on the jump latency of mice placed on a 50°C hotplate, using an automated apparatus (O'Neill et al., J. Pharm. Methods, in press). CD-1 male mice (Charles River) weighing 20-25 g served as subjects. Each animal was tested only once. Naloxone's time course was established using a dose of 3.2 mg/kg, s.c. Jump latencies were recorded at 7.5, 15, 30, 60, 120 and 240 min after treatment. Maximal hyperalgesia was observed at 15 and 30 min; at 1 hr after treatment, marginal hyperalgesia was observed; latencies returned to control values at 2 and 4 hrs after drug administration. There was no evidence of analgesia at these times.

Treatment with the enkephalinase inhibitor, thiorphan (100 mg/kg, s.c.; cf. Chipkin et al., Eur. J. Pharmacol., 83:283, 1982), 15 min prior to testing at 1, 2, or 4 hr after naloxone (3.2 mg/kg, s.c.), failed to produce analgesia. Thus, naloxone administration under this protocol does not appear to rapidly upregulate an enkephalinergic system.

In a dose-response study, 15-min pretreatment with naloxone at .5-log intervals over a dose range of 0.1 through 100 mg/kg, s.c. yielded hyperalgesia at doses of 1 mg/kg and above. No analgesia was observed at any dose.

Naloxone at low doses ($\leq$3.2 mg/kg s.c.), given in conjunction with morphine sulfate at a grossly analgetic dose (5.6 mg/kg), also resulted in hyperalgesia, and not just blockade of morphine's analgetic action. The combination of acute morphine + naloxone in fact appears to result in greater hyperalgesia than does naloxone alone. Similar results were observed in ancillary tests using phenylquinone-elicited writhing in mice as an endpoint.

These data suggest that in the hot-plate jump- and phenylquinone writhing-tests application of a nociceptive stimulus activates endogenous opiate systems. Blockade of opiate receptors by naloxone may result in hyperalgesia by displacement of endogenous opiates from receptors.

80.6 BRAIN MUSCARINIC CHOLINERGIC RECEPTOR CHANGES CORRELATED WITH RAT MORPHINE-SEEKING BEHAVIORS. J.E. SMITH, C. Co and J.D. Lane. Psychiatry Research Unit, Departments of Psychiatry and Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130.

The neurobiological substrates of opiate reinforcement have been under vigorous investigation. Pharmacological animal studies have shown agents that disrupt cholinergic, dopaminergic and noradrenergic neurons to attenuate intravenous opiate self-administration. Neurotransmitter turnover rate measurements in morphine self-administering rats have implicated cholinergic innervation of the frontal and pyriform cortices, nucleus accumbens, amygdaloid complex and ventral tegmental area (Smith et al, Neurosci. Abst. 8, 591, 1982). Acetylcholine turnover rates utilize short pulse times (5 and 10 min) with only the cholinergic neuronal activity during this period determining the turnover rates. Neuronal activity related to reinforcement processes may occur throughout the interinfusion interval (approximately 2 hours), therefore, cholinergic receptor parameters could be more representative of the total change in activity. Muscarinic cholinergic receptor binding was evaluated in membranes from seven brain regions of rats either intravenously self-administering morphine (10 mg/kg per infusion) or receiving yoked-infusions of morphine or vehicle. These were the same animals used for the acetylcholine turnover rate measurements. Membranes were prepared from frozen tissue powders by homogenization in 50 mM KH_2PO_4/Na_2HPO_4, pH 7.4 buffer and centrifugation (48 K x g for 30 min). Binding was evaluated (60 min at 25°C) with five to seven concentrations of [^{3}H]-quinuclidinyl benzilate (QNB) (0.375 nM to 2.4 nM) in the presence or absence of 9 uM atropine sulfate. The membranes were rapidly collected on Whatman GF/B glass fiber filters by suction filtration and the radioactivity determined by liquid scintillation spectrometry. Rosenthal plots and Scatchard analysis were used to determine QNB binding affinities (KD) and densities (Bmax). Binding affinities were not altered but densities were affected by morphine (yoked-morphine compared to yoked-vehicle group) and by self-administration (self-administration compared to yoked-morphine infused group). Morphine resulted in decreases in receptor densities in the cingulate and entorhinal-subicular cortices. Self-administration resulted in decreases in receptor densities in the frontal and entorhinal-subicular cortices and increases in the amygdaloid complex. The changes generally agree with turnover rate measurements but may be more indicative of overall neuronal activity. The frontal and entorhinal-subicular cortical and amygdaloid complex cholinergic neuronal innervations appear to be involved in components of opiate reinforcement processes.
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80.7 LACK OF EFFECT OF ADRENALECTOMY ON STRESS-INDUCED ANALGESIA.
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Recent studies have suggested that some forms of stress-induced analgesia (SIA) are mediated by opioid systems while others are mediated by nonopioid systems. MacLennon et al. (Science, 215:1530, 1982) recently reported that hypophysectomy or adrenalectomy (ADX) blocked the SIA observed after foot shock stress (FS), when the animals were tested upon reexposure to a brief FS. By contrast, Glusman et al. (Soc. Neurosci. Abstr., 6:321, 1980) reported that ADX potentiated SIA following cold water stress (CWS) or FS. Most investigators report using a 1-2 week recovery period after ADX before testing for SIA, which may result in the development of compensatory changes such as functional tolerance to the chronically high concentrations of B-endorphin observed in ADX animals. In the present study SIA was examined after only a 24-hour recovery period from surgery.

ADX, sham-ADX, and control rats were subjected to one of three treatments: CWS, CWS + naltrexone (10 mg/kg every 24 hours), or no-CWS. Each group was tested for SIA by the tail-withdrawal method (Miksec, S. and Lal H., Psychopharmacology, 54:217, 1977), immediately prior to, immediately after, 24 hours after, and 5 days after a single exposure to CWS (2°C, 3.5 min), in a 3 x 3 (surgery X stress) design with four repeated measures.

No differences in SIA were observed among surgical treatments at any time point. However, significant differences among stress treatments occurred immediately after CWS, when the CWS group showed longer latencies than the CWS + naltrexone group, which showed longer latencies than the no-CWS group. However, even the no-CWS group exhibited a tail-withdrawal latency significantly longer than its baseline response, which indicates that SIA occurred in response to the restraint procedure used in the analgesia testing. Twenty-four hours and 5 days after CWS, all groups again exhibited significantly longer latencies than their respective pre-stress baselines. These results indicate that animals are capable of exhibiting SIA 24 hours after surgery, and upon reexposure to treatment associated with stress, and that this SIA may, in part, be mediated by opioid mechanisms.

80.8 SELECTED OPIOIDS, PRESSING FOR INTRACRANIAL STIMULATION, AND THE CONDITIONED PLACE PREFERENCE TEST. G. A. Hunter Jr.,* A. M. Lindsay* and L. D. Reid, Dept. of Psychology, Rensselaer Polytechnic Institute, Troy, NY 12181.

Rats, fixed with chronically indwelling bipolar electrodes for intracranial stimulation (ICS) of the lateral hypothalamus, were given an opportunity to press a lever for a fixed intensity of ICS 30 min/day. Thirty min before some sessions, placebos (carriers of drugs) were administered. Before other sessions, injections of selected opioids were given. Doses of 10 mg/kg, as well as smaller doses, of naloxone (the prototypic antagonist of morphine and endogenous opioids' effects) reliably reduced pressing compared to placebo. Fentanyl (FEN, a potent analgesic) increased pressing at doses of .005 to 0.4 mg/kg (at .04 mg/kg by greater than 160%). Ethylketocyclazocine (EKC, an opioid that is a prototypic agonist for the kappa opioid receptor) decreased pressing at doses greater than .08 mg/kg. The levorotatory enantiomer of EKC also depressed rates at all doses tested while the dextrorotatory enantiomer and small doses of the racemic mixture increased pressing. Diprenorphine (DIP, an antagonist to many morphine-like effects) increased pressing at doses ranging from 5 to 250 microgm/kg (250 microgm/kg increased pressing by more than 180%).

It is presumed that a drug-induced increment in pressing for ICS reflects the ability of that drug to elicit heightened activity in neural substrates of reward or positive affect. Since DIP's effect on pressing for ICS was unexpected, it was decided to assess its capacity to elicit positive affect in another procedure, the conditioned-place-preference (CPP) test. The CPP test involves placing rats in one end of an alley under the drug's influence and the other end under placebo and, subsequently, seeing which end the rats prefer. Rats, after habituation to the entire alley, received doses of either DIP, EKC or morphine before being confined to one end of the alley. On alternate days, they were given placebo and limited to the other end. On test day, rats were given access to the entire alley while tabulating the time spent in each side. DIP, as does morphine, leads to a CPP. EKC, in large doses, only leads to a CPP with more extensive testing.

FEN, EKC, and DIP differ considerably in their ability to elicit analgesia and other morphine-like effects and in their capacity to engender signs of positive affect. There is no apparent relationship among these differing capacities, i.e., they seem to vary independently across opioids. This leads to the conclusion that opioid-analgesia and opioid's capacity to elicit positive affect are products of separate systems, and, perhaps, coded by way of different opioid receptors.

80.9 CONDITIONED TASTE AVERSION PRODUCED BY MORPHINE DOES NOT INVOLVE MU-TYPE OPIATE RECEPTORS IN BRAIN. M.T. Bardo, J.S. Miller*, D.F. McCoy* and M.E. Risner*. Dept. of Psychology, University of Kentucky, Lexington, KY 40506 and NIDA Addiction Research Center, P.O. Box 12390, Lexington, KY 40583.

The present experiments examined the involvement of μ-type opiate receptors in conditioned taste aversions produced by morphine. In the first experiment, adult male Sprague-Dawley rats were implanted subcutaneously with a slow-release pellet containing either naloxone (10 mg free base) or placebo. After 10 days of pellet implantation, the pellets were removed and the animals were sacrificed by decapitation at 1, 2, 4, 8 or 16 days after removal of the pellet. Brain and spinal cord regions were dissected, and the tissue homogenized in Tris HCl buffer, (pH 7.4) and incubated at 0°C for one hour with ^{3}H-naloxone in the presence or absence of 100 nM levallorphan tartrate. Each sample was then washed over GF/B glass fiber filters and the radioactivity was determined by liquid scintillation spectrometry. Using this in vitro assay procedure, it was found that naloxone-implanted animals exhibited a significant increase in specific binding of ^{3}H-naloxone. This increase in μ-type opiate binding was evident for varying durations after pellet removal, depending upon which region of the central nervous system was examined.

In a second experiment, animals were similarly treated with either naloxone or placebo pellets, except that these animals were given only 15-minutes daily access to tap water during the last seven days of pellet implantation. One day after the pellets were removed, these animals were then given 15-minutes access to .1% saccharin solution followed immediately by an injection of morphine sulfate (0, 1, 3 or 10 mg/kg, s.c.). Thirty minutes after the injection of morphine, each animal was also tested for pain sensitivity using a standard 50°C hot-plate test. The saccharin-morphine pairings were repeated once every third day for five days. During the intervening days, the animals were given 15-minutes access to water. The results from this experiment demonstrated that morphine produced a dose-dependent increase in paw-lick latencies (analgesia) and a dose-dependent decrease in saccharin consumption (taste aversion). While the naloxone pellet treatment enhanced the analgesic effect of morphine, the aversive effect of morphine remained unchanged. In addition, a third experiment demonstrated that when the naloxone pellet was left implanted during behavioral testing, the analgesic effect of morphine was blocked completely, whereas the aversive effect of morphine remained unchanged. Thus, unlike analgesia, the aversive effect of morphine does not depend directly upon μ-type opiate receptors in brain.

80.10 6-OHDA LESIONS OF THE VENTRAL TEGMENTAL AREA BLOCK MORPHINE FACILITATION OF SELF-STIMULATION. T.H. Hand* and K.B.J. Franklin. Psychology Dept., McGill Univ., Montreal, P.Q. Canada H3A 1B1

The rewarding properties of morphine and related drugs have been demonstrated to depend on the dopamine (DA) cells of the ventral tegmental area (VTA). Morphine is also known to facilitate self-stimulation (SS), and there is some indication (Broekkamp et al., Pharmac. Biochem. Behav. 11:289, 1979) that this facilitation is due to a direct influence of the drug on opiate receptors in the VTA. To test this hypothesis, we lesioned the VTA cells with 6-hydroxydopamine (6-OHDA), and subsequently evaluated the effects of morphine on SS.

Male hooded rats received bilateral microinjections of 6-OHDA (4ug in 1uL each side) or .05% ascorbic acid vehicle aimed at the VTA. Noradrenalin neurons were protected with protryptiline (15 mg/kg IP). Each rat was also implanted with a bipolar electrode aimed at the lateral hypothalamus. Rats were then trained to lever press for .2 sec trains of 100 Hz .2 msec pulses of brain stimulation delivered on a random interval 5 sec schedule. When stable response rates were established at 35-65% of maximal rate, drug testing was begun. Every second day for 7 days, rats were tested during 3 twenty minute sessions: immediately before, 1h after and 3h after subcutaneous injection of 10 mg/kg morphine sulfate. Morphine was administered daily.

Control animals showed the expected biphasic effect of the drug (rate suppression followed by facilitation), with gradual development of tolerance to the rate suppression. Lesioned animals showed neither the facilitation nor the tolerance to the rate suppression. These findings further substantiate the hypothesis that VTA DA cells are necessary for the facilitation of SS by morphine. This is consistent with the more general claim that the mesolimbic DA projection takes a significant part in the reinforcing properties of morphine and related drugs.

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80.11 PRE-EXPOSURE TO LOW DOSES OF MORPHINE BLOCKS MORPHINE-INDUCED CONDITIONED TASTE AVERSION. T.Hunt*, K.Spivak*, and Z. Amit, (SPON. M.Britt). Centre for Behavioral and Neural Biology, 1455 De Maisonneuve Blvd., Montreal, P.Q. H3G 1M7

Male Sprague-Dawley rats were placed on a 23h 40min water deprivation schedule. On days 2, 4, and 6, animals received pre-exposure injections of one of three doses of morphine hydrochloride (2.5, 5.0, or 15.0 mg/Kg) or saline following the 20min drinking period. On day 8, (conditioning day) animals were presented with a 0.1% saccharin solution in place of water. Within a minute after termination of the drinking period, animals in each of the four pre-exposure groups received one of the three morphine doses (2.5, 5.0, or 15.0 mg/Kg) or saline. There were thus 16 independent groups (7-8 animals per group). On ensuing days the water deprivation schedule was continued. On day 14, (conditioning day 2) saccharin presentation and drug administration were again given as before. This cycle was repeated until 5 conditioning days had been observed. On day 38 (test day), a final saccharin presentation was performed.

Results showed that saline pre-exposed animals in both the 15mg/Kg and 5mg/Kg conditioning groups exhibited significant reductions from baseline saccharin consumption levels over test trials, indicative of conditioned taste aversion (CTA). In contrast, rats pre-exposed to the three morphine doses failed to exhibit CTAs at either conditioning dose. Thus, pre-exposure to the 2.5 mg/Kg morphine, a dose which in itself did not induce a CTA, was as effective as a 15mg/Kg dose in blocking a morphine CTA. Previous studies have shown that morphine at low doses comparable to the 2.5mg/Kg dose can mediate positive reinforcement. This, then, suggests that pre-exposure to a morphine dose which does not mediate a CTA but has been shown to have positive reinforcing properties may serve to block a morphine CTA induced by higher morphine doses.

In a second experiment, it was shown that pre-exposure to the 2.5mg/Kg dose did not result in tolerance as measured by a hot plate test of morphine analgesia.

These data, then, may provide significant new insights into the discriminative properties of morphine as a potentially aversive agent.

80.12 THE CONDITIONING OF CHANGES IN LOCOMOTOR ACTIVITY INDUCED BY MORPHINE APPLIED TO THE VENTRAL TEGMENTAL AREA OF THE RAT BRAIN. P. Vezina* and J. Stewart. Center for Studies in Behavioral Neurobiology, Psychology Dept., Concordia Univ., Montreal, H3G 1M8.

Two experiments were conducted to determine whether the hyperactivity obtained from bilateral administration of morphine to the ventral tegmental area (VTA) of the rat brain (Joyce and Iversen, Neurosc. Lett., 1979) could be conditioned to the environment associated with the drug administration.

In Experiment I, locomotor activity was measured for 90 min in one group of rats immediately following a bilateral administration of morphine sulphate crystals to the VTA (18 μg tapped into the tip of 28 gauge cannulae), and sometime before morphine administration in another group. Each of these two groups was accompanied by a no-morphine control group that received a sham administration to the VTA. During a three-week period, morphine was administered daily for four days a week. A test for conditioning followed a 10-day morphine-free period; all animals were given sham administrations and placed in the activity boxes. Animals that had previously received morphine in the activity boxes were significantly more active than their sham controls; animals that had received an equivalent number of morphine administrations, but never associated with the activity boxes, had activity levels similar to their sham controls. These data show that the hyperactivity was conditioned to the environment associated with morphine administration, and that the conditioned response (CR) mimicked the unconditioned response (UCR) to this action of morphine and was not a compensatory CR that opposed the UCR.

In Experiment I, repeated administration of morphine to the VTA resulted in a progressive increase of the hyperactivity (see also Joyce and Iversen, 1979). Experiment II examined the possibility that this sensitization to the effect of morphine was due to conditioning. Two groups of rats were administered morphine sulphate crystals to the VTA on five occasions, once every fourth day. One group received morphine in the activity box; the other group received morphine in the home cage. A test followed on which both groups were administered morphine in the activity box. Animals that had previously received morphine in the activity boxes were significantly more active than animals that had received an equivalent number of morphine administrations, but in their home cages. Hence, the sensitization of the hyperactivity only appeared when animals were tested in the environment previously associated with morphine administration. These data show that the sensitization is due, in large part, to a learned association between the morphine effect and the administration environment and cannot be accounted for by changes brought about by the repeated exposure of opiate receptors in the VTA to morphine.

80.13 NON-NALTREXONE REVERSABLE HEAT-STRESS INDUCED ANALGESIA. Z.H.Galina*, F.Rogan* & Z.Amit. Center for Studies in Behavioral Neurobiology, Concordia Univ. Rm.H1013, Montreal, Canada, H3G1M7.

Using the same procedures that we have previously shown to induce a graded release of corticosterone (B) which was related to the intensity of the heat stress (higher intensity=greater release), we now report that a transient analgesia ca be produced by heat-stress; that it is not naltrexone (NTX) reversable, but does seem to be related to the amount of B release (as determined by previous study).

The prodedure consisted of placing male Wistar rats upon one of four hot-plates for thirty sec. Each hot-plate was set at a different temperature (21 (control), 47, 52, or 57°C). Two hrs. before hot-plate testing the rats were injected with either saline or NTX (10 mg/kg). After 30 sec on the hot-plate the rat was removed and placed in a plastic baby bottle (8 oz) that had the bottom cut out so that the rats tail projected from the end. This provides an unobtrusive restraining device with easy accsess to the tail. A modified tail-flick procedure was used to measure analgesia. A hot water bath was heated to 45 C into which 5cm of the rats tail was immersed. A 30 sec cutoff time was established. Analgesia was measured at 0, 5, 10, 15, 30, 60, 120 min and 24 hrs after the 30 sec hot-plate stress.

A three-way ANOVA (temp x time x NTX) revealed a sign. effect of Temp, time, and temp x time interaction. The main effect of NTX was not sign. Post-hoc analysis (Tukey) indicated that exposure to 57 induced a transient analgesia as compared to (21) controls which lasted for 15 min. Interestingly, though 52 was not sign different than control group, the group that recieved exposure to 52 and NTX , exhibited greater analgesia than the group recieving 52 alone. This effect was sign. at 5, 10, and 15 min. The sign. effect of time results from the slow decrease over time of the analgesia exhibited by the 57 group and the increase in analgesia over time of the control group.

This is a demonstration that another stressor (heat) can induce a transient analgesia in rats that is not effected by the NTX sensitive receptor.

80.14 THE INFLUENCE OF CONTEXTUAL CUES ON SUBSEQUENT PREFERENCE FOR ORAL ETONITAZENE. M. R. Lynch and J. H. Porter*. Psychology Dept., Va. Commonwealth University, Richmond, VA 23284.

In order to determine facilitory effects of environmental stimuli previously paired with oral opiate availability, on subsequent drug selection in a choice test procedure, daily ½ hr limited access to 5 μg/ml etonitazene solutions was paired with a set of discrete contextual cues. Of 80 male albino rats, 40 received this 'CS-complex' with drug pairing, while 40 were exposed to drug in the home cage as their sole available drinking solution. Groups were further subdivided into control rats which were pretreated daily with 3 mg/kg naltrexone and those injected ip. with saline. An additional factor of food deprivation was introduced in an attempt to obtain maximum drug intake during sole access, with half of the above groups gradually reduced to 75% ad lib body weight over 12 days of opiate availability. Following these drug-environment pairings, choice testing with water was conducted for all rats, both in the same environment as prior drug exposure, and in a different environment (i.e., distinctive vs. home cage). Tests were conducted during the daily ½ hr limited access period, with order of testing counterbalanced over the two environments.

While the predicted facilitation was not obtained, (no main effect for place of testing), food deprived rats did show a significantly greater preference for etonitazene than those maintained at 100% body weight. This finding indicates that food deprivation may indeed increase opiate reinforcement as measured by preference in a choice test situation, and thus extends previous findings of Carroll, France & Meisch (Science, 205: 319-321, 1979) and Carroll & Meisch (Pharmac. Biochem. Behav., 10: 155-159, 1979) who reported increases in absolute ml intake with 24 hr sole access to drug solutions. Overall, animals exposed in the familiar home cage also preferred drug solutions more than distinctive environment groups, this difference being significant for saline-injected but not naltrexone animals (an injection X environment interaction). Post-choice test tail-flick tests of morphine analgesia revealed cross-tolerance in all saline-injected rats, which was no longer apparent after a two-month drug free period. As contrasted with the previously reported inability to block opiate effects of oral etonitazene with naltrexone pretreatment, in a 24 hr drinking paradigm (Lynch, Porter & Johnson, Neurosci. Abstr., 8: 590, 1982), no signs of precipitated or spontaneous withdrawal were observed during these studies.

NEUROPEPTIDES AND BEHAVIOR: OPIATES

81.1 FACILITATION OF DEVELOPMENT OF RESISTANCE TO MORPHINE ANALGESIA BY OXYTOCIN AND THE ROLE OF BRAIN CATECHOLAMINES IN THE RAT. K. Sharifi Hossaini, and M.S. Shahid Salles. Department of Pharmacology and Physiology, School of Medicine, Shiraz University, Shiraz, Iran.

Rats were prepared with permanent electrodes for recording EEG and EMG. Morphine injections of 10 mg/Kg given S.C. were followed by the appearance of high voltage EEG slow bursts associated with stuporus behavior. This phase was suppressed by the appearance of behavioral arousal shown by EMG and behavior of rats and afterwards behavioral sleep became apparent on the EEG. Administration of 10 mg/Kg of morphine to rats pretreated with oxytocin (0.5 μg/0.5 ml/animal) strongly increased the above mentioned behaviors. The result of tailflick-tests in another series of animals treated with oxytocin show rapid facilitation of development of resistance to morphine analgesia. Administration of a 10 mg/Kg dose of morphine to rats pretreated with α-methylparatyrosine (α-MPT)50mg/Kg was followed by a significant decrease in morphine effect. Pretreatment with α-MPT and oxytocin before morphine injection was followed by a reduced effect of α-MPT treatment. The result may suggest an important role for neurohypophyseal hormone oxytocin in morphine analgesia. Furthermore it is suggested that the effect of oxytocin may depend on the integrity of the brain noradrenergic system.

81.2 IRON DEFICIENCY: MODIFICATION OF CIRCADIAN RHYTHM OF PAIN THRESHOLD-BRAIN DOPAMINE AND β-ENDORPHIN. S. Yehuda and M. B. H. Youdim.* Dept. of Psychology, Bar-Ilan Univ., Ramat-Gan 52100, Israel, and Rappaport Medical Research Ctr., Fac. Med., Technion, Haifa, Israel.

Rats were made nutritionally iron-deficient. Experimental iron deficiency (ID) selectively modifies the binding capacity of dopamine-D_2 receptors. Behavioral correlates of an ID state includes reversal of the circadian cycles of dopamine-mediated behaviors, such as d-amphetamine-induced hypothermia in rats kept at 4°C, d-amphetamine- and apomorphine-induced hypermotility, and stereotyped behavior. d-Amphetamine potentiates the reversal of the cycles, but TRH and its peptidase-resistant analogue CG 3703 prevent the reversal of the cycles. Pain threshold (Hot plate, 58°C) is also circadian cycle-dependent. In ID rats the pain threshold cycle was reversed. Peripherally injected β-endorphin (0.1, 1.0, 3.0 mg/kg) elevates pain threshold only in ID rats and not in control rats (no linear dose-response curve was found). Morphine (10.0 and 20.0 mg/kg) and haloperidol (2.0 mg/kg) elevate pain threshold both in ID and in control rats. This elevation was smaller than that induced by a 1.0 mg/kg dose of β-endorphin. No additive effect was found after the combined treatment of haloperidol and β-endorphin. Naloxone (1.0 mg/kg) induces a reduction in pain threshold and blocks the effect of β-endorphin. Neuroleptics are also able to increase pain threshold. These results indicate that dopamine (via the endorphin system) may play an important role in modifying pain threshold.

81.3 FRACTIONATION OF POSTURAL SUPPORT MECHANISMS WITH COMBINED SYSTEMIC ADMINISTRATION OF HALOPERIDOL AND MORPHINE. S.M. Pellis*, F. de la Cruz*, V.C. Pellis* and P. Teitelbaum. Psychology Dept., University of Illinois at Urbana-Champaign, Champaign, IL 61820

Haloperidol (a dopamine antagonist) and morphine have been used to isolate two separate, but complementary, complexes of reflexes used in posture and locomotion. Haloperidol, in rats, induces a state of akinesia/catalepsy in which the rats adopt a crouched posture with broad-based support. When pushed they will resist being displaced or rolled over by actively pushing against the displacing force. In contrast morphine eliminates these supporting reactions. Rather than oppose a displacing force, they allow themselves to be rolled over. The limbs are rigidly arrested in phases of the step cycle, compatible with a state preparatory to locomotion. (De Ryck et al., Brain Res., 201:143-172, 1980). EMG recordings from antagonistic muscles of the limbs support these conclusions (De Ryck and Teitelbaum, Expl. Neurol., 79:54-76, 1983).

When given haloperidol and morphine in combination, the rats adopt and actively maintain their limbs in an abducted and extended posture, while their bodies lie completely flat. Although most postural support mechanisms are abolished, some residual, fractionated ones remain. For example, in response to being pushed on the ground or placed on a vertical grid, the rats will grip with their digits. However, unlike when given haloperidol alone, the gripping does not involve flexion of the forearms and ventroflexion of the head, rather the body is limply held up by the flexed digits. The sensory guidance of remaining responses is also simplified. For example, while these rats do not respond to slowly occurring postural changes, they exhibit normal limb and body movements in response to rapid postural changes. With the use of visual occluders and labyrinthectomy, it was found that for either drug alone, these movements involved both proprioceptive and vestibular guidance. With haloperidol and morphine combined these movements were exclusively dependent on vestibular guidance.

The combined haloperidol and morphine preparation has proved to be useful in fractionating hitherto intact postural support reflexes. Furthermore, there appears to be an interaction between opiate and dopaminergic systems in postural and locomotor behaviour not previously described.

81.5 SELF-STIMULATION IN THE REGION OF LOCUS COERULEUS: OPIOID OR CATECHOLAMINERGIC MECHANISMS? S.E. Loughlin, J.D. Belluzzi, F.M. Leslie, and L. Stein. Department of Pharmacology, University of California, Irvine, Irvine, CA 92717.

The catecholamine reinforcement hypothesis, and, more specifically, the noradrenergic hypothesis, predicts that stimulation of nucleus locus coeruleus should support self-stimulation (SS). While many investigators report positive electrode sites in the locus coeruleus, some workers suggest that those electrodes located precisely in the nucleus are negative for SS. Furthermore, in many experiments, manipulation of noradrenergic transmission failed to affect SS at positive sites. Since the region surrounding the locus coeruleus contains dense opioid terminal fields, and a role of opioid systems in reinforcement has been suggested (Belluzzi and Stein, 1977), we postulated that opioid systems might play a role in SS in this area. To test this hypothesis, we examined the self-stimulation behavior of rats implanted with electrodes in dorsal pons. Animals which exhibited stable rates of self-stimulation were tested for sensitivity to the opiate antagonist, naloxone (0.5 to 40 mg/kg). Localization of electrode tips was accomplished by routine histological procedures or in brains prepared by immunocytochemical staining for various opioid peptides. In addition, maps of noradrenergic cell bodies and axons were generated and electrode placements were correlated with these. The results suggested the following tentative conclusions: First, one group of electrode tips supported high rates of self-stimulation, which was blocked or greatly decreased by administration of naloxone. These were located in, or in close proximity to, regions which exhibited opioid-like immunoreactivity. A second group of electrode tips supported low to medium rates of self-stimulation which was unaffected by naloxone. Many of these electrodes were located within or directly anterior to the nucleus locus coeruleus. These data may explain the results of other studies suggesting that self-stimulation in this region is unaffected by a variety of noradrenergic manipulations. Electrode tips in these studies may have been located in opioid-positive regions. These data further suggest that at least two independent systems may support ICSS in dorsal pons, an opioid system and a non-opioid, perhaps catecholaminergic, system.

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81.6 PERIPHERALLY ADMINISTERED NEUROPEPTIDES AND LATERAL HYPOTHALAMIC SELF-STIMULATION IN RATS. G. Meisenberg, S.A. Lorens and W.H. Simmons*. Loyola University Medical School, Maywood, IL 60153.

Peptides related to neurohypophyseal hormones, as well as [des-Tyr^1]-γ-endorphin, have been reported to alter responding for brain stimulation reward via electrodes implanted in the ventral tegmental area or the nucleus accumbens (Brain Res. 172, 367-371,1979; Pharmacol. Biochem. Behav. 10, 899-905,1979; Neuropharmacol. 19, 429-434,1980). In the present study, the effects of subcutaneously administered [8-arginine]vasopressin (1μg), [9-desglycinamide,8-lysine]vasopressin (1μg), Prolyl-leucyl-glycinamide (1μg) and [des-Tyr^1]-γ-endorphin (15μg) on lateral hypothalamic self-stimulation (LHSS) were assessed in rats using methods which have been detailed previously (Psychopharmacol. 48, 217-224, 1976). Briefly, the animals were tested daily for 10 min in an operant conditioning chamber. Each lever press delivered a 0.2 sec train of biphasic square waves (100 Hz) on a continuous reinforcement schedule. The current intensity was adjusted individually to yield response rates which were approximately 50% of the maximal response output. On drug days, the animals were tested before and 30 min after the injection. None of the peptides significantly altered LHSS responding. On the other hand, D-amphetamine (0.2-1.0mg/kg) produced a marked dose-dependent increase while haloperidol (0.25mg/kg) was followed by a profound reduction in LHSS response output. Our results to date, thus, have failed to support the view that peripheral administration of the aforementioned peptides induce a "neuroleptic-like" or "psychostimulant-like" effect on brain stimulation reward.

81.7 ENDORPHIN-CATECHOLAMINE INTERACTIONS IN NUCLEUS ACCUMBENS SELF-STIMULATION. K. A. Trujillo, J. D. Belluzzi, and L. Stein. Department of Pharmacology, University of California, Irvine.

Evidence suggests that the catecholamine-like drugs amphetamine and cocaine (Lyness, et al, Pharmacol. Biochem. Behav. 11:553, 1979; Roberts, et al, Pharmacol. Biochem. Behav. 12:781, 1980) as well as opiate drugs, such as heroin (Wise and Bozarth, Pharmacol. Biochem. Behav. 17:239, 1982; Spyraki, et al, Psychopharmacol. 79:278, 1983) exert their reinforcing effects by actions on mesolimbic dopamine neurons. These neurons project heavily to the nucleus accumbens, a region rich not only in dopamine, but also in enkephalins. There are at least two possible mechanisms by which catecholamine and opiate drugs might produce reinforcing effects in the mesolimbic system. The first is that they act on independent and parallel pathways—catacholaminergic drugs acting through the dopamine neurons, and opiate drugs acting through an independent set of enkephalin neurons. The alternative is that these pathways are interactive—opiates might act by affecting dopaminergic transmission. Since intracranial self-stimulation (SS) is a valuable tool for studying reward mechanisms, experiments were performed examining drug effects upon barpress rates for nucleus accumbens SS. Experiment I studied the dose-related effects of naloxone (0.2, 2.0, 20 mg/kg subcutaneous) upon this behavior. Consistent with previous studies that have shown supressive effects of naloxone in rats with electrodes in enkephalin-rich brain regions, nucleus accumbens SS rates were dose-dependently supressed by this opiate antagonist. This action of naloxone suggests that endogenous opioids play a role in SS behavior in the nucleus accumbens. Experiment II compared the effects of a single dose of naloxone (2.0 mg/kg subcutaneous) with those of a single dose of amphetamine (1.0 mg/kg subcutaneous) in individual rats (n=18). The supression of barpress rate produced by naloxone (calculated as percent of control) correlated well ($r=-.575$, $p < .02$) with the facilitation produced by amphetamine. This correlation is supportive of a common pathway for the rewarding effects of endogenous opioids and catecholamines. The results of these experiments are consistent with the hypothesis that the rewarding effects of these compounds are mediated by the mesolimbic dopamine system.

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81.8 INHIBITION AND FACILITATION OF HYPOTHALAMICALLY-ELICITED HISSING BY CENTRAL GRAY STIMULATION ARE DIFFERENTIALLY SENSITIVE TO NALOXONE. C. B. Pott*, S. Z. Kramer* and A. Siegel. Dept. of Neuroscience, UMDNJ, Newark, NJ 07103 and Dept. of Biology, Seton Hall Univ., So. Orange, NJ 07079.

Specific areas of feline central gray (CG) modulate intraspecific aggression elicited by hypothalamic stimulation. Recent studies in our laboratory (M. Brutus et al., 1982, Neurosci. Abst. 8:971) have demonstrated regional differences of modulatory sites. Stimulation of rostral-dorsal CG inhibits, while stimulation of caudal-ventral CG facilitates, attack. Central gray stimulation also produces analgesia, possibly mediated by enkephalins (Akil & Liebeskind, 1975, Brain Res. 94:279). The relationship between CG stimulus induced analgesia and aggressive behavior is unclear, although analgesic midbrain stimulation (rat) suppresses shock elicited target biting (Renfrew & Leroy, 1982, Physiol. & Behav. 30:169). The present study examines the possible role of endogenous opiates in CG modulation of feline intraspecific attack.

Adult cats were implanted with chronic electrodes aimed at the ventromedial hypothalamus (VM), which when used to stimulate, elicited hissing, growling, piloerection, mydriasis, and attack directed at conspecifics. Later, while restrained, control latency to hypothalamically-elicited hiss was established. Each animal was then implanted with CG cannula-electorodes. Response modulation was determined by dual stimulation of CG and VM electrodes, alternated with VM stimulation alone, in an A-B-B-A sequence. A Hamilton syringe was lowered into CG modulatory, cannula-electrodes to the tip, and 5 ul (1ug) of naloxone (in saline) injected. Hypothalamically-elicited hiss latency was again determined, followed by paired trials of single and dual stimulation. To date, three electrodes aimed at caudal-ventral CG produced facilitation of VM induced hissing, not significantly affected by naloxone. In contrast, three electrodes in the rostral-dorsal CG produced inhibition of hissing which was blocked by naloxone. Moreover, two of the sites became facilitatory.

These results demonstrate that CG inhibition of VM-elicited hissing can be blocked or reversed by the opiate antagonist, naloxone, and are consistent with the premise that both analgesia and inhibition of emotional responses produced by dorsal CG stimulation are interrelated.

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81.9 ARCUATE NUCLEUS LESIONS REDUCE OPIATE-MEDIATED STRESS-INDUCED ANALGESIA (SIA) IN RATS AND ENHANCE NONOPIATE-MEDIATED SIA. J. E. Kelsey and L. D. Kimball III*. Dept. Psychology, Bates College, Lewiston, ME 04240.

If the β-endorphin system in the rat's brain is involved in mediating stress-induced analgesia (SIA), then lesions of the arcuate n. of the hypothalamus, which contains the cell bodies of this β-endorphin system, should reduce SIA. In contrast to this expectation, Kelsey and Hoerman (1981) reported at these meetings that lesions of the arcuate n. enhanced the analgesia produced by inescapable shock (1 sec shock every 5 sec for 30 min) when the rats were tested more than 2 weeks following surgery. Indicating that this enhanced SIA represented a time-dependent compensatory change occurring within the damaged brain, we also reported that if the rats were tested within 3-7 days following surgery, the same lesions tended to reduce the SIA.

In this presentation, we report that neither the SIA observed in the control rats nor the enhanced SIA observed in the rats with arcuate n. lesions was blocked by the opiate receptor blocker, naltrexone (6 mg/kg). This finding indicates that this SIA was not produced by the release of opiates as expected, but was produced largely by the release of nonopiate substances. Furthermore, the enhanced SIA observed in the rats with arcuate n. lesions appeared to reflect enhanced activity within a non-opiate system. In contrast to these results, we found that if the mode of inescapable shock delivery was changed such that the SIA produced was blocked by naltrexone, the arcuate n. lesions reduced the SIA as originally expected.

If the ability of naltrexone to block the SIA is used as a criterion for opiate-mediation, these data suggest that arcuate n. lesions damage a β-endorphin system that is important for the production of opiate-mediated analgesia. In response to the loss of this opiate analgesic system, the brain apparently initiates a time-dependent compensatory (or overcompensatory) change in an undamaged nonopiate analgesic system. Hence, if the SIA is produced by the release of opiates, i.e., is opiate-mediated, arcuate n. lesions reduce the SIA. On the other hand, if the SIA is nonopiate-mediated, the compensatory nonopiate system is activated in rats with arcuate n. lesions, producing enhanced SIA.

81.10 HANDLING, ACTH, CORTICOSTERONE AND NALOXONE EFFECTS ON CHICKEN BEHAVIOR. S. Williams* and D. Scampoli* (SPON: L.N. Irwin). Biology Dept., Simmons College, Boston, MA 02115.

Intraventricularly administered ACTH has been associated with excessive grooming or preening behavior in cats, dogs and pigeons. The purpose of the present study was to see if handling, ACTH, $ACTH_{1-24}$, corticosterone and naloxone influence the behavior of domestic chickens, Gallus domesticus. Chickens were fitted with intraventricular cannulae (I.V.C.) using Ketalar and Chloropent anaesthesia and stereotaxic techniques. The behavior of four birds was then observed for 15 min after one of the following procedures: 10 min of handling, 2 µl saline (I.V.C.), 1 I.U. ACTH/2 µl saline (I.V.C.), 8 µg $ACTH_{1-24}$/2 µl saline (I.V.C.), 0.02 mg corticosterone/0.2 ml peanut oil (I.P.) plus handling, and no handling or other treatment. These procedures were repeated randomly five times using a double blind procedure where appropriate. Handling, ACTH, $ACTH_{1-24}$, and corticosterone were associated with significant increases in preening and pecking at $p < .05$ for two-tailed Mann-Whitney U tests when compared to saline-treated controls. Naloxone-treated birds showed significantly less preening and pecking than birds treated with ACTH, $ACTH_{1-24}$ and corticosterone.

81.11 CYCLIZATION OF α-MSH FRAGMENTS MARKEDLY INCREASES BEHAVIORAL POTENCY. R. Wilson#, M.D. Hirsch#, T.K. Sawyer#, V.J. Hruby#, M.E. Hadley# and T.L. O'Donohue (SPON: W. Mink). ETB, NINCDS, NIH, Bethesda, MD 20205 and Depts. of Chem. and Biol., Univ. of Arizona, Tucson, AZ 85721.

Alpha-melanocyte stimulating hormone (α-MSH) is an N-acetyl linear tridecapeptide-amide secreted by opiomelanotropinergic cells of the intermediate lobe of the pituitary gland and by neurons in the central nervous system. Previous structure-function studies have demonstrated that the 4-10 sequence is crucial for mediation of behavioral, melanotropic, steroidogenic, and lipolytic functions (Schwyzer, R. and Eberle, A., Front. Horm. Res., 18, 1977). It has recently been demonstrated that cyclization of α-MSH and α-MSH fragments via a disulfide bridge between cystine substitutions in the 4 and 10 residue positions (half-Cys 4, half-Cys 10) results in a superagonist on melanotropic activity (Knittel, J.J. et al., J. Med. Chem., 26: 125, 1983). Present studies evaluated the behavioral potency of cyclic α-MSH fragments.

Adult, male Sprague-Dawlwy rats (250-350g) received equimolar intracerebroventricular injections of 3nmol peptide/5µl saline. Grooming activity was quantitated over a 55 min. period as described previously (Gispen, W.H. et al., Life Sci., 17: 645, 1975). Excessive grooming behavior has been interpreted to be indicative of an increased state of arousal. The results of this study indicate that the linear α-MSH fragments α-MSH/ACTH4-10, Ac-α-MSH4-10-NH_2, Ac-(Nle4)-α-MSH4-10-NH_2, Ac-(Nle4)-α-MSH4-11-NH_2, Ac-(Nle4)-α-MSH4-12-NH_2, and Ac-(Nle4)-α-MSH4-13-NH_2 are inactive or weak agonists in eliciting grooming. Interestingly, whereas the linear fragments are relatively inactive, cyclization of these peptides to Ac-(half-Cys4, half-Cys10)-α-MSH4-10-NH_2, Ac-(half-Cys4, half-Cys10)-α-MSH4-11-NH_2, Ac-(Half-Cys4, Half-Cys10)-α-MSH4-12-NH_2, Ac-(half-Cys4, half-Cys10)-α-MSH4-13-NH_2 results in peptides that are approximately equipotent to α-MSH in inducing grooming. In addition, Ac-(half-Cys4, half-Cys10)-α-MSH-NH_2 was equipotent to native α-MSH in eliciting grooming. Cyclization of α-MSH fragments may increase potency by decreasing susceptibility to peptidase degradation or by changing the three-dimensional conformation of the peptide.

81.12 BEHAVIORAL EFFECTS OF AN ADRENOCORTICOTROPIC HORMONE FRAGMENT ANALOGUE IN SHAM, NEOCORTICAL, AND HIPPOCAMPAL LESIONED RATS. J. H. Hannigan, Jr., and R. L. Isaacson. Center for Neurobehavioral Sciences and Department of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

This research tested the ability of a potent analogue of the $ACTH_{4-9}$ fragment, ORG 2766 ($[Met\text{-}O_2]^4[D\text{-}Lys]^8[Phe]^9$ $ACTH_{4-9}$), to alter the behavioral effects of brain damage in rats. ORG 2766 (1 µg/rat) or saline was injected (s.c.) daily for 1 week after "sham," neocortical, or hippocampal ablations. Twenty-four hrs. after the last injection, the food-deprived rats were trained on a food search task for 7 consecutive daily sessions. The rats were again tested about 3 mos. later. Hippocampally lesioned rats, and shams treated with saline, easily learned to perform the task. Hippocampals showed specific deficits that suggested an impairment in attentional processes. Prior treatment with ORG 2766 attenuated behavioral changes related to these deficits, even those found 3 mos. later, but did not affect changes in lesion-induced hyperactivity. Rats with neocortical lesions were severely impaired in the food search task and were largely unaffected by ORG 2766 treatment. Shams that received ORG 27666 injections were impaired in all phases of the task. The results suggest that specific behavioral deficits following hippocampal damage in rats, perhaps related to attentional processes, may be attenuated by treatment with ORG 2766. However, these results must be evaluated relative to possible deleterious effects of ORG 2766 on adaptive behavior in nominally normal animals.

OPIATES II

82.1 IPSIVERSIVE TURNING IN RATS AFTER UNILATERAL MORPHINE INJECTION INTO THE VENTRAL TEGMENTAL AREA. M.R. Szewczak* and M.T. Spoerlein. (SPON: T. Jerussi). Rutgers University, P.O. Box 789, Piscataway, N.J. 08854.

Morphine has been reported to cause contraversive turning in rats when injected unilaterally into the substantia nigra (Iwamoto and Way, J. Pharmacol. Exp. Ther. 203: 347, 1977; Kaakola, Acta Pharmacol. et Toxicol. 47: 385, 1980), which was naloxone, haloperidol and pilocarpine sensitive, and atropine and muscimol insensitive.

We are now reporting that morphine causes dose-dependent (12.5-50.0 nMoles) ipsiversive turning when injected unilaterally into the ventral tegmental area (VTA) in the rat. This turning is not blocked by naloxone given systemically (10 mg/Kg, sc) or by simultaneous injection (50 nMoles) with morphine into the VTA. The turning is blocked by haloperidol (0.5 mg/Kg, ip), alpha-methyl-p-tyrosine (250 mg/Kg, ip), phentolamine (2 mg/Kg, ip), clonidine (0.01 mg/Kg, ip) and muscimol (1 mg/Kg, ip). Partial blockade is seen with atropine (10 mg/Kg, ip). while pilocarpine (10 mg/Kg, ip) has no effect. Neither levorphanol (50 nMoles) nor the delta receptor agonist, D-ala, D-leu enkephalin (50 nMoles) cause turning, when injected alone into the VTA; both, however, cause catalepsy.

The kappa receptor agonist, ethylketocyclazocine (10 nMoles), when injected into the VTA, shows ipsiversive turning; this is not blocked by simultaneous injection of naloxone (50 nMoles) into the VTA. The sigma receptor agonists, SKF 10,047 (50 nMoles) and phencyclidine (50 nMoles) cause no turning when injected centrally.

The VTA appears to have receptive sites which may be of the kappa opioid type.

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82.2 CHANGES IN SPINAL AMINE METABOLISM PRODUCED BY INTRATHECAL AND PERIAQUEDUCTAL GRAY INJECTIONS OF MORPHINE. T. G. Reigle and R. M. LoPachin*. Dept. of Pharmacology, University of Georgia, College of Pharmacy, Athens, GA 30602.

This study examined the ability of intrathecal (IT) injections of morphine to alter the concentrations of homovanillic acid (HVA) 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA), major metabolites of dopamine (DA) and serotonin (5-HT), in cervical, thoracic and lumbar spinal segments of male Sprague-Dawley rats. Changes in 3-methoxy-4-hydroxyphenylethylene glycol sulfate (MOPEG-SO_4), the major norepinephrine (NE) metabolite, were measured in the spinal cord after periaqueductal gray (PAG) injections of morphine as well as in the limbic system and cortex following IT morphine injections. Analgesia (tail-flick latency) was 89 ± 1.4% of maximal 15 min. after 5µg IT morphine and this effect was maintained for at least an hour. This dose also produced significant ($p < 0.01$) increases in cervical, thoracic and lumbar HVA and DOPAC which peaked 15 min. after injection and returned to control levels by 60 min. A significant ($p < 0.01$) increase in cervical and lumbar 5-HIAA was also maximal at 15 min. The PAG injection of morphine (3µg, bilateral) produced a significant ($p < 0.05$) increase in spinal MOPEG-SO_4 at 30 min. In addition, IT morphine (5µg) elicited significant ($p < 0.01$) increases in limbic and cortical MOPEG-SO_4 30 min. after injection. The increases in spinal and brain NE metabolism occurred in conjunction with significant analgesic effects and were antagonized by pretreatment (10 min.) with intraperitoneal naloxone (1mg/kg). Thus, interactions between spinal DA, 5-HT and NE systems may be involved in the mediation of opiate analgesia and both spinal and supraspinal mechanisms may contribute regardless of the initial site of opiate receptor activation.

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82.3 CHANGES IN LOCOMOTOR ACTIVITY AND IN THE DISCHARGE RATES OF MIDBRAIN DOPAMINE NEURONS WITH REPEATED ADMINISTRATIONS OF LOW DOSES OF MORPHINE. N.L. Ostrowski, I. Paul*, M. Drnach* and A.R. Caggiula. Psychobiology Program, Psychology Department, University of Pittsburgh, Pittsburgh, PA 15260.

We previously reported (Soc. Neurosci. Abstr., vol. 8, p. 230, 1982) that 10 ug/kg of morphine (IV) increased locomotor activity in awake rats, increased the discharge rates of putative Type A dopamine neurons in the midbrain within the same time period, and, produced a long-lasting suppression of discharge rates in a population of Type B dopamine neurons.

Here, we report that morphine (10 and 200 ug/kg, IV) produces a dose-dependent increase in locomotor activity in rats, and that pre-treatment with 0.1 mg/kg of naloxone antagonizes this increase. Similarly, in electrophysiological tests in chloral hydrate anesthetized male rats, the same doses increased the discharge rates of Type A neurons and suppressed firing rates in Type B cells. While the morphine-produced increase in Type A cell firing rates was antagonized by pre-treatment with 0.1 mg/kg of naloxone, the morphine-produced suppression of Type B neurons was not.

Across four consecutive days of morphine administration, the increases in locomotor activity during the first 10 min after drug delivery remained stable. Over repeated tests, however, morphine-treated rats showed a progressive increase in locomotor activity and stereotyped gnawing throughout the 30 min test sessions.

In electrophysiological experiments, the responses of Type A neurons to IV morphine did not change after 5 days of repeated administrations. However, the morphine-produced suppression of Type B neurons in rats previously exposed to morphine were of shorter durations than in animals receiving morphine for the first time. Specifically, Type B neurons recovered firing rates within 30 min of drug delivery and these neurons demonstrated an oscillatory pattern of discharge.

These data support the idea that dopamine subsystems in the substantia nigra pars compacta show differential responses to acute and repeated administrations of morphine.

82.4 MU AND KAPPA OPIOID MECHANISMS IN RAT AND TOAD. D. Aleman*, K. Carr, M. Holland* and E. Simon (SPON: J. Miller). Depts. of Psychiatry and Pharmacology, New York Univ. Med. Ctr., NY 10016.

We have recently shown that the predominant opiate receptor subtype in toad brain is the benzomorphan-preferring (κ/σ) type (Simon et. al., Life Sci. 31:1367, 1982). By contrast, rat brain is deficient in this type but has a high proportion of the morphine-preferring (μ) type. To examine the relation between these biochemical findings and the behavioral pharmacology of opioids we compared analgesic effects of a benzomorphan ($\pm$)ethylketocyclazocine (EKC), and morphine sulfate (MS) in rat and toad.

Threshold intensity of footshock (200 msec) to elicit a locomotor response was determined using the method of limits. Animals were then injected (s.c.) with EKC, MS, or vehicle. Thresholds were retested 30 min later. In rat, EKC and MS elevated threshold in a dose-related manner, though EKC was 6 times as potent. Naloxone was used to evaluate whether EKC and MS analgesia are mediated by different receptor types because, in vitro, agonists are displaced from the μ receptor by 4-8 fold lower concentrations than from the κ receptor. Separate groups of rats received EKC (1.5 mg/kg) or an equi-analgesic dose of MS (10.0 mg/kg) preceded by saline or one of four doses of naloxone (0.05-0.5 mg/kg). EKC and MS analgesia showed similar reductions as a function of naloxone dose, suggesting that EKC analgesia results from activity at the μ receptor. A unique behavioral effect of EKC, though, was a flaccid paralysis at doses greater than 2.0 mg/kg.

In toad, doses of EKC up to 50.0 mg/kg had no effect on response to footshock. Higher doses produced a flaccid paralysis as was observed in rats. MS in the toad elevated threshold with no signs of motor impairment, but doses of 100.0-200.0 mg/kg were required. MS analgesia in toad is blocked by pre-treatment with naloxone (5.0 mg/kg) and is therefore receptor-mediated.

The findings that EKC and MS analgesia are equi-sensitive to naloxone antagonism in rat, and that EKC at sub-paralytic doses fails to produce analgesia in toad while MS does, suggests that in both animals the locomotor response to footshock is modified by opioid activity at the μ receptor.

The observation that MS is 20 times less potent (analgesia) and EKC 30 times less potent (flaccidity) in toad than rat does not seem due to differential distribution or blood-brain barrier permeability to these compounds; rats and toads were injected (s.c.) with EKC (2.0 mg/kg) containing 30 μCi ^{3}H-EKC/kg and sacrificed at 30 min. Brains were removed and solubilized. In both animals, approximately 500 cpm/100 mg tissue were recovered, indicating that uptake into brain is similar. The possibility that these compounds are metabolized differently or have different levels of specific binding in rat and toad brain will be discussed. (Supported by NYS HRC Award 12-073 (K.C.) and NIDA grant DA-00017 (E.S.).

82.5 OPIATE SUBSTANCE RELEASED BY A WALKER-256 (W-256) DERIVED TUMOR. G.K.W. Yim, K.M. Johnson,* M.T. Lowy,* and P.V. Malven, Dept. of Pharmacology & Toxicology, Sch. of Pharmacy and Pharmacal Sci. and Dept. of Animal Sci., Purdue University, W. Lafayette, IN 47907.

The similarity in the feeding deficits of W-256 tumor bearing rats (TBR) and of rats treated with opiate antagonists or dexamethasone (to deplete pituitary β-endorphin) suggested that tumor-induced anorexia might result from depleted endogenous opioids(EO) (Neural Basis of Feeding and Reward, B. Hoebl and D. Novin, eds., p. 485, 1982). Since a depletion of EO might be expected to cause hyperalgesia, pain sensitivity (tail-flick) was tested in Sprague Dawley rats bearing Walker 256 carcinosarcoma which had been maintained in this laboratory for two years. Rather than hyperalgesia, we observed significant analgesia in TBR compared to controls. The degree of analgesia increased with tumor size, and was reduced by 1 mg/kg naloxone. Acid-ethanol extracts of the tumor, when injected i.c.v. in mice, also elevated tail-flick responses and induced Straub tail responses. Both responses were blocked by naloxone. Immunoreactive β-endorphin was not detected in tumor extracts nor was it elevated in plasma of anlagetic TBR. When new W-256 tumors were acquired from Mason Res. Inst., the significant tail-flick analgesia was not observed. In summary, these results document the secretion by a secondary W-256 tumor line of a potent EO capable of causing analgesia. (Supported in part by grants from the Amer. Cancer Soc., IN #194, and the Elsa Pardee Fdn.)

82.6 RX-336M, AN EXPERIMENTAL CODEIONE, HAS OPIATE ANTAGONISTIC EFFECTS IN TESTOSTERONE-TREATED FEMALE RATS. C-A. Hardy and R.L. Isaacson. Dep't of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, N.Y. 13901.

RX-336M, an experimental codeione with morphine-like effects has been shown to produce sex-related behavioral effects in periadolescent rats (age: 40 days of age). In general, the drug effects are greater in young male than in young female rats. The present study was designed to asses the possible contribution that testosterone may have on the opiate-related effects of RX-336M. Female Long-Evans rats, 33 days of age at the start of the study, received daily subcutaneous injections of either 1mg testosterone benzoate in .2ml oil or .2ml of the oil vehicle for 8 days. At 40 days of age each animal received either an IP injection of 6 mg/kg/ml RX-336M or the volume equivalent of the saline vehicle. Immediately after injection each animal was placed in a plexiglass observation chamber and during the next 40 min behaviors were recorded every 15 sec. The behaviors scored included locomotion, rearing, grooming, and scratching. "Wet-dog shakes" were scored independent of the 15 sec observation time interval. The following day the testing procedure was repeated with each animal receiving the opposite treatment (i.e. RX-336M or saline) from its previous test day. The RX-336M had no significant behavioral effects in the oil-treated female rats. However, in the testosterone-treated females RX-336M significantly increased grooming and "wet-dog shakes". Additionally, following RX-336M there was a tendency for decreased locomotion and increased scratching in testosterone-treated females. In conclusion, the present findings suggest a role of the sexual hormones in the opiate-like effects of RX-336M.

82.7 IN VIVO AUTORADIOGRAPHY: VISUALIZATION OF CHANGES IN OPIATE RECEPTOR OCCUPANCY DUE TO BEHAVIORAL MANIPULATIONS. T.F. Seeger, C.B. Pert and A. Pert (sponsored by R.U. Esposito). Biological Psychiatry Branch and Clinical Neuroscience Branch, NIMH, Bethesda, MD 20205

A method of in vivo autoradiography was utilized which allows the indirect visualization of functional opiate peptide release, based on the assumption that prior receptor occupation will exclude the binding of an exogenously applied, tritium-labelled, opiate ligand. Coupled with tritium-sensitive film autoradiography, it allows the mapping of relative levels of behavior-specific receptor occupancy throughout the whole brain.

We tested two types of stress manipulations which are known to release endorphins; forced swims in cold water (4°C for 3.5 min.), and prolonged intermittent footshock (1 ma. for 20 min., 1 sec. on, 5 sec. off). Both of these have been shown to increase nociceptive thresholds in a naloxone-reversible manner. Following the stress, the high affinity opiate antagonist, 3H-diprenorphine was injected via an indwelling jugular catheter (50 µCi/kg, 0.002 mg/kg). After twenty minutes (to allow washout from non-specific sites), the rats were decapitated and the brains frozen intact for slicing. Unstressed control rats were prepared identically and matched to stressed rats on the basis of cerebellar and liver (non-specific) binding levels. All comparisons made were between anatomically congruent sections from these matched pairs, exposed on the same sheet of film.

In the cold water swim group, this severe stress caused a greatly decreased binding to most caudal subcortical structures, while cortex, hippocampus, and more rostral structures were largely unchanged. Greatest decreases (up to 60%) were seen in brain areas such as periaqueductal gray, reticular formation, and midline-intralaminar nuclei of thalamus, reflecting opiate peptide release in areas which receive and modulate incoming pain information. Lesser decreases in binding were seen in hypothalamus and limbic areas such as amygdala, the bed nucleus, and preoptic area. Motor areas of thalamus and the basal ganglia showed still smaller effects. In the footshock group, decreases were similar but less extreme. Periaqueductal gray, reticular formation, and the midline thalamic areas showed decreases of up to 30%, while limbic changes were slightly smaller. No changes unique to the footshock treatment were seen, suggesting similar opiatergic responses to painful stress of differing modalities.

This in vivo autoradiographic technique holds great promise, both for the study of other behaviorally-induced changes in patterns of opiate release, and for adaptation to other neurotransmitter systems.

82.8 EFFECTS OF GLUTAMATE AND/OR BIPIPERIDYL MUSTARD TREATMENT ON HYPOTHALAMIC PROOPIOMELANOCORTIN NEURONS, FOOD INTAKE, OBESITY INDEX AND BLOOD INSULIN. A.C.Scallet and J.W.Olney, Washington Univ Sch Med, Dept Pathology & Psychiatry, St. Louis, MO. 63110

Glutamate (Glu) produces neurotoxic lesions of areas immediately adjacent to circumventricular organs, particularly the arcuate hypothalamus (AH). Bipiperidyl mustard (BPM) produces a lesion straddling the border between AH and the ventromedial hypothalamus (VMH). Rats treated neonatally with subcutaneous Glu or saline, received a subsequent intraperitoneal injection of BPM or vehicle as weanlings. Groups of such rats consisting of Glu alone, BPM alone, Glu/BPM, or controls were observed developmentally for linear growth, weight gain, food intake and plasma insulin. At 32 weeks of age some rats received intraventricular injections of colchicine and were sacrificed 48 hr later for immunohistochemical evaluation of the AH for loss of proopiomelanocortin (POMC) neurons employing an antibody to α-melanocyte stimulating hormone. There was a 77%, 70% and 92% loss of POMC neurons from the rostral 3/4 of AH in rats treated with Glu, BPM and Glu/BPM, respectively. The Glu/BPM lesion differed from the Glu lesion in deleting a larger number of POMC neurons from the lateral margins of AH. No treatment appreciably reduced the number of POMC neurons in the posterior 1/4 of the nucleus.

Glu but not BPM produced elevated fasting levels of plasma insulin ($p<.01$) and Glu but not BPM rats became obese as defined by elevated Lee Index scores, although both groups had lowered food intakes consistent with their shortened body statures. A subgroup of the Glu/BPM-treated rats became extremely obese and had elevated food intakes despite insulin levels no greater than for Glu alone rats. Thus hyperinsulinemia correlates with the normophagic obesity observed in Glu-treated rats but not with the more extreme hyperphagic obesity in some Glu/BPM rats which presumably stems from increased loss of neurons at the lateral border of AH. Supported in part by NIH Grants ES07066, DA00259 and RSA MH38894 (JWO).

82.9 Methadone Effects on Dopamine and Serotonin Systems of C57BL/6J and DBA/2J Mice: Relationship to Locomotor Activity. George E. Platt* and Lawrence D. Middaugh. Depts. of Biochemistry and of Psychiatry and Behavioral Sciences, Med. Univ. So. Car., Charleston, S.C., 29425.

Narcotics either elevate or reduce locomotor activity of C57BL/6J (C57) mice depending on dose and post-injection time, however, only reduce that of DBA/2J (DBA) mice. Limiting the availability of catecholamine neurotransmitter eliminates the narcotic induced activity elevation. Since morphine reportedly increases dopamine metabolism in C57 mice while having no effect or decreasing its metabolism in DBA mice, strain differences in dopaminergic systems have been hypothesized to account for the different effects of the drug on locomotor activity. Support for this hypothesis is currently restricted to one time period after morphine injection. To test the generality of this hypothesis, we determined the effect of methadone in these two strains on dopamine metabolism at intervals after injection when locomotor activity was either elevated, reduced, or no different from controls. Since serotoninergic systems are involved in narcotic induced activity reductions in rats, we also determined the effect of methadone on metabolism of this transmitter.

Activity was tested for five minutes beginning 15, 45, or 180 min. after subcutaneous injections of saline or methadone hydrochloride (7.0 or 21.0 mg/Kg). Immediately following this test animals were decapitated and their brains removed and dissected for determination of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in n. accumbens septi and neostriatum. Locomotor activity of C57 mice was elevated at early time periods by both doses, however, at later time periods was inversely related to dose. Activity of DBA mice was also inversely related to dose, however, was never elevated above control levels. Methadone elevated DOPAC concentration in the n. accumbens septi and the neostriatum of both mouse strains. The drug, however, decreased 5-HIAA concentrations in both structures of C57 mice and in neostriatum of DBA mice. Since similar changes in monoamine metabolites occurred under conditions where locomotor activity was increased, decreased, or not effected, the relationship of drug induced changes in locomotor activity and monoamine metabolism appears to be quite restricted and may be only spurious.

82.10 CHANGES IN BRAIN GLUCOSE UTILIZATION AFTER AN INJECTION OF MORPHINE OR EXPOSURE TO IONIZING RADIATION. G. Andrew Mickley, Karen E. Stevens, Gerald A. White* and Gregory L. Gibbs*. Department of Behavioral Sciences and Leadership, USAF Academy, CO 80840 and Penrose Cancer Hospital, Colorado Springs, CO 80903.

When C57BL/6J mice are exposed to ionizing radiation they exhibit a naloxone-reversible locomotor hyperactivity which is similar to that observed after an injection of morphine (Mickley et. al. Soc. Neurosci. Abstr., 7:166,1981). This finding, along with others, (Mickley et.al. Rad. Res. 93:381-387,1983) suggests that endogenous opiates may be involved in radiogenic behavioral change. The present study used the [^{14}C] Deoxyglucose (^{14}C-DG) method (Sokoloff et.al. J. Neurochem., 28:897-916,1977) to analyze glucose utilization in the brains of both morphine-injected and irradiated mice.

Male C57BL/6J mice received an i.p. injection of 30mg/kg morphine sulfate or saline followed, 15 minutes later, by an i.p. injection of 15µci of ^{14}C-DG. These subjects moved freely in their home cages for 45 minutes and were then sacrificed. Other mice were treated as just described but, after the morphine or saline treatment, they received an injection of gallamine(14mg/kg, i.p.) which inhibited their locomotion. Additional ^{14}C-DG-injected subjects were exposed to 1500 rads ^{60}Co or sham irradiated. After irradiation/sham half of these mice were permitted to move freely in their home cages while activity of the remaining animals was inhibited by an injection of gallamine. Brains were removed and treated as described by Sokaloff et.al. The density of brain radiographs and standards were analyzed photometrically.

Morphine enhanced glucose utilization in many brain areas. As compared to controls, the radiographs of morphine-injected brains reflected significantly higher ($p<.05$, ANOVA) energy metabolism in the Nucleus Accumbens, Lateral Septum, Medial Caudate and several sites in the cerebral cortex. These increases were not dependent on the behavior of the mouse since akinetic (gallamine-injected) subjects showed similar (albeit reduced) effects. These data agree with others which implicate portions of the basil ganglia and limbic system as locii of morphines' central actions (Olivero. Br. Res., 83:135-141,1975; Teitelbaum et.al. J. Comp. Physiol. Psychol. 93:745-751,1979;Ebel et.al. Neuropharm.19:423-427,1980).

Although the effect was less dramatic, exposure to ionizing radiation also produced significant changes in the Nucleus Accumbeus, Lateral Septum and Medial Caudate. However, irradiation decreased ($p<.05$) glucose utilization in these areas. The data suggest that although both morphine and ionizing radiation may effect similar areas of the brain morphine activates these areas while ^{60}Co inhibits energy metabolism.

This research was supported by Defense Nuclear Agency.

82.11 ANATOMICAL, PHARMACOLOGICAL AND PHENOMENOLOGICAL CHARACTERISTICS OF MORPHINE-INDUCED CIRCLING ELICITED FROM THE VENTRAL MESENCEPHALON. L.J. Holmes*, M.A. Bozarth and R.A Wise. Center for Research in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Quebec.

Crystalline morphine applied unilaterally throughout the ventral mesencephalon induced circling directed contralateral to the side of injection. Circling was seen after morphine application to either the ventral tegmental area or the zona compacta of the substantia nigra, however latency was shorter and rates higher following ventral tegmental stimulation. When morphine-induced circlers were placed in an open field they consistently followed the perimeter of the enclosure in a contralateral direction; the radius of the circle was determined by the size of the enclosure. Morphine induced little postural asymmetry and induced forward locomotion in all four limbs. The opiate receptor antagonists naltrexone and naloxone blocked morphine-induced circling whether given before or during the sessions. Pimozide pretreatment also blocked morphine-induced circling. Thus circling following unilateral morphine application was dependent on both an opiate receptor mechanism and dopaminergic circuitry. In contrast to morphine-induced circling, unilateral application of muscimol at the same sites resulted in more circumscribed contralateral circling that was resistant to dopamine receptor blockade. Muscimol-induced circling was qualitatively different from morphine-induced circling. Rather than travelling over the entire test area, muscimol-treated animals maintained a relatively fixed position within either the small test box or the large open field, circling with a diameter of travel on the order of 12 cm. In this case, the hind limb contralateral to the side of injection was engaged in backward stepping movements while the hind limb ipsilateral to the side of injection served as a pivot. A strong postural asymmetry accompanied muscimol-induced circling.

These studies demonstrate that two midbrain mechanisms can mediate circling. Morphine-induced circling results from dopaminergic activation and results in behavior that is compatible with forward locomotion. In contrast, muscimol-induced circling activates a mechanism that is independent of, or efferent to, the dopamine pathways and appears incompatible with simple forward locomotion. Muscimol-induced circling, unlike that induced by morphine, appears relatively independent of environmental objects and events.

82.12 AN ENKEPHALIN SYSTEM IN THE CHICK RETINA:
1. PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES; ENKEPHALIN CONTENT AND IMMUNOCYTOCHEMICAL LOCALIZATION. C. Watt*, D. Tavella*, Y.Y.T. Su, Y.W. Peng* and D.M.K. Lam. Clayton Foundation for Research, Houston and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

We have produced and affinity-purified monoclonal antibodies against enkephalins (Tyr-Gly-Gly-Phe-Met and Tyr-Gly-Gly-Phe-Leu) using ovalalbumin-conjugated enkephalins as antigens. These antibodies are specific for enkephalins, binding only slightly with Gly-Gly-Phe-Met and not at all with Tyr-Gly-Gly-Phe-Met-Arg (dynorphin), Tyr-Gly-Gly-Phe-Met-Arg-Phe or Tyr-Gly-Gly-Phe-Met-Arg-Gly-Leu. These results indicate the absolute requirement of a free carboxyl terminal in position 5 for binding of our monoclonal antibodies. We have used these as well as polyclonal antibodies for biochemical and immunocytochemical studies of putative enkephalinergic pathways in the chick retina.

Immunochemical assays demonstrate that the concentration of enkephalin-like immunoreactivity is about 0.2 μM in this retina. At the light microscope level, enkephalin-immunoreactive cells are localized to a subpopulation of amacrine cells with somas in the proximal half of the inner nuclear layer and processes ramifying in sublaminas 1, 3-5 of the inner plexiform layer. This result agrees well with the findings of Brecha and others. Additionally, all our monoclonal antibodies against enkephalins stain the same amacrine cells as the polyclonal antibodies.

At the ultrastructural level, the processes of enkephalin-immunoreactive amacrine cells are found to receive input from bipolar and other amacrine cells. Immunolabeled varicosities form conventional synaptic contacts onto other vesicle-filled profiles tentatively identified as amacrine cell varicosities. Bipolar cell terminals are not found to receive synaptic input from stained varicosities of enkephalin-immunoreactive amacrine cells. Moreover, all of the above synaptic relationships were identified in each of sublamina 1, 3-5 of the inner plexiform layer. Lastly, large enkephalin-immunoreactive varicosities often contain a number of large dense core vesicles in addition to small agranular vesicles.

We thank Professor W.M. Cowan of the Salk Institute for his valuable advice and collaboration in this project.

82.13 AN ENKEPHALIN SYSTEM IN THE CHICK RETINA:
2. BIOSYNTHESIS, RELEASE AND PHYSIOLOGICAL STUDIES. Y.Y.T. Su and D.M.K. Lam. Clayton Foundation for Research, Houston and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

Enkephalin-like immunoreactivity has been localized immunocytochemically to subpopulations of amacrine cells in both mammalian and nonmammalian retinas (Brecha et al. 1979; Stell et al. 1981; Altschuler et al. 1982). However, the localization of enkephalin-like immunoreactivity alone is clearly not sufficient to demonstrate that enkephalins are present in these neurons. In this report we present direct evidence that in the chick retina, Met^5-enkephalin is synthesized and released, and that exogenously applied enkephalins influence the release of certain retinal transmitters.

Isolated chick retinas were incubated in 0.2 ml of oxygenated (95% O_2 and 5% CO_2) Ringer's solution containing 40μCi of ^{3}H-methionine and the peptidase inhibitor, trasylol, for 30 min. The tissue was rinsed three times in large volume of Ringer's solution containing trasylol and incubated in the same unlabeled solution for at least another hour. The products synthesized in the tissue were extracted in acetic acid and assayed by high performance liquid chromatography (HPLC) and immunochemical assays. In addition to Met^5-enkephalin (35%), Gly-Gly-Phe-Met (12%) and Arg^1-Met^6-enkephalin (3%) were also detected.

High external K^+ induced ^{3}H-labeled compounds release from the labeled tissue was observed. On HPLC, the eluates contained all three Met^5-enkephalin related compounds with Met^5-enkephalin as the major component (95%). In the presence of 6 mM Co^{+2}, K^+-stimulated release of Met^5-enkephalin was significantly inhibited, indicating that the release may be Ca^{+2} dependent.

The relationship between enkephalins and other neurotransmitter systems were also studied. Our results show that exogenous enkephalins inhibit the K^+-stimulated release of GABA and dopamine from the chick retina and may therefore function as a presynaptic modulator to both GABAergic and dopaminergic amacrine cells.

83.1 MOTOR EVOKED POTENTIALS VIA TRANSCRANIAL CORTEX STIMULATION IN CAT AND MAN. W. J. Levy, M. McCaffrey*, D. H. York. Neurosurgery Div., Univ. of Mo. Sch.of Med., Columbia, MO. 65212.

Evoked potentials are increasingly important in testing of the central nervous system for injury. However, until now they have tested only sensory systems (visual, auditory, somatosensory). We have developed and reported a motor evoked potential based on one of two stimulation methods. During spinal cord surgery the cord is stimulated directly by placing an electrode directly on it between the intermediolateral sulcus and the dentate ligament. This stimulates the underlying motor tracts and can produce distal limb movement. The second method is non-invasive. A 3x5 cm plate is placed on the scalp over the motor cortex. Stimulation between this plate and one held against the hard palate will direct a mild current (3-15 m A, 500-1000 sec) through the cortex and produce an evoked potential in the spinal cord. This can be detected by skin recording or, more effectively, by deep or dural electrodes. This signal has the same properties as that described in the experimental literature on stimulation of the exposed motor cortex and can produce contralateral limb movement. In cats we have found with lesioning and depth electrode studies that the signal is primarily in the pyramidal-rubrospinal area with some component in the anterior cord near small motor tracts there. This work has extended to man where we found a similar wave. It correlates in clinical conditions better with motor function than the somatosensory evoked potential. On 60 cases to date no adverse effects have occurred and patients tolerance is good. We are continuing to evaluate his test. It offers the possibility of testing the motor pathways of cortex, brainstem and spinal cord for injury. The production of movement (detected by EMG or peripheral nerve recording) could assess functional capabilities as well as electrical continuity. The recording of cranial nerve signals could aid in localizing brainstem lesions. Further more this test could aid development of investigative and therapeutic efforts for spinal cord injury. We report new experimental data on the basis of this test and supporting clinical observations.

83.2 THE VENTRAL PART OF THE CAUDAL BRAINSTEM TEGMENTUM INCLUDING THE NUCLEUS RAPHE MAGNUS AND PALLIDUS IN THE CAT: EFFERENTS AND AFFERENTS. AN AUTORADIOGRAPHIC TRACING STUDY. Gert Holstege, Linda Meiners and Kiong Tan, Dept. of Anatomy, Erasmus University Rotterdam, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

For studying the efferent connections several ^{3}H-leucine injections (+ 0,5 µL containing 50 µCi) were made in the ventral part of the medial tegmentum of the caudal brainstem. For studying the afferent connections many other injections were made in parts of the central nervous system.

I. Efferent connections (rostralwards).

The nucleus raphe magnus (NRM) and adjoining tegmentum at levels of caudal pons and upper medulla distribute fibers to the PAG, mesencephalic tegmentum, zone incerta, the intralaminar and paraventricular thalamic nuclei, the posterior, paraventricular, lateral and anterior parts of the hypothalamus and the preoptic area. Yet, an injection in the nucleus raphe pallidus (NRP) resulted in only a very restricted projection to the PAG.

II. Efferent connections (caudalwards).

Injections in the caudal pontine medial tegmentum including the rostral NRM distributes fibers to the medullary lateral and medial tegmentum, the solitary and dorsal vagal nuclei and all parts of the spinal dorsal horn and intermediate zone. After injections in the upper medullary medial tegmentum including the caudal NRM the same distribution pattern in the caudal brainstem areas could be observed but in addition labeled fibers were present in the marginal layer of the caudal trigeminal nucleus. In the spinal cord labeled fibers were distributed to laminae I and II of the dorsal horn, the intermediate zone and the intermediolateral cell column at the thoracolumbar and sacral levels. In contrast, after injections in the NRP and in the medial tegmentum at the level of the inferior olive, labeled fibers were distributed to the brainstem motor nuclei V, VII and XII and to the intermediate zone and autonomic and somatic motoneuronal areas throughout the spinal cord.

III. Afferent connections.

The NRP received afferents from the dorsomedial hypothalamic area, the tegmentum just caudal to the red nucleus, the ventral PAG and the medullary lateral tegmentum at the level of the rostral pole of the hypoglossal nucleus.

The NRM and the adjoining caudal pontine and medullary tegmentum received afferents from these same areas but also from the lateral hypothalamus, the dorsal and lateral PAG, the nucleus subcoeruleus and the lateral pontine tegmentum. The lateral tegmentum of caudal pons and medulla did not distribute fibers to this area.

These results suggest that afferents to the ventral part of the medullary and caudal pontine medial tegmentum are mainly derived from limbic brain structures.

83.3 FACTORS AFFECTING ATONIA PRODUCED BY ELECTRICAL STIMULATION OF THE MEDIAL MEDULLA IN CATS. W.J. Wilson, J.M. Siegel, K.S. Tomaszewski† and R. Nienhuis*. Sepulveda VAMC, Sepulveda, CA 91343, and Dept. of Psychiatry, School of Medicine, University of California, Los Angeles, CA 90024.

Magoun first identified a large medial medullary region which when stimulated produced nonreciprocal inhibition of muscle tone. Later studies by Sprague, Gernandt, and others disputed this conclusion, contending that the predominant effect of medullary stimulation was excitation, or reciprocal inhibition and excitation of muscle groups. The nature of the medullary inhibition mechanism has generated increasing interest because of its presumed role in the atonia of REM sleep and narcolepsy. In the present series of experiments, we have seen both cats in which medullary stimulation generates bilateral inhibition of muscle tone, and cats in which the stimulation generates only excitation or reciprocal changes in muscle tone. We find that transection level is the variable most strongly correlated with the amount of inhibition.

Sixteen adult mongrel cats of both sexes received brainstem transections at varying levels from P 6.5 to A 8. After aspiration of the medial cerebellum, the medial medulla was stimulated electrically via a stereotaxically mounted electrode passed through at least 6 tracks in each cat, and the effects of the stimulation on the splenius muscles were noted. The proportion of sites yielding bilateral inhibition of muscle tone varied across cats, ranging from 0 to .78. Some cats showed small amounts of bilateral inhibition even after transections at the lowest levels. The best predictor of the number of inhibitory sites was the transection level: $r = .58$, $p<.05$. Of various physiological variables recorded, none was correlated with the amount of inhibition observed: heart rate, $r = -.01$; respiration rate, $r = .23$; percent expired CO_2, $r = -.01$; blood pressure($n=4$), $r = -.40$.

Although there is a large amount of variability, the data suggest that a region near AP 0 or A 1 contributes importantly to medullary inhibition. 7 of 8 cats with transections caudal to AP 0 showed atonia on 50% or less of the tracks tested, while all 8 cats transected rostral to A 1 showed inhibition on 50% or more of the tracks tested.

Rostral brainstem mechanisms could contribute to medullary atonia in a number of ways. Ascending pathways from the medulla to the pons and midbrain might be involved in a positive feedback loop the effect of which is the recruitment of medullary inhibitory neurons. Alternatively, descending projections from the rostral brainstem to medullary or spinal regions might gate interneuronal activity critical to the production of atonia.

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83.4 THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS PARS COMPACTA IN THE RAT: ORGANIZATION, EFFERENT PROJECTIONS AND CHOLINERGIC ASPECTS. T. Sugimoto and T. Hattori. Dept. Anat., Fac. Med., Univ. Toronto, Med. Sci. Bldg., Toronto, Ontario M5S 1A8 Canada.

The cellular organization and efferent projections of nucleus tegmenti pedunculopontinus pars compacta (TPC) were examined in the rat. From measurements of Nissl-stained TPC sections, three neuronal populations (large, medium and small neuron groups) were seen in the TPC. Although large neurons were the least encountered population, their volume occupancy rate apparently contributed to compact appearance of the nucleus. 3H-leucine injections into the TPC produced transported label in dorsally and ventrally coursing ascending fibers. The dorsally coursing fibers terminated in the centrolateral nucleus (CL) and centre median-parafascicular complex (CM-Pf) of the thalamus. The ventrally coursing fibers formed apparent terminal fields in the substantia nigra pars compacta (SNc) and subthalamic nucleus (STN), and less significantly in the ventral tegmental area, lateral hypothalamus and zona incerta. Much less anterograde labeling was seen in the equivalent terminal sites contralateral to the injection. The contralateral thalamus was innervated by crossed TPC fibers through the posterior commissure and thalamic midline nuclei. The ventrally coursing TPC fibers crossed through the supraoptic commissure of Meinert and then descended to reach the contralateral basal ganglia. Terminal sites of TPC axons in the SNc, STN, CL and CM-Pf were examined by EM autoradiography in rats injected with 3H-leucine in the TPC and later injected with HRP in the ipsilateral striopallidum. Statistical data showed preferential radiolabeling of asymmetrical terminals in the CL, CM-Pf and STN. Termination in the SNc was not supported by this analysis. In the CL and CM-Pf, asymmetrical radiolabeled terminals making synaptic contact with HRP-labeled dendrites verified direct TPC inputs to the striatal projection neurons. 3H-choline injections in the thalamus and STN produced retrograde perikaryal labeling of large TPC neurons; these neurons remained unlabeled after 3H-choline injections in the SN. The result strongly suggests the cholinergic nature of TPC-thalamic and TPC-STN projections. Large TPC neurons are responsible for this cholinergic innervation and probably provide the axon terminals of asymmetrical type in these target nuclei. (Supported by the Medical Research Council of Canada.)

83.5 **BENEFICIAL EFFECT OF MEGADOSE METHYLPREDNISOLONE ON POST-INJURY SPINAL CORD ISCHEMIA.** D.L.Wolf and E.D.Hall, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

Previous work demonstrated that a single large 30 mg/kg i.v. dose of methylprednisolone (MP) sodium succinate (Solu-Medrol^{R}), administered 30 min after injury, decreased free radical-induced lipid peroxidation and increased (Na^{+}+K^{+})-ATPase activity in the acutely contused cat spinal cord (Hall and Braughler, J. Neurosurg. 57:247, 1982). The present study has investigated the effects of MP given 30 min after injury on local spinal cord blood flow (SCBF) and somatosensory evoked potential (SEP) conduction.

A 500 gm-cm (50 gm dropped 10 cm) contusion was applied to the exposed L3 spinal segment (dura intact) of Na pentobarbital anesthetized cats. At various times before and after injury, SEP conduction was monitored and SCBF determinations were made in the dorsolateral funiculus using the hydrogen clearance method. Blood flow values (ml/100 g/min) were derived from the exponential slope of the initial 10 min of the hydrogen washout curve. Four animals received a 30 mg/kg i.v. bolus of MP at 30 min after spinal contusion while another 4 cats received an equivalent vehicle injection.

The mean pre-injury SCBF values for all animals was 12.33±1.04 (S.E.) ml/100 g/min. Following injury, SCBF within the injured segment was reduced approximately 40% in vehicle-treated cats by 1 hr and remained at that level over the subsequent 4 hr (table). In contrast, MP administration at 30 min significantly prevented the fall in SCBF ($p<0.001$ by ANOVA, F=103, df=1,6). Nevertheless, SEP conduction, which was completely lost upon injury, never returned in either group over the ensuing 4.5 hr. Administration of the MP dose at 4.5 hr in vehicle-treated cats did not affect the depressed SCBF over the subsequent 30 min.

Mean (±S.E.) SCBF in White Matter (ml/100g/min)

	Pre-Injury	Hours After Injury 0.5	1	2	3	4
V	11.57	7.08	6.15	6.25	6.78	6.95
	(2.60)	(1.33)	(0.83)	(0.88)	(0.83)	(0.81)
MP	13.09	11.68	12.75	12.90	13.80	13.55
	(1.71)	(1.85)	(1.29)	(2.20)	(2.32)	(2.65)

These results confirm the work of others (Young et al., J. Neurosurg. 57:667, 1982) in demonstrating the efficacy of the 30 mg/kg MP dose, if given early, to prevent post-traumatic spinal cord ischemia. Maintenance of SCBF may be important in attenuating the secondary hypoxia-related degenerative changes which occur following spinal cord injury.

83.6 ANATOMICAL EVIDENCE OF A POSSIBLE GLYCINERGIC RETICULOSPINAL PATHWAY, H. Nauta and D. A. Carter*. Playfair Neuroscience Unit, Univ. of Toronto, Toronto, Ontario, Canada and Div. of Neurosurgery, Univ. of Texas Medical Branch, Galveston, Texas 77550.

An autoradiographic study was carried out in rat comparing the retrograde cell labeling in the brain resulting from in vivo spinal cord injections of radiolabeled glycine and three other putative transmitter substances.

Following in vivo injections of ^{3}H-glycine into the lower cervical spinal cord, retrogradely labeled cells were consistently observed in the medullary gigantocellular reticular formation (GCRF). When the spinal injection locus was predominantly unilateral the labeled neurons were found predominantly contralateral in the GCRF. Injections of ^{3}H-glycine into the lumbar cord enlargement failed to produce such retrograde cell labeling. No retrogradely labeled cells were observed in the brains of rats receiving spinal injections of either ^{3}H-GABA or ^{3}H-D-aspartate. However, following injections of ^{3}H-serotonin, almost universal retrograde labeling of spinal cord afferents resulted.

Bearing in mind the many reservations to be discussed, the findings may provide anatomical evidence of a glycinergic reticulospinal pathway previously postulated on physiologic and pharmacologic grounds.

83.7 THE TERMINATIONS OF CORTICOSPINAL TRACT AXONS IN THE MACAQUE MONKEY. Diane D. Ralston*, Henry J. Ralston III and Robert E.W. Fyffe* Dept. of Anatomy, University of California, School of Medicine, San Francisco, CA 94143 and Depts. of Anatomy and Physiology, University of North Carolina, Chapel Hill, NC 27514

The corticospinal tract (CST) projections of the motor and somatosensory cortices have been examined in monkeys using orthograde transport methods, or intraaxonal staining of single, physiologically characterized axons.

Following injections of ^{3}H-leucine or horseradish peroxidase conjugated to wheat germ agglutinin (HRP-WGA) into either the sensory or motor cortex, terminal fields could be identified with the dominant projection to the cervical enlargement. There are more labeled large diameter axons from motor than from sensory cortex. Motor cortex projects widely to the spinal gray, from laminae V through IX, the heaviest projections being to the mid regions of laminae VI and VII. Some axons cross the midline to reach contralateral VI, VII and VIII. Sensory cortex has its chief projections to the dorsal horn, with significant numbers of terminal fibers to II (substantia gelatinosa), and a few fibers as dorsally as lamina I. Ventrally, the sensory cortex projections are dense in laminae III-VI, with some into dorsal VII.

Electron microscopic autoradiography reveals a heterogenous synaptic population labeled following cortical injection. CST axons make axodendritic contacts upon dendrites of all sizes. No axosomatic or axoaxonal contacts involving CST terminals have been found. The CST synaptic profiles usually contain rounded vesicles, but some contain pleomorphic vesicles, suggesting different types of transmitters being utilized. The terminals vary in size, and some in the dorsal horn make multiple contacts with postsynaptic dendrites, thus resembling the "C" type terminals of dorsal root afferents.

Individual CST axons were impaled with micropipettes in the lateral column white matter at lumbar levels. Bipolar stimulating electrodes were placed in either the precentral or postcentral cortex. Spinal axons which followed 100 Hz stimulus trains with fixed latency were judged to be CST axons. Intraaxonal labeling with HRP reveals that single CST axons, arising from sensory or motor cortex, may arborize over several laminae, and thus make synaptic contact with different types of dorsal and ventral horn neurons. (Supported by NS-11614 from NIH.)

83.8 A DOUBLE LABELLING STUDY DEMONSTRATING THAT MOST SPINALLY PROJECTING NEURONS OF THE NUCLEUS RETICULARIS GIGANTOCELLULARIS DO NOT PROVIDE COLLATERAL INNERVATION TO THE DIENCEPHALON IN THE RAT. R.P. Waltzer* and G.F. Martin, Dept. of Anat., Coll. of Med., The Ohio State Univ., Columbus, Ohio 43210. (Spon: A.O. Humbertson)

The interest of our laboratory in descending systems had led us to investigate the extent to which reticulospinal neurons provide collateral innervation to other targets in the brain. HRP injections into either the spinal cord or diencephalon label neurons in the nucleus reticularis gigantocellularis (Gi) in the rat. In light of observations made from Golgi studies of the reticular formation in young animals (Scheibel, '58), it is often assumed that many spinally projecting neurons of the Gi provide collateral innervation to the diencephalon. In order to determine whether that is the case we have employed fluorescent markers in double labelling experiments. Multiple injections of True Blue (TB) were made into the cervical spinal cord of adult rats, followed six days later by injections of Nuclear Yellow (NY) into the diencephalon. The latter injections were centered in the medial forebrain bundle (MFB) and adjacent areas, but also included reticular targets of the dorsal thalamus. The animals were sacrificed 24 hours or less after the second injection by intracardiac perfusion with 30% formalin in cacodylate buffer. Sections were cut at 32um on a freezing microtome and viewed with a fluorescence microscope using an excitation wavelength of 360nm. Spinally projecting neurons i.e. those containing TB, as well as neurons projecting to the MFB, which contain NY, were found intermingled in the Gi. Less than 3% of the neurons in the Gi were double labelled. Similar results were obtained when the NY injections were made into the midbrain reticular formation.

In a previous communication (Waltzer and Martin, Soc. for Neurosc., Abst. Vol. 8, 1982) we presented evidence that Gi neurons projecting to the spinal cord are intermixed with those projecting to the cerebellum but that few neurons provide collaterals to both targets. Such results, plus those reported herein, lead us to the hypothesis that spinally projecting neurons of the Gi (and other nuclei of the reticular formation, unpublished observations), are for the most part a separate population of cells within a nucleus which is connectionally heterogeneous. (Supported by BNS-80-08675)

83.9 THE ORIGINS AND TRAJECTORIES OF DESCENDING PATHWAYS IN THE SPINAL CORD OF THE STINGRAY, DASYATIS SABINA. C.A. Livingston, B.J. Williams, T.C. Ritchie and R.B. Leonard. Marine Biomedical Institute and the Department of Physiology & Biophysics, University of Texas Medical Branch, Galveston, Texas.

Cells of origin and funicular trajectories of descending systems have been studied in vertebrates. We are interested in identifying the pathways capable of initiating locomotor activity. We used a combination of whole cord HRP injections caudal to spinal lesions and small injections into the white matter. Large injections of HRP were made several segments caudal to subtotal spinal cord lesions while small injections were made by inserting an HRP coated insect pin into the white matter.

Cells were labeled in the reticular formation from the spinomedullary junction to the rostral rhombencephalon by pathways in the dorsolateral (DLF), lateral (LF) and ventrolateral (VLF) funniculi. Cells of the reticulospinal system are predominantly located ipsilateral to the injections at all levels except the spinomedullary junction. Here labelled cells are located bilaterally along the midline and in the medial and lateral portions of the reticular formation. Cells labelled through the DLF are located mostly within the medial reticular formation and include many larger cells. LF pathways labeled cells within both the medial and lateral reticular formation. Injections of the VLF labeled a large reticulospinal component in the raphe as well as in the medial and lateral zones. Large cells located in the ventrolateral margins of the medulla are labeled through the VLF.

Injections of the DLF or LF occassionally result in labeled cells in the vestibular complex. With involvement of more ventral pathways, larger numbers of vestibulospinal cells are found in the ipsilateral magnocellular vestibular nucleus. With larger injections involving portions of the ventral gray matter, cells located medially within this nucleus are labeled. These cells may be projecting through the ventromedial portions of the cord. A band of cells extending across the base of the cerebellar peduncle is observed following large ventral injections. This may also be a vestibulospinal component.

In the caudalmost portions of the mesencephalon cells were occassionally observed along the lateral boundary of the PAG following DLF injections. Ventral pathways labeled occassional cells in the tegmentum of the caudal mesencephalon. A contralateral rubrospinal pathway can be demonstrated projecting through the DLF. Injections of either the DLF or VLF labeled cells of the interstitiospinal pathway. These were located predominantly on the ipsilateral side. Labeled cells are only rarely observed in the tectum following spinal cord injections of HRP. Supported by grant NS11255.

83.10 TRIGEMINO-HYPOGLOSSAL PROJECTIONS IN THE RAT. T.B. Boone* and L.D. Aldes*. Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, Texas 77025 (SPON: R. Wiggins)

Secondary trigeminal afferent projections to the hypoglossal nucleus (XII) in the rat were investigated with retrograde and anterograde labeling methods. The significant finding in this study was that only the rostral trigeminal nuclear complex, consisting of the principal trigeminal nucleus, supratrigeminal nucleus and subnucleus oralis of the spinal trigeminal nucleus, was found to send axons to XII.

Microiontophoretic injections of horseradish peroxidase into XII resulted in retrograde labeling of large neurons (35-40u) in the dorsal one-third of the principal trigeminal nucleus. The density of labeling was greater in the caudal two-thirds of the nucleus, and labeled neurons were found equally in both medial and lateral regions. The supratrigeminal nucleus, located dorsal to the motor nucleus of V, contained small (15-20u),fusiform shaped labeled neurons. Similarly shaped, labeled neurons occasionally were seen in the reticular formation medial, lateral and ventral to the motor nucleus of V, and in two animals, a single labeled, small neuron was identified within the motor nucleus of V. Medium and small sized neurons (25-30u) were found labeled in the dorsal and rostral one-half of the subnucleus oralis. The greatest number of labeled neurons was found near the level of the exiting facial nerve. In all components of the rostral trigeminal complex, labeled neurons were found bilaterally, although a clear preponderance for ipsilateral distribution was evident.

Each of the projections observed in the retrograde labeling experiments was confirmed autoradiographically. Small microiontophoretic injections of tritiated amino acids into the dorsal regions of the principal trigeminal nucleus, subnucleus oralis, and the supratrigeminal nucleus, resulted in predominately ipsilateral terminal fields within XII. The density of silver grains over XII, however, consistently was greater following injections into the supratrigeminal nucleus than into the principal trigeminal nucleus or subnucleus oralis. Additional injections into the ventral portion of the principal trigeminal nucleus and subnucleus oralis, as well as into the subnucleus interpolaris and caudalis, did not result in labeling in XII. However, following a large injection into the dorsal subnucleus caudalis that included the parvicellular component of the lateral reticular formation, bilateral labeling of XII was observed.

The significance of these projections are discussed in relationship to the reflex and central control of the tongue.

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83.11 EFFECT OF SARIN AND SOMAN ON SPINAL REFLEXES IN THE CAT. Qin Zhao Yang* and Jordan E. Warnick. Dept. of Pharmacol. & Exp. Ther., Univ. of Maryland School of Medicine., Baltimore, MD 21201.

The effects of sarin and soman were investigated on spinal reflexes of cats anesthetized with α-chloralose (70 mg/kg, i.v.). Stimulation of either the peroneal or tibial nerves evoked an afferent volley in the intact L_7 dorsal root, a monosynaptic reflex (MSR) in the cut L_7 ventral root and dorsal root reflex (DRR; polysynaptic reflex) in the cut L_6 dorsal root. The afferent volley, MSR and DRR were each evoked at 0.2 to 10 Hz and the input/output (I/O) relationship of the MSR determined at 0.2 Hz. From 30 min to 5 hr after injection of sarin (10 μg/kg, i.v.), the MSR gradually increased in amplitude to 196% of control while the DRR decreased to 85% of control with no change in blood pressure. Sarin was equally effective in producing facilitation after high cervical (C_1) transection as in cats with intact neuraxes. Atropine (1 mg/kg) reduced the facilitatory effect of sarin in the intact cat but had little or no effect on sarin-induced facilitation of the MSR in spinal transected cats. At higher doses sarin caused hypotension and anoxia which, despite artificial respiration, led to a rapid loss of the reflexes that could be reversed with atropine. Sarin reduced the frequency-dependent depression of the MSR elicited by peroneal or tibial nerve stimulation at 0.2, 2 and 5 Hz by 20 to 24% such that the ratios of the 10th/1st potentials in the train are increased from 0.9, 0.18 and 0.03 to 1.1, 0.5 and 0.27, respectively. Changes in the I/O relationship of the MSR with sarin suggest a possible alteration in motoneuron threshold. The plasma levels of cholinesterase after injection of sarin were reduced by 30 to 70% within 3 min and this lasted for at least 4 h. The time course and degree of inhibition of cholinesterase by sarin did not correlate with the effects on the MSR and DRR. In the intact cat, soman (5 μg/kg) depressed the MSR and DRR after a brief (< 20 min) period of potentiation. At 10 μg/kg, soman rapidly depressed the spinal reflexes but atropine (1 mg/kg) allowed gradual recovery of the spinal reflexes which remained smaller than (70% of) control. Atropine pretreatment (1 mg/kg) prevented the usual depressant effect of soman. The plasma levels of cholineseterase were reduced by 50% with 5 μg/kg. Soman and sarin have different effects on the MSR which do not appear to be directly related to an inhibition of cholinesterase but the alteration induced by atropine suggests that some muscarinic component is involved in their effects. Additionally, these agents may have a direct action on neurons of the lumbar spinal cord as indicated by the persistance of sarin's effect in the transected cat. (Supported by U.S. Army Medical Research and Development Command contract DAMD17-81-C-1279.)

83.12 ULTRASTRUCTURAL DEMONSTRATION OF NORADRENERGIC SYNAPSES IN THE RAT SPINAL CORD BY DOPAMINE-β-HYDROXYLASE IMMUNOCYTOCHEMISTRY. E.W. Akeyson*, M.S. Lewis*, M.E. Molliver and R. Grzanna. Dept. of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Noradrenergic (NA) varicosities have been demonstrated throughout the spinal cord gray matter and descending NA projections have been implicated in the control of visceral, somatomotor and sensory spinal cord functions. To understand the function of these NA projections it is necessary to identify the neuronal elements within the spinal cord which are influenced by these NA axons and to characterize the fine structure of the appositional sites. In this study we have employed antibodies to dopamine-β-hydroxylase (DBH) to determine the ultrastructural features of NA terminals in the dorsal horn, the intermediate zone and the ventral horn of the rat thoracic spinal cord. DBH is a specific marker for NA neurons and their processes, but does not distinguish them from epinephrine containing neurons. DBH varicosities were visualized using the peroxidase-anti-peroxidase immunocytochemical method with pre-embedding staining.

Light microscopic inspection reveals three regions exhibiting a high density of DBH varicosities: the outermost portion of the dorsal horn, the region containing the preganglionic sympathetic neurons and the ventral horn. All other regions of the spinal gray contain a lower density of DBH varicosities. In the ventral horn, DBH varicosities were found in close apposition to large multipolar neurons, their proximal dendrites and the surrounding neuropil.

At the ultrastructural level, DBH immunoreactive varicosities are seen in the three regions described above, and inter-varicose segments are rarely stained. The diameter of DBH-positive boutons varies from 0.4 μm to 1.9 μm ($\bar{x}$ = 0.91 μm ± 0.25 μm). In individual thin sections, more than half of all DBH-positive varicosities exhibit distinct synaptic membrane specializations. The majority of contacts are axo-dendritic on medium sized dendrites and are typically asymmetric with a moderate but varied amount of post-synaptic specialization. Axo-somatic contacts are infrequent and have symmetric membrane specializations.

The finding that DBH terminals in the spinal cord make synaptic contacts indicates that NA neurons have a precisely targeted and restricted influence on spinal cord neurons via specialized and discrete junctions. Additionally, the existence of morphologically heterogeneous DBH synapses in all major subdivisions of the spinal cord indicates multiple types of synaptically mediated influences of NA neurons on diverse spinal cord functions. (Supported by grants NS-15199, RR-5378 and UCP R-340-83)

83.13 ELECTROPHYSIOLOGICAL CHARACTERIZATION OF THE IN VITRO SPINAL CORD OF DASYATIS SABINA, AN ELASMOBRANCH FISH. B.J. Williams and R.B. Leonard. Marine Biomedical Institute and the Dept. of Physiol. and Biophy., Univ. Tex. Med. Branch, Galveston, Texas.

We have been studying the spinal cord of an elasmobranch fish, the Atlantic stingray, as a comparative model of spinal cord organization. Isolated portions of the nervous system maintained in vitro have several advantages for neurophysiological analysis. We now report the development of an in vitro preparation of the stingray spinal cord. Small stingrays (15 cm fin span) were anesthetized in tricaine methanesulfonate. The peripheral nerves innervating one pectoral fin were exposed and a laminectomy was performed. A length of spinal cord (7-10 segments) in the vertebral column, along with the peripheral nerves to one fin, was removed and placed in cold elasmobranch Ringer's solution. The composition of the Ringer's solutions was (in mM): 280 NaCl, 4.5 KCl, 8.5 $CaCl_2$, 1.4 $MgCl_2$, 25 $NaHCO_3$, 11 glucose, containing 28g/l urea and bubbled with 95% O_2- 5%CO_2. In most experiments, most of the cord contralateral to the intact nerves was removed. After completing the dissection the cord was superfused with the same Ringer's solution and allowed to come to room temperature.

Isopotential contours were computed from signal-averaged records of field potentials which were recorded with NaCl filled electrodes and evoked by stimulation of the separated motor and sensory nerves. The evoked volleys were monitored with suction electrodes on the sensory or motor nerves close to the spinal cord. As in the in vivo preparation (Leonard, Rudomin & Willis, J. Neurophysiol., 41: 108, 1978), late negative field potentials were recorded in the substantia gelatinosa. These field potentials are attributed to activation of second order neurons in this area by the small myelinated fiber population in the sensory nerves. In addition, an N1 wave was recorded in the nucleus proprius, and is attributable to activation of interneurons by the large myelinated sensory fibers. This field potential inverts in the ventral horn in both the in vitro and in vivo preparations. Antidromic stimulation of the motor nerves evokes a small negative field potential in the ventral horn with a maximum negativity at the level of the motoneuron somata. This negativity is delayed and appears rounded when the electrode is dorsal and dorsolateral to the motoneurons, which suggests electrotonic conduction along the dendritic trees. Intersegmental interactions are also viable in the isolated spinal cord. Conditioning stimuli applied to adjacent sensory nerves depress the segmental field potentials with a time course similar to the depression seen in vivo which is attributed to PAD (Rudomin, Leonard & Willis, J. Neurophysiol., 41: 126, 1978). Supported by NIH grants, NS11255 and NS16093.

83.14 LIGHT MICROSCOPIC STUDY OF THE INFERIOR OLIVARY COMPLEX IN NORMAL MICE AND REELER AND WEAVER MUTANT MICE. G.J. Blatt* and L.M. Eisenman (Spon: D. Goldowitz). Daniel Baugh Institute, Jefferson Medical College, Philadelphia, PA 19107.

This is a preliminary report of a study of the inferior olivary complex (IO) in normal (+/+: +/rl), reeler (rl/rl) and weaver (wv/+; wv/wv) mouse strains. We have begun our analysis by looking at serial sections through the IO, cut in the coronal and parasagittal planes and stained with cresyl violet. The rostrocaudal and mediolateral extent of the inferior olive as well as the morphology of its three major subdivisions, the principal olive (PO), dorsal accessory olive (DAO) and medial accessory olive (MAO) were examined at the light microscopic level.

The organization of the IO of the neurologic mutant mice remains remarkably preserved, despite gross cerebellar abnormalities including reduced numbers of Purkinje cells in the cerebellar cortex.

The inferior olive of the normal mouse (+/+) and heterozygous reeler (+/rl), which has no known mutant phenotype, possess well defined olivary subdivisions with distinct borders.

Subtle differences in the homozygous mutant mice were found suggesting some minor alterations in olivary structure. This is especially apparent in reeler (rl/rl) where it is difficult to distinguish PO from DAO in mid-rostral sections of the olive. There is also an approximate 20-25% reduction of olivary cells in this animal. Preliminary counts of the IO suggest that there are similar numbers of olivary cells in +/rl, wv/+ and wv/wv mice. We plan to do Purkinje cell counts in these same strains to determine the ratio of Purkinje cells to olivary cells which in the normal mouse is approximately 6:1 (Caddy and Biscoe, 1978).

83.15 THE RELATIONSHIP OF THE HYPOGLOSSAL NUCLEUS AND ITS ADJACENT ENVIRONMENT. M.H. Cooper and T.G. Spangler*. Depts. of Otolaryngology and Anatomy. St. Louis Univ. Sch. of Med., St. Louis, MO. 63104.

The hypoglossal nucleus (XII Nu) and its relationship to surrounding structures has been studied in the laboratory rat (Rattus norvegicus albinus). A variety of techniques were used including the horseradish peroxidase (HRP) intraaxonal transport technique of Mesulam (J. Histochem. Cytochem. 26:106-117, 1978), the autoradiographic anterograde transport technique of Cowan et al. (Br. Res. 37:21-51, 1972), the degeneration technique of Fink and Heimer (Br. Res. 4:369-374, 1967) and the Golgi technique of Adams (Stain Technol. 54:225-226, 1979). Tetramethylbenzidine (TMB) was used as the chromagen for the HRP technique and tritiated leucine was used in the autoradiographic technique.

The most striking feature of the XII Nu and its surrounding environment is the intricate relationship of the XII Nu and the adjacent reticular formation (RF). This is most remarkable at the ventro-lateral aspect of the nucleus. Following an iontophoretic injection of tritiated leucine or an electrolytic lesion in the RF either silver grains or fragments of degenerating axons were observed bilaterally in this area. Ejections or electrolytic lesions in the medial part of the RF caused the greatest amount of input to occur. This included the RF from medullary through mid-pontine levels. The amount of grains or fragments was heaviest on the ipsilateral side.

Following injections of HRP into the musculature of the tongue, numerous dendrites of HRP positive neurons of the XII Nu were observed to extend into the adjacent RF laterally and ventro-laterally. These dendrites extended into the same portion of the RF in which the input from the RF, previously described, was observed. Similar dendritic extension was observed in Golgi preparations.

HRP injections in the musculature of the tongue resulted in HRP-positive neurons not only within the XII Nu, but also neurons outside the nucleus. In the area ventral and somewhat lateral to the XII Nu and possibly in one of the perihypoglossal nuclei (nucleus of Roller) large multipolar neurons were observed to be HRP-positive. Dendrites of these neurons were occasionally observed to extend into the XII Nu proper. Similar neurons including the dendritic extensions were observed in Golgi preparations. Frequently in HRP preparations an axon of one of these neurons was observed to enter fascicles of the hypoglossal nerve.

(Supported by The Whitehall Foundation.)

83.16 **COERULOSPINAL EFFECTS ON THE RHEOBASE OF CAT SPINAL MOTONEURONS.** S. J. Fung and C. D. Barnes, Department of Physiology, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

Previous investigations from our laboratory have demonstrated an excitatory coerulospinal action on lumbar monosynaptic reflexes as well as on afferent impulse transmission. The present study on precollicularly decerebrate cats examined the excitability change of the motoneuron membrane evoked by locus coeruleus stimulation. The excitability of motoneuron membrane was tested by measuring the threshold current to induce a discharge 90% of the time with long (20-50 ms) depolarizing current pulses. Single units of locus coeruleus demonstrating the typical firing response to pinch stimuli were searched for with either glass micropipettes filled with 0.5 M sodium acetate and 8% Alcian Blue or stainless steel microelectrodes. These sites were subsequently stimulated with cathodal volleys at low intensities (100-500 μA). These recording/stimulation sites were subsequently shown with fluorescent techniques to be located in close proximity to noradrenergic neurons. With the locus coeruleus electrode in place intracellular recordings were made with 3 M KCl or 4 M K acetate-filled micropipettes from ipsilateral hindlimb motoneurons. A total of 25 motoneurons (both flexors and extensors) with antidromic spike heights above 60 mV were used for the present analysis. A majority of motoneurons (20/25) exhibited a lowering of rheobase (up to 40%) upon locus coeruleus stimulation. This could explain the findings that activation of noradrenergic locus coeruleus neurons resulted in an augmented somatomotor outflow at the level of spinal cord. This work was supported by NIH grant NS 17040.

83.17 **INDOLEAMINERGIC PROJECTIONS FROM LOCUS COERULEUS TO THE LUMBAR CORD IN THE CAT.** Y.-Y. Lai and C. D. Barnes, Department of Physiology, Texas Tech Univ. Health Sci. Ctr., Lubbock, TX 79430.

In the cat, the nucleus locus coeruleus is a loosely arranged group of neurons in the pons. Although the majority of neurons are, as in the rat, noradrenergic, a number of them are serotonergic. Several studies using a retrograde and anterograde axonal transport have demonstrated a projection of noradrenergic neurons to lumbar cord from locus coeruleus in rat and cat. The question of whether the serotonin cells project into the cord remains unanswered. The present study was designed to answer that question. Descending projections from the locus coeruleus were studied by using retrograde fluorescence labeling combined with monoamine fluorescence histochemically. Under pentobarbital anesthesia, 0.5 µl of Evans blue (10% w/v) was injected into the L_7 ventral horn of adult cats. After 4 days, the cats received 50 mg/kg of nialamide and were given 20 mg/kg of L-tryptophan 1 hr later. After another 1 hr the cats were perfused with Ringer-Locke solution (pH 7.4) followed by a solution of 4% formaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate buffer solution (pH 7.0) at room temperature. The brainstems and spinal cords were then removed and stored separately in 30% sucrose Faglu solution overnight at 4°C. The tissues were cut into 12 µm slices on a cryostat. The sections were placed in Faglu solution and then transferred to precoated microslides. Wet sections were photographed under the light microscope, dried over phosphorus pentoxide for 1 hr, and mounted in mineral oil. Fluorescence was examined under a fluorescence microscope system equipped with V/Y475 (420/480 nm) filter combination for visualizing catecholamine, V/0515 (420/520 nm) filter combination for visualizing indoleamine, and G/R610 (500/590 nm) filter combination for visualizing Evans blue. After fluorescence photomicrographs were taken, the sections were stained with neutral red. The fluorescence of Evans blue could be seen in both catecholamine and serotonin neurons. Thus, this study demonstrates both catecholaminergic and indoleaminergic neurons within the locus coeruleus projected to the lumbar spinal cord. This work was supported by NIH grant NS 17040 and the Tarbox Parkinson's Disease Institute of TTUHSC.

OPIATES, ENDORPHINS, AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS I

84.1 MEASUREMENT OF ENKEPHALINS IN BIOLOGICAL TISSUE EXTRACTS WITH UNAMBIGUOUS MOLECULAR SPECIFICITY BY MEANS OF MASS SPECTROMETRY, D.M. Desiderio, F.S. Tanzer*, and M. Kai*, Charles B. Stout Neuroscience Mass Spectrometry Laboratory, University of Tennessee Center for Health Sciences, Memphis, Tennessee,38163.

This research program aims to elucidate molecular processes involved in pain. This study is based on peptide measurement with an off-line combination of reverse phase high performance liquid chromatography (HPLC) and fast atom bombardment mass spectrometry (FAB-MS) to determine antagonism between methionine enkephalin (ME), leucine enkephalin (LE), and prostaglandins (PGE_2) in control (untreated) and stimulated (treated) tooth pulp tissue. The hypothesis to be tested is: in control (untreated) tissue, neuropeptide (ME and/or LE) levels are elevated while PGE_2 levels are lowered whereas in stimulated (treated) tissue, the converse is true.

In the first phase of this study, the two opioid pentapeptides, methionine enkephalin (YGGFM) and leucine enkephalin (YGGFL), are measured in tissue extracts from canine tooth pulp and brain regions. The peptides are separated using acidified acetone for protein precipitation, low resolution mini-column (sep-pak) chromatography, and high resolution analytic reverse phase HPLC utilizing acetonitrile as organic modifier and 0.04 M triethylamine formate as volatile buffer. While ultraviolet detection at 200 nanometers is used as a preliminary screen for peptides, the detector for this system is a fast atom bombardment (FAB) mass spectrometer. A Finnigan MAT 731 double focusing mass spectrometer is utilized in the fast atom bombardment mode to produce a protonated molecular ion of each individual peptide. While increased molecular specificity is achieved for quantification utilizing the protonated molecular ion and an ^{18}O-incorporated enkephalin internal standard, a much higher level of molecular specificity is achieved by novel processes to be reported here. The protonated molecular ion of the peptide is subjected to collision activated dissociation processes to produce structurally unique amino acid sequence-determining ions. The amino acid sequence-determining ions are analyzed in a linked field scan mode. By alternatively jumping between two linked field scan values, B/E corresponding to the amino acid sequence-determining carboxy-terminus tripeptide ion GGM from the peptide of interest and B'/E' for the corresponding fragment for the internal standard, we achieve for the first time an extraordinarily high level of molecular specificity coupled with that analytical measurement. Measurements will be presented for canine tissue extracts - anterior pituitary, posterior pituitary, caudate nucleus, unstimulated tooth pulp, and electrostimulated tooth pulp. This work is supported by NIH (GM 28611 and 26666).

84.2 COMPARISON OF RAT HIPPOCAMPAL TISSUE CONTENTS AND IN VITRO RELEASE OF PRO-DYNORPHIN-RELATED OPIOIDS. C. Chavkin[1], C. Bakhit[1], E. Weber*[2], and F.E. Bloom[1]. [1]A.V. Davis Center, The Salk Institute, P.O. Box 85800, San Diego, CA 92138 and [2]Department of Psychiatry, Stanford University, Palo Alto, CA 94305.

The pro-dynorphin molecule can potentially be cleaved to yield several active opioids: dynorphin-A-(1-17) [dynA], dynorphin-A-(1-8) [dyn8], dynorphin-B [dynB], α-neoendorphin [αneo], β-endorphin [βneo], and possibly leucine-enkephalin [LE]. Since these products differ in opioid receptor selectivites and potencies, the determination of which peptides are naturally present and released at nerve terminals is important for understanding the physiological role of the pro-dynorphin containing neurons.

Previous immunohistochemical and radioimmunoassay (RIA) studies have demonstrated that the pro-dynorphin-derived opioids are present in rat hippocampus. Using separate, selective RIAs for each of the six opioids listed above, we have measured their contents in 1M acetic acid extracts of rat hippocampus. Tissue contents were found to be (pmoles immunoequivalents/gm protein): dynA, 250; dyn8, 100; dynB, 925; αneo, 200; βneo, 20; and LE, 290. DynB immunoreactivity (IR) had the highest concentration and βneo the lowest.

In order to determine the molecular forms of the IR, hippocampal extracts were resolved on C18 HPLC columns and then eluate fractions were assayed using selective RIAs. Dyn8, αneo, βneo and LE-IR present in hippocampal extracts were each homogeneous and coeluted with appropriate synthetic standards. DynA-IR and dynB-IR however, were both hetereogenous with roughly 20-40% of each coeluting with synthetic standards and the remainder of each eluting as higher molecular weight material.

Calcium-dependent release of each peptide-IR listed above from fresh hippocampal tissue slices was stimulated by 50 mM K+ induced depolarization. The rates of stimulated release from 200 mgs of tissue were found to be (fmoles/min): dynA, 1.6; dyn8, 16.6; dynB, 23; αneo, 9.6; βneo, 6.1; and LE, 84. The release of each opioid is not strictly consonant with their relative tissue contents; dyn8, βneo and LE were each released at a ten fold higher fraction of their contents than dynA, dynB or αneo. Potential differences in peptide recoveries from the release chambers needs to be considered. Taken at face value however, our results not only reflect the relative peptide abundances at nerve terminals but may provide the means to determine the sequential steps by which the pro-dynorphin molecule is processed to its agonist products in the hippocampus. (Supported by NIAAA Grants 03504 and 07273.)

84.3 DISSOCIATION OF MORPHINE'S ANALGESIC AND RESPIRATORY DEPRESSANT ACTIONS. G.S.F. Ling, K. Spiegel, S.L. Nishimura* and G.W. Pasternak. The George C. Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021 USA

The high affinity (mu_1) opioid binding site plays a role in opiate and opioid peptide analgesia, as well as prolactin release and catalepsy but not lethality, growth hormone release or sedation. To further explore the relationship between morphine induced respiratory depression and analgesia, we examined the effect of naloxonazine, a selective mu_1 antagonist, on both of morphine's actions. Arterial and venous cannulae were placed and the rats treated with either saline or naloxonazine (10 mg/kg i.v) 24 hours prior to receiving morphine (i.v.). Respiratory function was evaluated in serial arterial blood samples (100 µl) and analgesia by radiant heat tail-flick. Morphine (3.5 mg/kg) elevated the mean control tail-flick latencies from 3.4 ± 0.2 sec to 9.2 ± 0.8 sec ($p<0.001$) while depressing the pO_2 about 17 mm Hg and raising pCO_2 approximately 12 mm Hg. Naloxonazine pretreatment had virtually no effect on morphine's respiratory depression, with a 23.8 mm Hg drop in pO_2 and a 10 mm Hg rise in pCO_2. However, naloxonazine treatment effectively blocked morphine's analgesic actions with a peak latency of 3.4 ± 0.4 sec compared to a 3.0 ± 0.1 sec baseline. Full dose response curves for both respiratory effects and analgesia using four morphine sulfate doses (3.5,5,10, and 15 mg/kg) have been studied. In contrast to analgesia whose dose-response curve was shifted to the right, no significant change in respiratory parameters was observed following naloxonazine treatment. These results suggest that morphine's effect on respiration can be dissociated from it's analgesia activity. Although these results confirmed earlier claims regarding the role of mu_1 sites in morphine analgesia, it is as yet unclear which opiate receptor subtype mediated morphine's respiratory depressant actions.

84.4 POSSIBLE PARTICIPATION OF ENDOGENOUS CHOLECYSTOKININ 8 (CCK) IN THE TOLERANCE TO MORPHINE ANALGESIA IN THE RAT. J. Tang*, J. Chou*, M. J. Iadarola, H.-Y.T. Yang and E. Costa (SPON: An-Zhong Zhang). Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

Numerous axons containing cholecystokinin (CCK)-like immunoreactivity are located in laminae I and II of the posterior horn of the spinal cord (Brain Res. 165:201, 1979). Intrathecal injections of CCK-8 antagonize opiate analgesia (Science 219:310, 1983) but the molecular events and physiological mechanisms underlying this action are unclear. In rats, we have replicated the CCK-8 (15 pmol) antagonism of morphine analgesia (30 µg) by injecting the two compounds intrathecally and estimating analgesia by measuring the tail flick latency in response to radiant heat. Moreover, the CCK-8 antagonism of morphine analgesia was inhibited by pretreatment with proglumide (25 mg/kg s.c.), a blocker of CCK-8 action at CCK receptors. Using a push-pull pump we infused (1 ml/10 min) the subarachnoid spaces of the rat spinal cord with artificial spinal fluid containing captopril (10^{-6}M) and bestatin (10^{-6}M). The addition of morphine (10^{-6}M) to the artificial spinal fluid releases endogenous CCK-8-like immunoreactivity into the perfusion fluid. This effect of morphine was curtailed or abolished by naloxone ($2x10^{-6}$M). Rats receiving one injection of morphine sulfate (10 mg/kg s.c.), repeated every two hours exhibited a progressive decrease of morphine-elicited analgesia. Complete morphine analgesia usually appeared after the 7th or 8th injection. If morphine injections were given concurrently with injections of proglumide (15 mg/kg s.c.), the morphine tolerance was greatly reduced and usually failed to occur. These results suggest that release of endogenous cholecystokinin may participate in the onset of morphine tolerance in rats.

84.5 ACUTE AND CHRONIC MORPHINE MODIFIES THE IN VIVO RELEASE OF METHIONINE ENKEPHALIN-LIKE IMMUNOREACTIVITY FROM THE CAT SPINAL CORD AND BRAIN. K. Jhamandas*, T.L. Yaksh and V.L.W. Go* (SPON: R.J. Boegman). Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada; Departments of Neurological Research and Gastroenterology, Mayo Clinic, Rochester, Minnesota, 55901.

Interaction between exogenous and endogenous opioids has been proposed as the basis for narcotic dependence. Occupation of opiate receptors by morphine is hypothesized to trigger a negative feedback mechanism which leads to reduced biosynthesis or release of the endogenous opioids. This study was performed to test whether the release of methionine enkephalin-like immunoreactivity (MELI) in vivo is modified by acute or chronic morphine treatment.

Release of MELI from the spinal cord and the brain of chloralose-urethane anesthetized cat was investigated using the technique of spinal superfusion and ventrocisternal perfusion. To evoke release of MELI bilateral sciatic nerve stimulation was applied at intensities known to activate small diameter fibers. The released material was identified as methionine enkephalin by radioimmunoassay with two different anti-sera, parallel displacement with serial dilutions of perfusates and co-migration with authentic methionine enkephalin in a Sephadex G-25 column.

In control experiments release of MELI occurred spontaneously from the spinal cord and the brain; bilateral sciatic stimulation significantly increased this release. Local application of morphine (5×10^{-4} M) to the spinal cord decreased evoked release of MELI from the spinal cord and the brain. Naloxone (2 mg/kg i.v.) administered during morphine treatment increased both the spontaneous and the evoked release of MELI. In morphine naive animals naloxone did not modify MELI release. In cats chronically exposed to morphine by implantation of morphine pellets (2 x 75 mg) the spontaneous release of spinal and brain MELI was higher than that seen in control animals. Naloxone treatment resulted in a large and sustained increase in spontaneous and evoked release of MELI.

The results show that acute morphine suppresses MELI release. Chronic treatment does not produce this effect. The enhanced release of MELI in chronically morphine treated animals may be due to a loss of an autoinhibitory mechanism or an excessive drive in enkephalinergic neurons by other excitatory systems.

[Supported by the MRC (Canada) and NS Grant 16541.]

84.6 THE EFFECTS OF EXERCISE TRAINING ON BRAIN ENDOGENOUS OPIOID SYSTEMS. M. J. Blake, E. A. Stein and A. J. Vomachka*. Dept. of Biology, Marquette University, Milwaukee, WI 53233.

It has been postulated that endogenous opioid peptide (EOP) systems are involved in reproductive functions since: 1) exogenous morphine can inhibit ovulation in rats, 2) Naloxone (Nx) reverses this drug effect, and 3) Nx also elevates serum luteinizing hormone (LH) levels in rats. The observations that trained female athletes are often amenorrheic, and that treadmill running increases plasma β-endorphin (βE) levels, led us to investigate the role of the EOPs in exercise training using a rat animal model. We report here the effects of treadmill running on serum gonadotropin and EOP levels in several discrete brain nuclei.

Thirty-two female Holtzman rats were randomly divided into two identical groups. One group received daily exercise training consisting of running on a treadmill on an 8° incline at 28 m/min for 60 min/day. The other group received no exercise training, serving as controls. Following 8 weeks of training and just prior to sacrifice each group was subdivided such that ½ of each group received a final fatiguing bout of exercise. Thus, the final four groups consisted of a trained-fatigued (TF), trained-nonfatigued (TN), control-fatigued (CF), and control-nonfatigued (CN). Animals were sacrificed on diestrus based on vaginal smears. Serum was collected and brains removed. Citrate synthase was assayed in plantaris muscle to determine the level of training. The nucleus accumbens (NA), cortex, caudate putamen, septum, amygdala (AMY), anterior and posterior hypothalamus, substantia nigra, and ventral tegmentum (VT) were microdissected according to the method of Heffner et al. Levels of βE and leucine enkephalin (LE) in all nine brain areas and serum levels of LH and follicle stimulating hormone (FSH) were determined using standard radioimmunoassay procedures.

Two-factor ANOVAs were performed with significance at $p \leq .05$. Fatigued rats showed an increase of βE in the NA which may have resulted from either an increased synthesis and transport from cells of origin or a decrease in release from this terminal field. Fatigued rats also showed an increase of LE in VT which may be due to local synthesis and accumulation in this intrinsic system. In addition, TF rats had less LE than TN rats in the AMY. This again may be due to intrinsic accumulation. Finally, serum RIA results showed a reduction of LH in fatigued vs. nonfatigued rats which may reflect an inhibition of LHRH. It thus appears that acute exercise (or stress) may influence brain EOP system. Supported in part by grants from Marquette University, Committee on Research, and the Dr. William Scholl Foundation.

84.7 GROWTH-INDUCED CHANGES IN THE PULSATILE PATTERN OF GROWTH HORMONE (GH) SECRETION IN THE HAMSTER : THE INVOLVEMENT OF ENDOGENOUS OPIATES. D.R.Nicoski* and K.T.Borer,Dept.Kinesiology,Univ.Mich., Ann Arbor,MI 48109.

Mature golden hamsters can be induced to grow rapidly by exposure to voluntary running (Borer,K.T. & Kelch,R.P.Am.J.Physiol. 1978,234:E611) or by lesions of rostromedial septum (Borer,K.T.et al.Neuroendoc.1977,23:133),and such acceleration of growth is accompanied by increases in serum GH concentration.

To determine whether acceleration of growth is accompanied by changes in the pulsatile pattern of GH secretion, mature female hamsters were induced to grow rapidly by exposure to voluntary running (n=18) or septal lesions (n=9).Sedentary and sham-operated hamsters (n=16) served as slowly-growing controls.Two to 3 weeks after the onset of exercise and 7 to 13 days after neurosurgery, hamsters were implanted with a jugular cannula and were subjected to sequential blood sampling every 20 minutes for 6 hr the next day.The 0.5-ml blood samples provided serum for measurement of hamster GH and prolactin (PRL) by homologous radioimmunoassays (Borer,K.T. et al.,Neuroendoc.,1982,35:349 and 13).

To determine whether the possible changes in the pattern of GH and PRL secretion was due to the release of endogenous opiates, 20 mg/kg of naloxone was administered I.P. to half of the animals in each of the experimental groups,while the remaining hamsters received an equal volume of saline.

Both exercise (0.8+0.1 g/day,$p<0.001$) and septal lesions (3.4±0.4 g/day,$p<0.001$) accelerated growth relative to control groups (0.4±0.04 and 0.2±0.2 g/day).In rapidly-growing hamsters, there was a reduction in GH pulse frequency from a pulse every 63 to 68 minutes to a pulse every 92 minutes. Naloxone administration normalized pulse frequency in all rapidly growing animals ($p=0.05$).Rapidly-growing hamsters displayed increased amplitude of GH pulses (28.5±6.2 vs 13.4±3.6 ng/ml,$p<0.05$).Naloxone suppressed GH amplitude only in neurologically intact animals ($p<0.05$).Basal GH levels were elevated in rapidly-growing exercising (17.6±1.3 ng/ml) and septal-lesioned hamsters (21.6-23.4 ng/ml) relative to controls (12.6±1.5,$p<0.05$).Naloxone was effective in suppressing basal GH levels in neurologically-intact,but not in septal -lesioned animals ($p<0.05$).No changes were noted in the pattern of PRL secretion as a function of growth or naloxone.

Thus in the hamster,endogenous opiates control frequency, amplitude,and baseline levels of circulating GH.Furthermore,rostromedial septum may be the site of inhibitory input over opiate release directed toward facilitation of GH secretion.

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84.8 INCREASED BRAIN AND CEREBELLAR GROWTH IN INFANT RATS TREATED WITH AN OPIATE ANTAGONIST. P.J. McLaughlin and I.S. Zagon. Department of Anatomy, The M.S. Hershey Medical Center, Hershey, PA 17033.

Exposure to exogenous opioids such as heroin and methadone in perinatal life inhibits somatic and neurobiological development. This interference in growth by opioids is stereospecific and reversed by narcotic antagonists, with the locus of opioid action postulated to reside at the opiate receptor (see review by Zagon and McLaughlin, Neurobehavioral Teratology, J. Yanai, ed., in press). The present study was designed to explore the relationship of endogenous opioids and opiate receptors to brain development. Naltrexone, a long-acting narcotic antagonist, was given to preweaning rats in order to examine the neuro-ontogenetic effects of blocking the opiate receptor from interaction with endorphins.

Newborn Sprague-Dawley rats (8/litter) were injected (SC) daily with high dosages of naltrexone or sterile water until 21 days of age. At 21 days of age, rats were perfused with formalin, brains and cerebella removed, weighed, and macroscopic dimensions recorded. Brain weight, width, length, and height of naltrexone-treated pups were increased 2-11% over control levels. Cerebellar weight and width were elevated 7% and 5%, respectively, above control values. Morphometric analyses of hematoxylin-eosin stained sections from the somatosensory cortex (coronal sections) and cerebellum (sagittal sections) showed an enlargement of both regions for naltrexone-treated offspring in contrast to control animals. Cerebral area, cortical thickness, and cerebral width of naltrexone rats were 17%, 18%, and 9%, respectively, greater than control levels. Areal measurements of total,molecular, internal granule, and medullary layers were increased 41-45% in naltrexone-treated rats relative to controls. Analysis of cellular content in the pyramidal lobe of the cerebellum indicated 30% more internal granule neurons/section and 70% more glial cells/section (oligodendrocytes and astrocytes) in the medullary layer from naltrexone-treated rats relative to controls. The total population of neural cells, including glia, basket and stellate neurons in the molecular layer was increased 169% above control levels in the naltrexone-injected animals. These results indicate that an opiate antagonist, administered at dosages known to block the opiate receptor for 24 hr, stimulates brain and cerebellar development in young rats. Moreover, these data suggest that endorphins and opiate receptors are involved in cellular events such as cell proliferation, migration, and differentiation.

This research was supported by NIDA grant DA-01618.

84.9 PROGRESSIVE DECREASE IN SEIZURE SEVERITY PRODUCED WITH INTERMITTENT ELECTROCONVULSIVE SHOCK: INVOLVEMENT OF ENDOGENOUS OPIOID SYSTEMS. F.C. Tortella, J.B. Long, L. Robles*, and J.W. Holaday. Neuropharmacology Branch, Department of Medical Neurosciences, Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Given the recent findings that a) electroconvulsive shock (ECS) elicits a spectrum of opiate-like effects, b) ECS increases brain opioid peptide content and receptor numbers, c) opioid peptides protect against a variety of experimentally-induced seizures, it was proposed that central opioid peptide systems may function postictally as endogenous anticonvulsants (Tortella, et. al., Eur. J. Pharmacol., 1981). It has since been demonstrated that endogenous opioid systems play a selective role in the rise in seizure threshold occurring after ECS (Tortella and Cowan, Life Sci., 1982). Additional studies on opioid peptides as endogenous anticonvulsants are reported here.

Male S.D. rats (275-350g) were given either a single transauricular ECS (2 sec at 60 Hz, 50 mA) or a series of 6 intermittent ECSs (I-ECS) separated by 10 minute intervals. The duration of tonic extension and seizure pattern were recorded as measures of seizure severity for each ECS-induced motor seizure. The duration of posticta1 depression (PID) was also recorded. A single ECS resulted in a tonic-clonic seizure characterized by tonic extension of forelimbs and hindlimbs (duration = 14.58 ± 0.45 sec), a seizure pattern score of 2.9 (on a scale of 0 to 3), and a PID lasting 2586 ± 211 sec. Naloxone (NX,10 mg/kg,sc) given 10 minutes prior to ECS did not affect seizure severity, but markedly decreased the duration of PID (1386 ± 294 sec). I-ECS caused a progressive decrease in seizure severity. Following 6 I-ECS treatments the duration of tonic extension and the seizure pattern score declined from 13.8 ± 0.6 to 4.7 ± 0.8 seconds and from 2.2 to 0.78, respectively. It is interesting to note that, despite the decrease in seizure severity, I-ECS was associated with an enhanced duration of PID (4032 ± 507 sec). NX, administered 10 minutes prior to the sixth ECS, partially reversed the decline in seizure severity (duration of tonic extension = 9.91 ± 1.36 sec, and seizure pattern score of 1.45) as well as the duration of PID (1536 ± 305 sec) produced by I-ECS.

These data support the premise that following a seizure there exists a refractory period during which time the activation of a subsequent seizure becomes increasingly more difficult. Alteration by naloxone of the decrease in seizure severity and PID associated with I-ECS suggests that activation of enkephalin and/or endorphin systems contributes to these changes in seizure susceptibility. Additionally, it was assumed that the duration of PID was a direct function of seizure severity; the above findings indicate that these phenomena are not interdependent. The possible role of endogenous opioid systems in the self-regulation of seizure activity during postictal states will be discussed.

84.10 DISCRIMINATIVE STIMULUS EFFECTS OF RECEPTOR-SELECTIVE OPIOID PEPTIDES IN THE RAT. K. W. Locke* and S. G. Holtzman*, (SPON: B.A. Faraj). Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The morphine-like discriminative stimulus effects of μ- and δ-receptor selective opioid peptides were evaluated following intracerebroventricular (ICV) administration. Rats were trained to discriminate between SC injections of morphine (M) (3.0 mg/kg) and saline in a two-choice discrete-trial avoidance paradigm. Behavior was considered to be under stimulus control when the rats could reliably complete at least 18 trials of a 20-trial session on the appropriate choice lever. ICV administration of the relatively selective δ-receptor agonist D-Ala^2-D-Leu^5 enkephalin (DADL) produced dose-related increases in the number of trials completed on the M-appropriate lever, and 1.0-3.0 μg DADL produced M-appropriate responding comparable to the training dose of M. β-endorphin, which has equal affinity for μ- and δ-receptors, also engendered M-appropriate responding over the dose range of 0.01-10 μg. Complete generalization to ICV β-endorphin occurred at 1.0-10 μg. We have shown previously that the discriminative stimulus effects of 1.0-3.0 μg ICV M are comparable to the systemic M training dose (Locke, K.W. and Holtzman, S.G., Pharmacologist 24: 230, 1982). Thus, DADL and β-endorphin appear to be equipotent or more potent than M on a molar basis in serving as a discriminative stimulus. The stimulus effects of both DADL and β-endorphin were blocked by low doses of SC naltrexone (0.1 mg/kg). In contrast, the relatively selective μ-receptor agonist D-Ala^2-NMePhe^4-Gly^5(ol) enkephalin (DAGO) engendered almost exclusively saline-appropriate responding even though behaviorally active doses (0.1-3.0 μg) were tested. Although M is considered to be a relatively selective μ-receptor agonist, these data suggest that δ receptors are involved in mediating the discriminative stimulus effects of M in the rat. (Supported by US-PHS Grants 5T32-GM07594, DA 00541, and by KO2DA 00008.)

84.11 FACILITATION OF ENKEPHALINERGIC RECEPTOR FUNCTION BY THIORPHAN, AN ENKEPHALINASE INHIBITOR, K. Blum, L. DeLallo*, and A. H. Briggs*. Div. Subs. and Alc. Mis. University of TX, Health Sci. Ctr., San Antonio, TX 78284.

It is well established opioid-like compounds act through their interaction at opiate receptors. There is strong evidence for a multiple opioid receptor system and consensus of the literature indicates the endogenous enkephalins preferentially bind to the δ-receptor. Enkephalins as neurotransmitters are catabolized in vivo by enkephalinase which is inhibited by thiorphan (TH), a high affinity thiopeptide. In this study, a comparison was made between normorphine (NM), methionine enkephalin (ME) and D-ala^2-met^5-enkephalinamide(DAMEA) and the ability of TH to potentiate their in vitro activity to inhibit the electrically induced contractions of mouse vas deferens. Pretreatment with (TH) ($1X10^{-7}$M) for 15 minutes did not significantly effect the twitch inhibition of NM ($5X10^{-7}$M), but did significantly ($P<0.05$) facilitate the twitch inhibition of ME ($7X10^{-9}$M) as well as $1.7X10^{-9}$M DAMEA. Since the decay profile of TH - ME treated tissue was not different than the ME control it was inferred this potentiation was not the result of enkephalinase inhibition but through a direct δ-receptor mechanism. This is the first report showing that TH (or any other agent) facilitates enkephalinergic receptor function via a direct mechanism independent of enkephalinase inhibition. Analogs of thiopeptides may become potent useful therapeutic agents for the treatment of addictive diseases. (This research is supported in part by the Raleigh Hills Foundation.)

PEPTIDES: ANATOMICAL LOCALIZATION I

85.1 DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY IN RAT BRAIN. T.L. O'Donohue, B.M. Chronwall*, D.A. DiMaggio*. Experimental Therapeutics Branch, NINCDS, NIH, Bethesda, MD 20205.

Neuropeptide Y (NPY) was recently discovered and sequenced from extracts of porcine brain (Tatemoto, Proc Natl Acad Aci, USA 79:5485, 1982). The peptide is part of a family of peptides that include pancreatic polypeptide (PP) and peptide YY (PYY). Recent results demonstrated a system of neurons in brain that stains with bovine PP antisera (Olschowka et al, Peptides 2:309-331, 1981), yet the peptide was found to be distinct from PP (DiMaggio et al, Soc Neurosc Abst, 1983).

In this study, antisera were raised to NPY by conjugating synthetic peptide to bovine serum albumin. The indirect immunofluorescence method was used on 20 μM cryostat sections from colchicine treated adult Sprague-Dawley rats perfused with 0.5% sodium nitrite in phosphate buffered saline, pH 7.4, (PBS) followed by 4% formaldehyde in PBS (pH 7.4) which was, in some cases, followed by 4% formaldehyde in borate buffer (pH 10).

An extensive system of intensely staining NPY immunoreactive neurons were observed throughout the rat telencephalon. Regions containing highest densities of NPY-positive terminals were the lateral septum, nucleus (n.) of the stria terminalis, suprachiasmatic n., paraventricular n. and the dorsomedial n. Moderate densities were observed in cortical regions, n. tractus diagonalis, medial preoptic n., dorsomedial n. and amygdala centralis. Lower densities were observed in the striatum, hippocampus and most thalamic n. A large number of perikarya containing NPY immunoreactivity were observed in the arcuate n., periventricular n. and in the septum. The staining pattern seems identical to that of PP. Preabsorption of the NPY antisera with 1 μg/ml of NPY completely blocks staining, as does preabsorption with 1 μg/ml of bovine PP. Because PP immunostaining is also blocked by both PP and NPY, the identity of the peptide is unclear. Further studies are in progress to characterize this peptide in brain.

85.2 NEUROTENSIN AND DOPAMINE ARE NOT CO-LOCALIZED IN RAT BRAIN. G. Bissette, L. Jennes*, A.J. Prange, Jr., G.R. Breese, and C.B. Nemeroff. Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med. Chapel Hill, NC 27514.

Neurotensin (NT) localization in the central nervous system has been extensively studied by radioimmunoassay (RIA) and immunohistochemical (IHC) techniques. Many brain areas that contain high concentrations of NT are known to contain dopamine (DA) cell bodies or nerve terminals. In an attempt to determine if NT and DA are co-localized, DA neurons were chemically lesioned with 6-hydroxydopamine (6-OHDA). Adult, male Sprague-Dawley rats (200 g, n=16) were injected with desmethylimipramine (30 mg/kg, IP) one hour prior to injection with 6-OHDA (240 μg, IC). Desmethylimipramine (DMI) is used to protect norepinephrine-containing cells from 6-OHDA-induced neuronal destruction. Controls (n=16) received DMI and 6-OHDA vehicle (ascorbic acid). Three weeks later, animals were killed by decapitation and their brains quickly removed and placed on an ice-cooled petri dish. The following brain regions were rapidly dissected: nucleus accumbens, septum amygdala, ventral tegmental area of Tsai (VTA)-substantia nigra, hypothalamus, striatum, dorsal hippocampus, and olfactory tubercles. Brain samples from control (n=8) and 6-OHDA-treated rats (n=8) were homogenized in 1 N HCl, centrifuged, and duplicate supernatant aliquots were frozen and lyophilized for measurement of NT by RIA. An additional set of brain samples from control (n=8) and 6-OHDA-treated (n=8) rats were homogenized in 0.1 N perchloric acid and extracted with alumina for determination of DA concentration by HPLC with electrochemical detection. Protein concentration was measured with the Folin reagent. Data are expressed as pg NT/mg protein or ng DA/mg protein, and the degree of statistical significance ($p < .05$) was assessed with Student's t-test (two-tailed). Animals treated with 6-OHDA exhibited a 99% deplection of DA, as compared to controls in the septum, amygdala, VTA-substantia nigra, and olfactory tubercles, and an 89% and 83% depletion in the striatum and nucleus accumbens. Hypothalamic DA was reduced approximately 50% in the 6-OHDA-treated rats. The concentration of NT was not significantly reduced by 6-OHDA treatment in any brain region; in fact, small but significant increases in NT levels in septum and olfactory tubercles ($p < .01$) were observed. Immunohistochemical studies using an antiserum to tyrosine hydroxylase (as a marker of DA neurons) and an antiserum to NT confirmed the findings obtained with the neurochemical methods described above. These data demonstrate that DA and NT are not co-localized in rat brain. (Supported by NIMH MH-34121, MH-33127, MH-22536, and NICHHD HD-03110.)

85.3 SECRETIN LIKE IMMUNOREACTIVITY IN THE CEREBROSPINAL FLUID OF RAT AND HUMAN. C.G. Charlton*, R.L. Miller*, P.A. LeWitt*, T.N. Chase and T.L. O'Donohue (SPON: M.E. Goldman). Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health, Bethesda, MD 20205 and Division of Clinical Pharmacology, Howard University, Washington, DC 20059.

Secretin like bioactivity and immunoreactivity (SLI) in extracts of rat and pig brain have been reported (Mutt et al. Life Sci 25: 1073, 1979; Charlton, et al. Peptides 2: Suppl. 1, 45, 1981 and O'Donohue, et al. Proc Natn Acad Sci USA 78:5221, 1981). This report describes the identification and characterization of SLI in the cerebrospinal fluid (CSF) of rat and human.

CSF was obtained by lumbar tap from normal human and by ventricular perfusion in rats. A stainless steel cannula was stereotaxically placed in the lateral ventricle of each rat. Another cannula was placed in the cisterna magna. Artificial CSF (O'Donohue et al., 1981) was infused into the lateral ventricle while withdrawn through the cisterna magna at 15 ul/min for 20 min periods. CSF was acidified to 0.5 M acetic acid and analyzed utilizing reverse phase high pressure liquid chromatography (HPLC) with a μBondapak C_{18} column and a specific radioimmunoassay for secretin.

Aliquots of rat CSF perfusate and synthetic porcine secretin showed similar HPLC retention characteristic and superimposable curves for the inhibition of ^{125}I-secretin binding to secretin antibody. The concentration of SLI in human CSF was 0.72 ± 0.05 ng/ml. In the rats the mean SLI for each 20 min collection period was 3.6 ± 0.48 ng/ml.

85.4 IMMUNOHISTOCHEMISTRY OF RAT INTERPEDUNCULAR NUCLEUS(IPN): SUBNUCLEAR DISTRIBUTION. G.S.Hamill,J.A.Olschowka,N.J.Lenn & D.M. Jacobowitz. Dept.of Neurol.and Clinical Neurosci.Research Center, Charlottesville,VA 22908 and Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

The distribution of cell bodies and processes within the IPN, containing substance P(SP),vasoactive intestinal polypeptide(VIP), cholecystokinin(CCK),somatostatin(SST),leu-enkephalin(L-ENK), serotonin(5HT) and dopamine beta hydroxylase(DBH) was determined in male rats treated with colchicine 48 hrs prior to perfusion. Serial 16μm frozen sections cut coronally, were examined for immunofluorescence. Variations in the density of terminal fluorescence were rated 1+(sparse) to 4+(intense).

Rostral subnucleus contained SP,SST,and L-ENK reactive somata. SP and VIP varicosities(2+) were present throughout Rostral, but were concentrated in 2 ovoid areas located dorsally in the caudal region of this subnucleus. CCK(2+) and L-ENK(3+) varicosities were also present, but were absent from these ovoid areas. 5HT(1+) SST(1+) and DBH(2+) processes were present diffusely.

Central subnucleus contained SST reactive somata distributed in a narrow band at its dorsal margin. Fine SP(1+) varicose processes extended as 2 vertically oriented bands adjacent to the lateral margins of the subnucleus, and in horizontal bands across its width. 5HT(1+) varicose processes were evenly distributed. Fine DBH(3+) varicose processes were evenly distributed, except for 2 laterally placed, vertical bands of decreased density. VIP(2+)and SST(1+) processes were observed only in the dorsal margin of Central. A very fine L-ENK(2+) terminal plexus was distributed throughout the Central subnucleus.

Intermediate subnuclei contained fine 5HT(1+) and DBH(3+) varicose processes, which were evenly distributed.

Lateral subnuclei contained evenly distributed fine SP(4+) varicosities that extended medially, ventral to the Intermediate subnuclei. 5HT varicosities(1+) were present but concentrated(3+) in the ventral lateral corners, which also contained 5HT somata. Fine VIP(3+),CCK(3+),and L-ENK(1+) varicose processes were concentrated along the medial edge of the subnuclei. A narrow,horizontal band without staining for SP,VIP,and CCK was observed in the Lateral subnuclei.

Dorsal subnucleus contained 5HT and L-ENK reactive somata. SP (2+),5HT(3+),CCK(2+),L-ENK(3+),VIP(2+) and DBH(1+) varicosities were present. Dorsal lateral subnuclei contained SP(2+),L-ENK(2+), DBH(2+) and SST(1+) varicosities. Interstitial subnuclei contained SP(4+),5HT(1+), and DBH(1+) varicosities.

The data demonstrate precise localization for various peptides, noradrenergic and 5HT systems within the IPN whose distribution and density of somata, and processes differ amongst the subnuclei of IPN.

85.5 IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROPEPTIDE Y (NPY) IN THE RAT BRAIN. J. Guy*, Y.S. Allen*, J.M. Polak* and G. Pelletier. MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada and Dept. of Histochemistry, Royal Postgraduate Medical School, London, U.K.

Recently, a 36 amino acid residue peptide, named neuropeptide Y (NPY) has been purified from porcine brain and fully characterized (Tatemoto, K., Proc. Natl. Acad. Sci. USA 79: 5485, 1982). In order to identify the nervous structures containing NPY, we performed the immunocytochemical localization of this peptide in the rat brain. For this purpose, adult rat brains were perfused with 4% paraformaldehyde or a mixture of 4% paraformaldehyde and 0.2% glutaraldehyde. Immunocytochemistry involving use of the peroxidase-antiperodixase complex was performed on both 30 μm thick Vibratome and ultrathin plastic sections (post-embedding technique). The Vibratome sections were used for light microscopic observations and also embedded for subsequent ultrastructural studies (pre-embedding technique). Antibodies against porcine NPY, raised in rabbits, did not show any significant crossreactivity with related peptides including peptide YY (PYY) and pancreatic polypeptide. Light microscopic immunocytochemistry revealed that immunostaining was widely distributed throughout the brain. Positive cell bodies and fibers were found in large number in the cortex, caudate-putamen nucleus, hypothalamic arcuate nucleus and hippocampus. High concentrations of fibers were also observed in the paraventricular and suprachiasmatic nuclei of the hypothalamus, the paraventricular nucleus of the thalamus and the periaqueductal gray. All these regions were examined for ultrastructural localization of NPY immunoreactivity. With the pre-embedding technique, immunostaining could be detected in axon profiles and endings. In these structures, the reaction product was located within the large dense core vesicles (70-100 nm in diameter) and also to some extent throughout the axoplasm. With the post-embedding technique, the staining was restricted to the large dense core vesicles in axons and endings. Only a small proportion of positive endings were seen making synaptic contacts with other neural elements. These results indicate that NPY could act as a neuromodulator or neurotransmitter of which the role remains to be clarified.

85.6 CO-LOCALIZATION OF CYCLIC GMP IN SUPERIOR CERVICAL GANGLION WITH PEPTIDE NEUROTRANSMITTERS. M.A. Ariano & E.L. Tress.* Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Synaptic activation of ganglionic afferents produces significant elevations in cyclic nucleotide concentrations in rat superior cervical ganglion (Briggs, et al, Cell Molec. Neurobiol. 2: 129-142, 1982). Immunofluorescent localization of cyclic AMP after stimulation is greatly augmented within satellite cells of the ganglion, while cyclic GMP immunofluorescence is increased within postganglionic neurons (Ariano, et al, Cell Molec. Neurobiol. 2: 143-156, 1982). This investigation has examined the coincidence of immunofluorescence of the peptide neurotransmitters, substance P, somatostatin, and met-enkephalin with cyclic GMP in rat superior cervical ganglion.

Pairs of ganglia were isolated from 17 Sprague-Dawley rats, and maintained in oxygenated Locke's solution for 30 min at 23°C, then frozen at -25°C in a cryostat and sectioned at 8 μm. Serial sections were mounted sequentially onto chrom alum-coated glass slides, and processed for neuropeptide and cyclic GMP immunoreactivity. Substance P, somatostatin, and met-enkephalin (Immuno-Tech, Inc., Chapel Hill, NC), and cyclic GMP antisera, synthesized in our lab and extensively characterized, were used at 1:500 to 1:1000 dilution in PBS, incubated overnight at 4°C in a moisture box. Unbound primary antisera were removed with PBS washes, and secondary, fluorescein-conjugated antisera (Cappel Labs, W. Chester, PA) was applied at 1:50 to 1:100 dilution in PBS for 1 hour. Following a final wash, sections were examined with ultraviolet optics, using a 485 nm/520 nm FITC filter combination. Photomicrographs of serially-identified regions of ganglia were studied and tabulation of all cyclic GMP-staining cells were compared to substance P, or somatostatin, or met-enkephalin immunofluorescent neurons. The percentage of peptide neurotransmitter-reactive cells versus the cyclic nucleotide was also determined.

Cyclic GMP-immunofluorescent postganglionic neurons were evenly distributed among the neuropeptide-reactive cells. 36.9% of the cyclic GMP-positive cells demonstrated co-localization with substance P, 36% of cyclic GMP-stained cells exhibited somatostatin reactivity, and 37.7% of the cyclic GMP-fluorescent elements also contained met-enkephalin reaction. 43% of the substance P-positive neurons demonstrate cyclic GMP-staining. 41% of somatostatin and met-enkephalin fluorescent neurons also demonstrate co-localization of cyclic GMP immunofluorescence. The results suggest cyclic GMP is not preferentially associated with one of the three peptide neurotransmitters studied. This work also suggests that some 60% of the neuropeptide-staining neurons utilize non-cyclic GMP mechanisms.

Supported by NSF grant BNS 81-02648.

85.7 INCREASE IN IMMUNOREACTIVE VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN THE MAJOR PELVIC GANGLION FOLLOWING INTERRUPTION OF THE PELVIC NERVE. W.G. Dail, M.A. Moll* and R.A. Dziurzynski*. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.

Postganglionic autonomic neurons are subject to a variety of influences mediated by their presynaptic input. For example, the appearance of certain enzymes in the postganglionic neuron during development depends upon the acquisition of functional connections by preganglionic cells. Preganglionic nerve activity is also known to elevate the activity of synthetic enzymes in adrenergic neurons. The effect of removal of the preganglionic input on the level of neurotransmitter in the postganglionic neuron is less clear, but the majority of studies indicate a slow, but significant, rise in catecholamines. In contrast, a very rapid and pronounced rise in peptides may occur in autonomic neurons following deafferentation (Schultzberg, et al. Neurosci. 3, 1978; Lewis, et al. J. Neurosci. 1, 1981; Kessler and Black, Brain Res. 234, 1982). In a series of studies to define the VIPergic innervation of male pelvic viscera, we have noted a rapid rise in immunoreactive VIP in the major pelvic ganglion of the rat after surgical section of the pelvic nerve, the presumed preganglionic parasympathetic input to this ganglion. In the intact ganglion, most of the neurons are VIP positive, as determined by immunofluorescent and immunoperoxidase methods (Dail, et al. in press, Neuroscience 1983). VIP staining appears as a nuclear halo in the Golgi zone. Only a few VIP-IR fibers are present in the neuropil of control ganglia. However, at three days following surgical interruption of the pelvic nerve, a rich plexus of VIP fibers appeared in the ganglion in close relationship to neurons. The plexus of fibers was notably sparse in the region of the ganglion known to contain adrenergic neurons. Some neurons stained intensely and evenly throughout their cytoplasm. This staining pattern was rarely seen in control tissue. Although no quantitative study was performed, the number of neurons which stained for VIP did not seem to increase after deafferentation. The changes we have described were present at five weeks after deafferentation. The remarkable increase in VIP staining may have several explanations: (1) preganglionic nerve activity is inhibitory to VIP and its removal causes an increase in VIP synthesis; (2) peripheral transport of VIP is merely slowed, resulting in loading of VIP neurons in the MPG; (3) aberrant axons of VIP neurons have been sectioned; and (4) the effect is non-specific, perhaps related to interruption of blood supply or stimulatory effects of degenerating tissue. Some of these possibilities are now being investigated. Supported by NIH RR-08139-09.

85.8 Hippocampal Cholecystokinin-like Neurons - A Fluorescence Retrograde Labeling Study, R. S. Greenwood and K. K. Winstead*. Dept. of Neurology and Neurobiology Program. U.N.C. Sch. of Med., Chapel Hill, N.C. 27514.

The hippocampal formation contains numerous intensely cholecystokinin-like (CCK-L) immunoreactive neurons. These neurons are found in most layers and subdivisions of the hippocampal formation. It is not known if these CCK-L neurons are only short-axon cells or if they have connections outside the hippocampal formation or to another subdivision of the hippocampal formation. In this study retrograde fluorescent dye tracing was combined with the indirect immunofluorescence technique to label hippocampal neurons and determine the course of axons of CCK-L immunoreactive neurons.

True Blue (5%, wt/vol) was injected into the lateral septal nucleus, the medial septal nucleus, the subiculum, or the entorhinal cortex of pentobarbital anesthetized rats. After four to six days for dye transport and after colchicine injection the brain was sectioned. The sections were examined for True Blue distribution and processed for cholecystokinin immunocytochemistry by the indirect immunofluorescence technique (Coons 1958) using an antiserum to the C-terminal octapeptide of cholecystokinin.

Previously described features of the efferent and intrahippocampal pathways of the hippocampal formation were observed. No definite CCK-L immunoreactive hippocampal neurons were labeled with True Blue following lateral septal nucleus, medial septal nucleus or entorhinal cortex injections. True Blue injections in the dorsal subiculum, however, did result in retrograde labeling of CCK-L immunoreactive neurons in regio superior and subiculum. These CCK-L immunoreactive neurons were located in stratum pyramidale of regio superior and layer III of the subiculum. The CCK-L immunoreactive neurons labeled with True Blue constituted a minority of the CCK-L immunoreactive neurons in regio superior and the subiculum. Adjacent to the sites of True Blue injection, CCK-L immunoreactive cells were frequently found to contain True Blue.

We conclude that the majority of CCK-L immunoreactive neurons in the hippocampal formation are short axon neurons. However, some CCK-L immunoreactive neurons in septal regio superior and the dorsal subiculum have axons that extend caudally to the subiculum.

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85.9 LOCALIZATION OF GASTRIN, CHOLECYSTOKININ, SRIF, INSULIN, AND GLUCAGON IN FELINE NERVOUS SYSTEM. L. Eldridge*, T. Yamada*, and W.F.H.M. Mommaerts. Department of Physiology, University of California at Los Angeles, CA 90024.

The distributions of five peptides in the nervous system of the cat were studied with previously published radioimmunoassay techniques. In thirteen normal adult cats of both sexes, gastrin, cholecystokinin (CCK), somatostatin (SRIF), insulin, and glucagon were assayed in spinal cord, dorsal root ganglia, and brain. The cord and ganglia tissues were divided into cervical, thoracic, lumbar, and sacral sections, and the cord sections were then split into dorsal and ventral portions. The brain tissues analyzed were cerebellum, brainstem, thalamus, cortex, hypothalamus, and pituitary. In the spinal cord as well as the dorsal root ganglia, the peptides were found in the greatest concentrations in the sacral tissue. The dorsal portions of the spinal cord usually were higher than the ventral. For all peptides, highest levels were found in the brain, but certain regions of the brain had much lower levels than were observed in many cord and ganglia tissues. Of the brain tissues, the cerebellum, brainstem, and thalamus were relatively low in peptides, while cortex, hypothalamus, and pituitary were moderate to high, depending on the peptide.

	GASTRIN	CCK	SRIF	INSULIN	GLUCAGON
			femtomols/ gm tissue		
Dorsal Ganglia					
Cervical	194	2860	1990	543	873
Thoracic	1230	3930	3750	1040	996
Lumbar	295	3200	3680	623	878
Sacral	776	11300	1200	3820	2220
Dorsal Cord					
Cervical	58	2060	6540	0	81
Thoracic	60	1530	7210	0	98
Lumbar	60	1950	11600	0	96
Sacral	314	9970	46100	1820	657
Ventral Cord					
Cervical	52	880	4730	0	74
Thoracic	41	721	4480	0	107
Lumbar	37	695	7780	0	99
Sacral	215	3850	33700	675	424
Brain					
Cerebellum	70	458	3630	0	81
Brainstem	90	2970	9120	0	139
Thalamus	105	9780	11500	0	104
Cortex	1000	49600	30300	0	194
Hypothal.	788	17700	59300	328	349
Pituitary	6900	15200	94800	6970	2190

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85.10 CCK NEURONS OF THE HIPPOCAMPUS AND SUBICULUM: POSSIBLE EXTRA-HIPPOCAMPAL PROJECTIONS. J.B. Nelson*, G.E. Handelmann*, M.C. Beinfeld, and T.L. O'Donohue. (SPON: D. Symmes). Lab. Cell Biology, NIMH, Bethesda, Md.; Dept. of Pharmacology, St. Louis Univ., St. Louis, Mo.; Experimental Therapeutics Branch, NINCDS, Bethesda, Md. 20205.

Cholecystokinin (CCK), a peptide hormone originally identified in the gut, is also contained in neurons in the brains of a number of vertebrate species and appears to act as a neurotransmitter. In the rat brain, the hippocampus contains a high concentration of CCK. Neuronal cell bodies containing CCK-immunoreactive material were identified in the hippocampus, dentate gyrus, and subiculum [1,2]. These polymorphic cells were located subjacent to the pyramidal cells in stratum radiatum in both regio superior and inferior of the hippocampus. A small number were located in other cell layers. In the dentate gyrus, they were located subjacent to the granule cells. CCK-positive terminals were visible in the hippocampal pyramidal layer and dentate granule layer.

Destruction of the major hippocampal afferents had no effect on the CCK content of the hippocampus, indicating that the intrinsic cells are responsible for the hippocampal CCK content. Destruction of the septo-hippocampal pathway, however, produced decreases in CCK content of the septum and hypothalamus of 59% and 24%, respectively. A microdissection analysis indicated that the loss of CCK occurred only in regions receiving direct projections from the hippocampus and subiculum: lateral septum, nucleus of the stria terminalis, mammillary bodies, and anteroventral nucleus of the thalamus. These results suggest that CCK-containing neurons of the hippocampus and subiculum project to extra-hippocampal regions. CCK may therefore be important in mediating neural function in the limbic system.

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2. Greenwood, R.S., Godar, S.E., Reaves, T.A., and Haywood, J.N. J. Comp. Neurol., 1981, 203: 335.

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85.11 THE PREFERENTIAL DISTRIBUTION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN THE SACRAL SPINAL CORD OF THE RHESUS MONKEY. J.R. Roppolo, I. Nadelhaft, W.C. deGroat. Depts. of Pharmacology and Neurosurgery, Univ. of Pittsburgh and V.A. Hosp., Pittsburgh, PA

Immunohistochemical (IHC) studies have localized VIP containing neural processes in various peripheral structures including the GI tract, gentiourinary tract, and in spinal and autonomic ganglia. In addition recent IHC studies in the cat spinal cord revealed that the distribution of VIP is restricted to the sacral segments of the cord. This distribution of VIP resembles closely the distribution of pelvic nerve afferents in the cat sacral spinal cord. The present study was undertaken to determine whether VIP has similar localized distribution in the monkey spinal cord.

The distribution of neuronal processes containing VIP-immunoreactivity (VIPIR) was studied in five rhesus monkeys using standard immunohistochemical techniques (either PAP or FITC methods). In two monkeys the sacral dorsal roots on one side were transected 10-14 days prior to sacrifice.

VIPIR neuronal processes were localized primarily in the sacral segments of the spinal cord and in the sacral dorsal root ganglia (DRG). VIPIR processes within the DRGs were seen as single fibers or thick bundles leaving the DRG and passing into the dorsal root. VIPIR cell bodies in the DRGs were not seen in the present study since visualization of cells usually requires pretreatment with an axonal transport inhibitor. Labelled fibers were seen projecting from the sacral dorsal rootlets, to their spinal entry zone and into Lissauer's tract (LT). The VIPIR in LT was arranged in thick bundles (5-20 μm) and in the sacral segments was distributed throughout LT. In horizontal sections these thick bundles could be seen as groups of long rostrocaudal projecting fibers. From LT fibers projected in the superficial laminae of the dorsal horn (DH). The projection along the lateral edge of the DH reached lamina V. Here some of the fibers ended abruptly near the sacral parasympathetic nucleus (identified by AchE stain) while a few fibers could be seen directed medially in laminae V and VI to the dorsal commissure (DC). A much less dense projection was seen along the dorsomedial border of the DH to DC. In lumbar and coccygeal spinal segments, the VIPIR in the DH disappeared. However, the VIPIR in LT could be detected as far rostral as T_{13}. The fibers in LT at the more rostral segments of the spinal cord occupied a more medial position. Section of the sacral dorsal roots markedly reduced the VIPIR fibers in the ipsilateral cord while ventral root section had no effect.

These results suggest that the majority of VIP fibers seen in the lumbosacral spinal cord are afferent fibers entering at the sacral level and that the distribution of these fibers resembles very closely the distribution of pelvic nerve afferents reported previously in the rhesus monkey.

85.12 THE DISTRIBUTION OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN THE LUMBOSACRAL SPINAL CORD OF THE RAT. I. Nadelhaft, V.A.Med. Centr. and Dept. of Neurosurg., Univ. of Pittsburgh, Pittsburgh, PA 15261

Recent immunohistochemical studies of the location of VIP immunoreactivity (VIPIR) in cat, monkey and human spinal cord have demonstrated a rather unique and restricted distribution for this polypeptide; 1) VIP was relegated to afferent fibers entering the cord via the sacral roots. 2) In the sacral cord, VIPIR was found in Lissauer's tract (LT), the superficial layers (I and II) of the dorsal horn, and characteristically in a prominent collateral projection around the lateral border of the dorsal horn into the area containing preganglionic neurons forming the sacral parasympathetic nucleus (SPN). 3) Outside the sacral region VIPIR was located mainly in LT. In contrast to these findings, the present study in the rat exhibits a quite different VIPIR distribution. Sprague-Dawley rats (300-400 gm) of either sex were used and the peroxidase anti-peroxidase immunohistochemical technique employed on 30 micron cryostat sections. Eight segments (S2-L2) were examined including the region containing the SPN (S1-L6). VIPIR was found in LT and dorsal horn laminae I and II (outer) in all these segments. The reaction was intense in LI and appeared to be contained in fibers running parallel to the boundaries of the lamina. In LII a less intense reaction was noted organized in varicosities along short fiber fragments in a random fashion characteristic of a terminal field. The intensities of VIPIR did not vary greatly from segment to segment. In segments L6 and L1 a collateral afferent projection extended along the lateral margin of the dorsal horn and into the area containing SPN neurons. This is homologous to the sacral lateral projection noted in cat, monkey and human. A prominent but small (diameter 20 microns) longitudinal fiber bundle was located on the midline just beneath the central canal in all segments. Other studies in the rat have revealed Substance P immunoreactivity in this same location and experiments in which horseradish peroxidase was applied to the rat pelvic nerve have shown that this fiber bundle contains visceral afferent projections from that nerve. Radially oriented strings of VIPIR varicosities were located in the lateral and ventral funiculi and a few fragments were noted in the dorsal columns. VIPIR fibers with varicosities were also found sparsely distributed throughout the gray matter.

In summary, this study has shown that although there is some specialization of the distribution of VIP in the region of the sacral parasympathetic nucleus in the rat as in the cat, monkey and human, the intensity and location of VIP within the cord outside this area is quite different from those other mammals. Thus, VIP in the rat spinal cord may be associated with pathways and functions other than those of the pelvic viscera.

85.13 EDINGER-WESTPHAL NUCLEUS: IMMUNOCYTOCHEMISTRY AND DESCENDING PROJECTIONS IN THE CAT. B.S. Phipps, R. Maciewicz, B.B. Sandrew*, and J.B. Martin, The Stanley Cobb Lab. for Psychiatric Res. and Depts. of Neurology, Neurosurgery, and Psychiatry, Mass. General Hospital and Harvard Med. Sch., Boston MA 02114.

The Edinger-Westphal nucleus (EW) is a well-defined collection of medium-sized neurons lying dorsal and anterior to the oculomotor nucleus. Although classically thought to principally contain preganglionic parasympathetic neurons that innervate the orbit, axoplasmic transport studies demonstrate that the projections of EW are considerably more complex. In the cat, EW has descending projections to spinal dorsal horn and several brain stem nuclei, including the superficial layer of the spinal trigeminal nucleus (nV). Recent studies also show that the majority of neurons in EW demonstrate substance P-like immunoreactivity (SP-LI). Since SP and cholecystokinin (CCK) are found together in some central and peripheral neurons, we examined EW cells for CCK-like immunoreactivity (CCK-LI) and compared the distribution of EW neurons with CCK-LI to the pattern of cells in the same region that stain for SP-LI. Immunoperoxidase methods in colchicine pretreated animals demonstrated cells with CCK-LI throughout the length of EW. The frequency, appearance and distribution of cells stained for CCK-LI was similar to that of EW cells that demonstrated SP-LI. The pattern of cells with CCK-LI also closely corresponded to the distribution of EW neurons that project to spinal cord, but was quite different from the pattern of preganglionic cells that project to the orbit. A combined retrograde transport-immunocytochemical method was next used to determine whether EW neurons that project to spinal cord also demonstrate CCK-LI. Simultaneous co-localization of retrogradely transported horseradish peroxidase (HRP) and CCK was accomplished by using 3,3'-diaminobenzidine (DAB) for the HRP histochemistry and alpha-napthol/pyronin B for the peroxidase-antiperoxidase (PAP) immunocytochemistry. In sections exposed to CCK antibody, cells showing CCK-LI were found throughout the rostrocaudal extent of EW, and many CCK-LI positive neurons throughout EW also contained retrogradely transported HRP.

In companion experiments, HRP was injected into spinal cord and either nuclear yellow or DAPI was injected into nV on either side. In these studies numerous EW neurons were retrogradely labeled with both HRP and a fluorochrome, evidence that some EW cells project to both nV and spinal cord. These findings demonstrate a direct pathway from midbrain to dorsal horn that contains CCK and probably also SP. As many EW neurons project to both nV and spinal levels, this system may have a widespread rather than regional effect on somatosensory transmission.

85.14 THE DISTRIBUTION OF MET-ENKEPHALIN (ME), SEROTONIN (5HT) AND SUBSTANCE P (SP) IMMUNOREACTIVITIES IN THE AREA POSTREMA (AP) OF THE RAT AND CAT. B. W. Newton*, B. Maley and H. H. Traurig*. Dept. of Anatomy, Univ. Kentucky Med. Ctr., Lexington, KY 40536.

The area postrema, the proposed emetic center, lies on the dorsal surface of the brainstem at the level of the obex. It is a midline structure in the rat, but is bilateral in the cat. Using the peroxidase, antiperoxidase technique, ME, 5HT, and SP immunoreactivities were localized in the AP of the two species. Eight cats and eight rats were sacrificed by vascular perfusion transcardially with 4% paraformaldehyde in 0.1 M Sorenson's phosphate buffer, pH 7.2.

In both the rat and the cat, ME had the greatest accumulation of immunoreactive fibers, followed by 5HT and SP. The rat AP contained ME and 5HT immunostained neurons, while the cat AP possessed only ME immunostained cell bodies. ME immunostained fibers, in the rat, were present along the ventrolateral border and the dorsal surface of the AP. ME immunoreactive cell bodies were found mainly along the ventrolateral and ventral borders. The cat's AP had the majority of ME immunoreactive fibers at its periphery in caudal sections with fewer fibers more rostrally, while the central region of the AP contained few immunoreactive fibers. ME immunostained cell bodies were located principally in central portions of AP with the cat having fewer cell bodies than the rat. For the rat, 5HT immunoreactive fibers were present along the AP's ventral and ventrolateral borders, with the fibers becoming more dense rostrally. 5HT immunoreactive cell bodies were seen centrally throughout the entire extent of the AP. In the cat, 5HT immunostained fibers were most prominent along the lateral border. The medial border contained fewer fibers which decreased in number from a caudal to rostral direction. In the rat the heaviest concentration of SP immunoreactive fibers was along the ventrolateral border in caudal sections, while the fiber numbers decreased rostrally. SP immunoreactive fibers, which increased in more rostral sections, were found mainly along the lateral border. There was consistent but low immunostaining along the medial border with some central AP regions lacking SP immunoreactivity. For all of the neuropeptides examined, in both species, the central region throughout the AP had the least numbers of immunostained fibers.

The heterogeneous distribution of immunoreactive fibers and neurons in the AP suggests possible functional divisions within the region. Differences between the cat and the rat may be due to species differences in their respective emetic and non-emetic behavior.

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85.15 VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IS LOCALIZED TO THE LUMBOSACRAL SEGMENTS OF THE HUMAN SPINAL CORD. M. Kawatani*, I. Lowe, J. Moossy*, J. Martinez*, I. Nadelhaft, R. Eskay* and W.C. deGroat. (Spon: B. Dixit). Depts.of Pharmacol.,Pathol.and Neurosurg.,Univ. Pittsburgh and V.A. Hosp.,Pittsburgh, PA 15261

Recent immunohistochemical (IHC) studies of the cat spinal cord revealed that VIP is located primarily in the sacral segments where it is contained in afferent projections to Lissauer's tract (LT) and lamina I on the lateral edge of the dorsal horn (DH). Dye tracing combined with IHC showed that VIP was present in sacral afferent pathways from the pelvic viscera. The present experiments were undertaken to determine whether VIP has a similar localized distribution in the human spinal cord.

Spinal cords were removed at autopsy (8 hrs post mortem) and fixed by immersion in Zamboni's solution. The tissue was sectioned in a cryostat and processed by indirect immunofluorescence or the PAP method. VIP-immunoreactivity (VIPIR) exhibited a very restricted segmental distribution in the human spinal cord being localized primarily to the sacral region, where the highest concentrations were present in LT and the superficial layers of the DH. Smaller amounts of VIPIR were detected in LT as far rostral as L_2 and caudally into the coccygeal segments, however, relatively few VIP terminals were present in the DH of the lower thoracic, lumbar and coccygeal segments.

In LT at the sacral level VIPIR was present in thick (6-25 μm) bundles of rostrocaudally oriented axons. In lamina I of the sacral dorsal horn fine VIP axons and varicosities were heavily concentrated at the apex and lateral edge of DH, with relatively small numbers on the medial side of the DH. This contrasts with the distribution of substance P (SP) terminals which were located throughout laminae I-III of the DH. Some of the VIP and SP terminals were present in the sacral parasympathetic nucleus which was identified by AchE stain in S_3 and S_4 segments. Large numbers of L-Enk terminals were also present in this area. In addition, a few VIP axons projected from lateral lamina I through lamina V to end in the region of the dorsal commissure and central canal. Horizontal sections showed that VIP terminals at the ventral end of lateral lamina I exhibited a periodic organization in which clusters of terminals occurred at regular intervals (300 μm) along the length of the cord. In horizontal sections VIP was also detected in rostrocaudally oriented axons in the dorsolateral funiculus.

In summary the present study has shown that in the lumbosacral and coccygeal segments of the human spinal cord VIP is localized primarily in LT and on the lateral edge of the dorsal horn. This distribution suggests that VIP may be associated with pelvic visceral afferent pathways as demonstrated previously in the cat.

85.16 SUBSTANCE P-CONTAINING SENSORY NERVES OF THE RAT IRIS: NORMAL DISTRIBUTION, ONTOGENY, REINNERVATION OF INTRAOCULAR IRIS GRAFTS AND DENERVATION BY CAPSAICIN. A. Seiger*, C. Ayer-LeLievre*, U.-B. Selin*, I. Black and J. Kessler. Karolinska Institute, Stockholm, Sweden, Cornell Univ. Med. Coll., New York, U.S.A., and Albert Einstein Coll. Med., New York, U.S.A.

Substance P (SP) in sensory nerves of iris stretch preparations was visualized using immunohistochemical fluorescence. SP innervation exhibited a characteristic 2-dimensional pattern with irregular plexuses and scattered arcades of non-terminal axons. Both sphincter and dilator areas were invested by SP nerves, whereas blood vessels were not associated with such fibers.

During ontogeny, scattered SP nerves were already present in newborn irides. Density of the fiber plexuses increased by 4 and 8 days postnatally, and approximated adult levels by 14 days. To study re-innervation, iris grafts were placed in oculo: no SP was detectable 5 days post-operatively, however, reinnervation occurred rapidly. Scattered ingrowing fibers were apparent at 2 weeks; slightly irregular plexuses and axons of normal density were present after 4 weeks and remained unchanged for 2 and 3 months. In host irides, SP fibers were initially normal, but moderate hyperinnervation developed 4-8 weeks after grafting. Capsaicin (C) treatment (50mg/Kg s.c.) of adults resulted in total disappearance of iris SP fibers within 3 days, and no reappearance for at least 12 weeks. After identical treatment of neonates, normal numbers and patterns of SP fibers were present at 4 and 8 weeks. However, administration x 4 at weekly intervals caused disappearance of all SP fibers by 4 and 8 weeks of age. Moreover, single intraocular injections of C (5μl, 2.5mg/ml.) caused a 70-80% decrease in iris SP fibers at 10 days. After 8 weeks SP fibers had reappeared with a normal distribution. Our results suggest that SP may be used to examine normal sensory innervation of targets as well as ontogenesis and regeneration.

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85.17 THE IDENTIFICATION OF LEUCINE ENKEPHALIN AND SOMATOSTATIN IN THE SACRAL PARASYMPATHETIC PREGANGLIONIC OUTFLOW TO THE COLON OF THE CAT. I.P.Lowe, M.Kawatani*, W.C.deGroat, Dept. of Pharmacology, Medical School, University of Pittsburgh, Pittsburgh, PA 15261

The presence of leucine-enkephalin (L-Enk) and somatostatin (SS) in neurons in the region of the sacral parasympathetic nucleus has raised the possibility that these peptides may be co-transmitters with acetylcholine in the sacral preganglionic outflow to the pelvic viscera. Support for this view was obtained in recent studies on the innervation of the cat urinary bladder where it was shown that L-Enk is localized in vesical preganglionic pathways and may function as an inhibitory transmitter in bladder ganglia. The present experiments were undertaken to examine the relationship of SS and L-Enk with the sacral preganglionic outflow to the colon of the cat.

Ganglia on the surface of the distal colon were removed from normal animals and from animals in which the sacral ventral roots had been transected unilaterally for 4-8 weeks to produce degeneration of the preganglionic input to one side of the colon. Frozen sections of ganglia were processed by indirect immunohistochemical methods to identify SS, L-Enk, substance P and vasoactive intestinal polypeptide (VIP).

Colon ganglia were characterized by dense networks of SS and L-Enk terminals surrounding the ganglion cells. Axons and axonal varicosities containing SS and L-Enk were also present between the ganglion cells and in nerves outside the ganglia. Pericellular L-Enk terminals occurred in a greater density than SS-terminals. In animals where the ventral roots were transected the L-Enk and SS terminals were eliminated in ganglia ipsilateral to the transection but were normal in the contralateral ganglia. Axons containing SS were also eliminated on the transected side, however, some axons containing L-Enk remained.

Axons and varicosities exhibiting VIP and substance P immunoreactivity were distributed throughout colon ganglia, although not in pericellular networks similar to L-Enk and SS. VIP was also present in some ganglion cells. The distribution of VIP and substance P immunoreactivity was not changed by ventral root transection.

In summary, the present findings indicate that SS and L-Enk terminals in extramural colon ganglia are of central origin, and most likely are a component of the parasympathetic preganglionic pathway arising in the sacral parasympathetic nucleus. Further studies will be necessary to determine whether SS and L-Enk have synaptic modulatory functions in colon ganglia as previously demonstrated for L-Enk in bladder ganglia.

85.18 SUBSTANCE P-CONTAINING FIBERS IN MIDDLE CEREBRAL ARTERIES - ORIGIN AND ULTRASTRUCTURE. L.Y. Liu-Chen, T. Liszczak, S.A. Gillespie*, M.R. Mayberg*, & M.A. Moskowitz. Neurosurgery and Neurology Services, Massachusetts General Hospital, Boston, MA. 02114 U.S.A.

Direct evidence for a substance P (SP)-containing trigeminovascular pathway was found in cats using the techniques of labeling two antigens both within a single section and in adjacent sections. Wheat germ agglutinin (WGA), a retrogradely transported marker, was applied to the proximal segment of the right middle cerebral artery. Forty eight to sixty four hours after the above application, cats were treated with colchicine intracisternally, and 6 hours later perfused with buffered 4% paraformaldehyde. Successive immunocytochemical localization of WGA and SP on single sections was performed using avidin-biotin-peroxidase (ABC method) and dual color reactions. Diaminobenzidine was used to identify immunoreactivity attributed to WGA (brown); 4-chloro-1-naphthol was used to identify cells containing immunoreactive SP (ISP) (blue). Cells containing blue were found in all three trigeminal divisions; small numbers of cells containing brown or brown and blue were found in the ipsilateral first division only. Results of the immunocytochemical experiments using alternate adjacent thin sections confirmed the coexistence of WGA and SP immunoreactivities in the same neuron.

Immuno-electron microscopy of ISP-containing afferent processes were performed in rat and cat cerebral arteries combining pre-embedding and ABC methods. Preliminary results indicated that fibers containing electron dense reaction product were associated with Schwann cells and were unmyelinated; axons contained neurotubules (24nm), microfilaments (10nm), mitochondria and vesicles; varicosities contained numerous clear vesicles (40-60 nm) and some dense core vesicles (60-120 nm), but few, if any, neurotubules. No membrane specializations were observed within varicosities. Control tissues incubated either with normal rabbit serum, or SP antiserum preadsorbed with SP did not contain reaction product within nerve processes.

In summary, trigemino-vascular projections to middle cerebral artery contain ISP in vesicle-containing small unmyelinated fibers.

85.19 EVIDENCE FOR THE PRESENCE OF NEUROTENSIN-LIKE IMMUNOREACTIVITY IN A SCHWANNOMA CELL LINE. A. Nath*, B. Haber, G. Bissette, L. Jennes*, P.J. Manberg*, and C.B. Nemeroff. Marine Biomed. Inst., Univ. Tex. Med. Branch, Galveston, TX 77550 and Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514.

Neurotensin (NT) is an endogenous tridecapeptide that is heterogenously distributed in the mammalian CNS and gastrointestinal (GI) tract. It is believed to be present in neurons of the CNS and in endocrine-like cells in the GI-tract. The purpose of the present study was to determine whether NT is present in a variety of glial and neural cell lines. To this end, NT-like immunoreactivity (NTLI) was determined by radioimmunoassay (RIA) as previously described (Manberg et al., J. Neurochem. 38:1777-1780, 1982) using an antiserum directed toward the mid-portion of the molecule. In addition, a C-terminally directed antiserum to NT, as previously described (Jennes et al., J. Comp. Neurol. 210: 211-224, 1982), was utilized to immunohistochemically (IHC) visualize NTLI in the cell lines. Using the RIA, NTLI was found to be present in the RN-22 Schwannona cell line but not in several neuroblastoma cell lines (BN41A, SK5Y and N2A), a C6 glioma line or B110, a rat glioma line. The immuncytochemical method, for visualization of NT was conducted after the cell lines were fixed in acetone: methanol (1:2) and incubated with NT antiserum (1:500 dilution) for 16 hrs. Using an indirect immunohistofluorescent method (fluoroscein isothiocyanate or thodamine), NTLI was visualized in the cytoplasm of the RN-22 Schwannona cells but not in a variety of other cell lines including the SK5Y human neuroblastoma, C6 glioma and bovine aortic endothelial and fibroblast cells. The staining of NTLI was abolished by addition of exogenous NT. Although the molecular nature of the NTLI is unknown, these findings raise the question as to whether NT occurs in the nervous system in cells other than neurons.

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METABOLISM OF TRANSMITTERS AND MODULATORS

86.1 FORMATION AND METABOLIC DISPOSITION OF NE METABOLITES IN MOUSE BRAIN. P.P.LI, J.J. Warsh and D.D. Godse*, Dept. of Biochemical Psychiatry, Clarke Institute of Psychiatry, University of Toronto, Toronto, Ontario.

Although 3,4-dihydroxyphenylethyleneglycol (DHPG) and 3-methoxy-4- hydroxyphenylethyleneglycol (MHPG) are the major brain NE metabolites, the metabolic interrelationship between these metabolites has not been completely clarified. In this study we have examined the kinetics of formation and disappearance of brain DHPG and MHPG to estimate the degree of conversion of DHPG to MHPG and the amount of DHPG eliminated unchanged from the brain. Studies were undertaken in the mouse, a species in which these brain metabolites occur principally in unconjugated form, as in humans. Turnover estimations were obtained with the technique of synthesis inhibition following monoamine oxidase (MAO) and/or catechol-O-methyltransferase (COMT) inhibition. Quantitation of DHPG and MHPG was performed simultaneously by GC-MS.

Preliminary studies indicated immediate and maximal inhibition of MAO-A and COMT following clorgyline (10 mg/kg i.p.) and tropolone (75 mg/kg i.p.), respectively. During the first 30 min following tropolone, brain DHPG levels accumulated linearly at a rate of 1340 pmol/g/h, whereas MHPG disappeared exponentially at a rate of 411 pmol/g/h, with an estimated fractional rate of elimination (k) of 1.5/h. Following clorgyline administration, brain DHPG declined exponentially at a rate of 1200 pmol/g/h (k = 10.9/h). In contrast, the elimination of MHPG followed first-order kinetics only when COMT was also inhibited in addition to MAO. Thus combined clorgyline and tropolone treatment resulted in an exponential decline of MHPG levels at a rate of 520 pmol/g/h (k = 1.8/h), whereas DHPG levels were slightly but significantly elevated compared to control values. As it was possible that a small but significant amount of NE might be deaminated through MAO-B in the presence of MAO-A and COMT inhibition, the animals were treated with a combination of clorgyline, tropolone and pargyline (75 mg/kg). Under this condition, brain DHPG and MHPG disappeared at rates of 40 and 660 pmol/g/h (k = 0.4 and 2.0/h), respectively.

The results suggest that mouse brain DHPG is cleared primarily through O-methylation with minimal direct elimination from the brain. Assuming the disposition of NE metabolites are similar in mouse and human brain, then peripherally measured DHPG in humans is likely derived principally from extracerebral sources and more likely reflects peripheral sympathetic function.

This work has been supported in part by the Medical Research Council of Canada.

86.2 TRYPTOPHAN AND SEROTONIN METABOLISM AFTER SUSTAINED INFUSION OF TRYPTOPHAN BY MINI-OSMOTIC PUMP. R. I. Peters and B. R. Buhr*. Dept. of Biological Sciences, Wichita State University, Wichita, KS 67208.

In an attempt to further delineate the regulatory role of tryptophan in serotonin synthesis and turnover, mini-osmotic pumps charged with L-tryptophan or vehicle were implanted subcutaneously in albino mice. After 24 or 96 hours of infusion, plasma and brain tryptophan concentrations, brain serotonin and 5-hydroxyindoleacetic acid concentrations, and hepatic tryptophan pyrrolase activities were measured. After 24 hr of infusion there was little difference in plasma tryptophan values of treated and control animals, presumably because of the elevated activity of the hepatic degradative enzyme seen in the treated animals. At this time serotonin concentrations were elevated in treated animals, although there were no apparent differences in brain tryptophan or 5-hydroxyindoleacetic acid concentrations. After 96 hours of infusion the activity of hepatic tryptophan pyrrolase was reduced in treated animals, and both plasma and brain tryptophan concentrations were elevated. Despite the elevated brain tryptophan at this time, serotonin and 5-hydroxyindoleacetic acid concentrations were not significantly elevated in treated animals. Thus, although even a very low dose of tryptophan (c.a. 0.47 mg/Kg-hr) infused for 24 hr was sufficient to elevate brain serotonin concentrations, sustained infusion of tryptophan at this rate resulted in a dissociation of serotonin synthesis from precursor availability.

86.3 EFFECT OF LOW pH ON CHATECHOLAMINES (CA) SYNTHESIS AND RELEASE IN THE CAT CAROTID BODY. (c.b.) R.Rigual*, E.González*, S.Fidone and C.González. Dept. of Physiol Fac. de Med. Valladolid, Spain, and Dpt. of Physiol. Sch. of Med. Univ. of Utah, Utah. U.S.A.

The c.b. is a CA containing organ, being dopamine (DA) the most abundant. DA has been suggested to play some role as modulator of chemosensory activity. Recently, Fidone et al (J.Physiol.333:69-110) have shown a close relationship among hypoxic stimulations, synthesis and release of DA and activity in the carotid sinus nerve (c.s.n). In order to define the precise role of DA in the chemoreception it is necessary to know if the relationship found for hypoxia exits for other natural stimuli. The present data show that it is also the case for acidic stimuli.

The c.bs. were isolated and cleaned of surrounding tissues. To study the effect of low pH on CA synthesis, the c.bs. were incubated for 3h in a modified Tyrode (pH 7.4 controls, pH 7 experimentals) equilibrated with 100% O_2 and containing 5mM glucose, 1mM ascorbic acid 0.1 mM 6-MPH_4 and 0.04 mM ^{3}H-Tyr. To study the relationship among the intensity of acidic stimulation, the c.s.n. activity and ^{3}H-DA release, the c.bs. were incubated with ^{3}H-Tyr and then superfused (Fidone, 333:69-110) with 100% O_2 equilibrated Tyrode at different pH. The effluents were collected for ^{3}H-DA analisis and the electrical activity was simultaneously monitored.

The c.bs. incubated at low pH showed a 190% increase in total synthesis of CA, most of these being ^{3}H-DA. Superfusion of the c.bs. with Tyrode of increasing acidity (pH 7.4 to 6.6) promotes a progresive increase in both c.s.n. activity and ^{3}H-DA release. The acidosis-induced release showed a marked Ca^{++} dependence. In chronically deafferented c.b.s. the acidosis-induced release was moderately reduced. Our data, as a whole, are consistent with a role for DA in the response to acid. Specifically our data suggest that low pH activates tyrosine hydroxylase and explain previous findings of increased stores of DA in acidotic animals. The released data show a direct effect of acidosis on type I cells and, consequently, the assumption that the acidic stimuli acts only on the chemosensory nerve endings seems untenable.

This work was supported by a Grant from the C.A.I. C.T.

86.4 SENSITIVITY OF MITOCHONDRIAL MAO-A AND RESISTANCE OF MAO-B TO INHIBITION BY TRYPSIN AND OTHER PROTEASES: COMPARISONS ACROSS TISSUES AND SPECIES. Helen L. White* and Deborah K. Stine* (SPON: R.M. Ferris). Dept. of Pharmacology, Wellcome Research Labs, Research Triangle Park, NC 27709.

A selective inhibition of MAO-A (monoamine oxidase-A) activity by trypsin has been observed in human brain or liver extracts [White & Tansik (1979) in Monoamine Oxidase: Structure, Function, and Altered Functions (eds. T.P. Singer, et al.) Academic Press, 129-144]. This inhibition occurs in both crude mitochondrial extracts and in solubilized preparations of the enzyme. In the present study the protease sensitivities of MAO-A and B in different tissues and species (rat, rabbit, dog, man) have been compared in an effort to explore factors that may influence the wide variations in MAO-A and B activities that are found in brain and peripheral tissues of different species. MAO-A/B ratios in mitochondrial extracts from brain, heart, and liver were estimated by determination of substrate affinities and selective inhibitions at low concentrations of clorgyline or deprenyl. Effects of incubations with trypsin, chymotrypsin, thermolysin or papain on MAO-A/B activities were interpreted in terms of known specificities of these proteases. Trypsin, which tends to attack basic peptide residues, was the most effective in causing loss of MAO-A activity, while MAO-B was remarkably resistant to the action of this protease in all MAO extracts studied except those from rabbit. For example, MAO-A of human brain, human liver, and dog heart were inhibited 76 - 86% after incubation at 37°C with 10 U/ml of trypsin, while MAO-B activity was unaffected. MAO-A of rat mitochondria was somewhat less sensitive to trypsin than were human and dog enzymes. On the other hand, MAO-B in mitochondrial preparations from rabbit was also sensitive to trypsin. MAO inhibition by trypsin was prevented by soybean trypsin inhibitor and was diminished in the presence of MAO substrates at concentrations that can protect enzyme active sites and, indirectly, enzyme conformation.

Although species differences were observed in the responses to various proteases, the sensitivity to trypsin hydrolysis of MAO-A from all sources examined leads to a conclusion that MAO-A may contain basic residues that are important for enzyme activity, either as part of the enzyme protein sequence or in an associated regulatory protein. The resistance of MAO-B in most extracts may indicate a lack or inaccessability of such basic residues. No interconversion of A and B activities was observed. In general, the results of this study support the concept that MAO-A and B are two distinctly different independent enzymes that may be embedded in the same mitochondrial membranes.

86.5 ESTRADIOL OR 2-OH-ESTRADIOL TREATMENT ALTERS SEROTONIN UPTAKE AND METABOLISM IN RAT BRAIN. S. Kowalik* and A.I. Barkai* (SPON: M. Baron) N.Y.S. Psychiatric Inst. and Dept of Psychiatry Columbia University Coll. of Physicians & Surgeons, N.Y. 10032.

The influence of the steroid sex hormone estradiol (E) on brain function and behavior is presumably mediated by E receptor sites which are located in certain brain areas of either females or males. Variations in circulating E levels which affect behavior are associated with changes in monoamines. Because of the apparent antidepressive effects of E and because the availability of serotonin (5-HT) has been implicated as an important factor in depression, the effects of chronic E and its catechol metabolite 2-OH-estradiol (2-OH-E) on the metabolism or uptake of 5-HT were studied. Male rats (180-220g) were treated with E or 2-OH-E (5 µg/kg) daily for 5 days. For 5-HT uptake studies animals were sacrificed 30 min. after the last injection and the hypothalamus or cortex homogenized in Krebs-phosphate buffer. Homogenate aliquotes were incubated for 5 min. at 37° C with ^{14}C-5HT concentrations ranging from 0.2 - 2 µM. Vmax and Km were determined using the Wolfe-Augustinson-Hofstee method. Changes in 5-HT metabolism in the intact brain were estimated from the concentrations of 5-Hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF). Cisternal CSF was withdrawn from the urethane-anesthesized animal at a rate of 2 µl/min. and 5-HIAA concentrations were determined using LCEC. In the uptake studies, treatment with either E or 2-OH-E significantly decreased Vmax values in the hypothalamus (2.1 ± 0.4 or 2.9 ± 0.3 µmol/5 min/mg tissue, respectively) compared to controls (3.7 ± 0.2) whereas no significant change was seen for cortex tissue. The level of 5-HIAA in the CSF was significantly higher in animals treated with 2-OH-E compared to controls (330 ± 20 vs 267 ± 16 ng/ml) but was not significantly different in E-treated rats (289 ± 7). These results indicate that E and 2-OH-E may act to decrease 5-HT uptake in the hypothalamus and that 2-OH-E may also enhance the metabolism of 5-HT to 5-HIAA presumably by increasing its availability in the synapse.

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86.6 ENZYMATIC DEACETYLATION OF N-ACETYLHISTAMINE IN RAT BRAIN. L.R. Hegstrand, T. Kalinke*, R. J. Hine* and C. Barksdale*. Univ. of Wisconsin and Middleton Vet. Admin. Hospital, Madison, WI 53706.

There is substantial evidence supporting a neurotransmitter role for histamine (HA) in brain. However, the mechanisms involved in the regulation of the biosynthesis of HA are poorly understood. Possible points of regulation are histidine uptake, histidine decarboxylase (HDC) and N-acetylhistamine deacetylase (NAcHAD). Chudomelka and Murrin (J. Neurochem. 40, 830, 1982) concluded from their studies on synaptosomal histidine uptake that this probably is not an important regulatory step in HA synthesis. Although the conversion of histidine to HA via the specific HDC is the most important biosynthetic pathway for HA formation, maximal in vivo doses of 2 HDC inhibitors, brocresine and α-hydrazinohistidine, cause less than a 50% HA depletion in brain (Dismukes and Snyder, Brain Res. 78, 467, 1974), whereas in vitro these compounds inhibit HDC 100% (Schwartz et al., J. Neurochem. 21, 1301, 1973). Perhaps when HDC is inhibited in vivo, the activity of NAcHAD prevents HA from being depleted further. Endo and Ogura first described the enzymatic deacetylation of N-acetylhistamine (NAcHA) in rat brain (Jap. J. Pharmacol. 25, 161, 1975). They determined NAcHAD activity by measuring the HA formed fluorometrically after a column separation. This method is very insensitive and time-consuming. We have modified the assay by coupling it to a radioenzymatic assay for HA. This results in an assay for NAcHAD that is at least 1000 times more sensitive as well as 5 to 10 times faster. Our results confirm their basic observations, but some of our findings differ. The source of our enzyme is fresh or frozen whole rat brain which has been homogenized on ice in 25 mM HEPES at pH 8.0, and 1 mM GSH in a glass homogenizer with a Teflon pestle that is motor driven. The homogenate is centrifuged for 15 min at 16,000xg. The supernatant is then assayed immediately or stored frozen at -80°C. NAcHAD activity is linear with time for 60 min and with tissue up to 10 mg in 200 µl. All enzyme activity is destroyed by boiling for 3 min. NAcHAD activity is maximal at pH 8.0 in HEPES buffer. This activity is dependent on the buffer as well as its pH. $MnCl_2$ more than doubles NAcHAD activity. For brain NAcHAD, the K_m for NAcHA is 140 µM and the V_{max} is 13.2 pmoles/hr/mg tissue. We synthesized and tested iodo-NAcHA for substrate and inhibitor activity. It is neither a substrate nor an inhibitor of NAcHAD. We have synthesized ^{3}H-NAcHA and are in the process of developing a direct radioenzymatic assay. Future studies will include additional biochemical characterizations, more complete kinetic studies, ontogenetic, regional and subcellular distribution of brain NAcHAD activity, as well as purification of the enzyme to generate antibodies which will be used to do immunocytochemical localizations.

86.7 HPLC ASSAY FOR 3,4 DIHYDROXYPHENYLGLYCOL (DOPEG) AND 3-METHOXY-4 HYDROXYPHENYLGLYCOL (MOPEG) IN GUINEA PIG. G.A. Davis* & R.W. Goy (Spon: P. Lipton). Wisconsin Regional Primate Research Center, University of Wisconsin, Madison, WI 53715-1299.

We have been engaged in studies on the role of norepinephrine (NE) in controlling sexual receptivity in the guinea pig. In order to gain an estimate of the rate of release of NE in vivo we have developed an HPLC assay for the NE metabolites, DOPEG and MOPEG. The method also provides values for the bioamines and their acidic metabolites.

Brain tissue is homogenized in 20 vol of acetone/1N formic acid (v/v:85/15) and centrifuged. 0.5 ml of the supernatant is extracted with 0.2 ml of heptane/chloroform (v/v:8/1), then dried under N_2. To obtain free MOPEG and DOPEG, the residue is reconstituted in 0.2 ml of 0.2M citrate-phosphate buffer (pH 3.6) and extracted 3X with 0.2 ml of water-saturated ethyl acetate/n-butanol (v/v:1/1). To obtain the total metabolites (free plus sulfate conjugated), the residue from the tissue supernatant is first digested for 5 h at 37° with 1.8 units of sulfatase (Sigma Type H-1) in 0.1 ml of 0.2M acetate buffer (pH 5.0). Before extraction, the pH is adjusted by adding 0.1 ml of 0.2M citrate-phosphate buffer (pH 2.5). The dried organic phase is reconstituted in 0.1 ml of HPLC mobile phase and 0.02 ml of it is injected onto a μBondapak C18 column, which is run at 1.9 ml/min with a buffer of pH 4.7 (1% acetic acid adjusted with NaOH, 1mM EDTA). For the assay of NE, the dried tissue supernatant is reconstituted and analyzed under the conditions described by Mayer & Shoup (J. Chrom. 255: 533, 1983).

Recoveries range from 68% for DOPEG to 85-90% for the acidic metabolites. The sulfatase treatment produces no significant change in recoveries of internal standards. As a check on the method, whole rat brain was assayed and values for amines and metabolites were found which were very close to those reported in the literature. Further, clonidine (0.3 mg 3 h before sacrifice) was found to reduce total DOPEG and MOPEG in rat hypothalamus by 40% and 28% respectively. In the guinea pig, as in the rat, a significant proportion of brain DOPEG and MOPEG was found to be conjugated with sulfate (SO_4). For guinea pig hindbrain the following values were obtained (in ng/g tissue): free DOPEG, 22 ± 2; DOPEG-SO_4, 73 ± 4; free MOPEG, 87 ± 3; MOPEG-SO_4, 34 ± 3. Supported by NIH grant HD14821.

86.8 HALOPERIDOL INDUCES HIGHER HOMOVANILLIC ACID CONCENTRATIONS IN CEREBROSPINAL FLUID IN FEMALE THAN MALE NON-HUMAN PRIMATES. R. F. Seegal and K. O. Brosch.*. Ctr. for Labs. & Res., New York State Dept. of Health, Albany, NY 12201.

Evidence suggesting sexually dimorphic responsiveness to agents that modify central dopaminergic (DA-ergic) function includes: (1) male/female differences in dextro-amphetamine induced release of DA from in-vitro perfused rat striata (Becker & Ramirez, Brain Res. 204, 361, 1981); (2) alterations in striatal DA receptor density with estrogen treatment (Hruska & Silbergeld, Science, 208, 1466, 1980); and (3) sexual differences in the development of haloperidol-induced catalepsy in the rat (Hardy and Spear, NSA 7, 287, 1981; Fujii and Ikeda, Jpn. J. Pharm. 32, 247, 1982).

The following study was undertaken to determine if sexually-dimorphic changes in lumbar cerebrospinal fluid (CSF) concentrations of homovanillic acid (HVA) (3-methoxy-4-hydroxy-phenylacetic acid) would occur in adult male and female Macaca Nemestrina (pig-tailed Macaques) following acute exposure to haloperidol.

Lumbar CSF concentrations of HVA were determined by high-performance liquid chromatography with electrochemical detection under no-drug (baseline), probenecid only (50 mg/kg IP, to inhibit transport of HVA from CSF), or probenecid + haloperidol (.25 and .5 mg/kg). The drug administrations and CSF collections occurred under the following schedule:

IP Probenecid	IM Haloperidol	CSF TAP
0	+2	+5
	HOURS	

Baseline CSF-HVA levels were higher in the females than in the males ($\bar{X}$=43 ng/ml vs 20 ng/ml). Probenecid led to a large increase in HVA concentrations in both sexes due to inhibition of a probenecid sensitive acid-metabolite transport system. Treatment with probenecid and haloperidol elevated CSF-HVA concentrations, with a significantly greater response seen in the females ($\bar{X}$=196 ng/ml vs. 66 ng/ml) than in the males.

These results demonstrate, in a species closely related to man, that adult female monkeys are hyper-responsive to a widely used anti-psychotic DA-receptor blocking agent. The sexually dimorphic differences in CSF-HVA concentrations induced by probenecid-haloperidol might be due to differences in drug distribution or metabolism, but evidence in rodents demonstrating increased DA-receptor density in striata following estrogen treatment and greater sensitivity in females to haloperidol-induced catalepsy suggests that the observed differences may be due to the influence of gonadal or pituitary hormones on central DA-ergic processes.

86.9 DIRECT FEEDBACK CONTROL OF GLUTAMATE DECARBOXYLASE BY GABA. T. G. Porter* and D. L. Martin. Center for Labs and Research, NYS Dept. of Health, Albany, NY 12201.

Studies from this laboratory have shown that glutamate decarboxylase (GAD), the enzyme responsible for GABA synthesis, is regulated by a cycle of inactivation and reactivation which is influenced by a number of regulatory factors including ATP, inorganic phosphate (Pi), the cofactor, pyridoxal 5'-phosphate (PLP), and the substrate, glutamate. Evidence is now presented that GABA, at concentrations within the range reportedly occurring in nerve endings, promotes inactivation of GAD by direct interaction with this cycle, thereby providing a potentially important molecular mechanism for negative feedback control of presynaptic GABA biosynthesis. Inactivation proceeds by an alternative transamination reaction catalyzed by GAD in which the active holoenzyme containing bound PLP is converted to apoenzyme with the release of pyridoxamine phosphate (PMP) and succinic semialdehyde. Subsequent reactivation of the apoenzyme by PLP completes the cycle.

Inactivation experiments were performed with purified rat-brain GAD. To measure inactivation, GAD was preincubated with GABA for various lengths of time after which the remaining GAD activity was measured in a short (5 min) radiometric assay with 10 mM glutamate as substrate. The effect of PLP, Pi and ATP were determined by their inclusion in the preincubation mixture. The results showed that in the absence of added PLP, GAD was rapidly inactivated by GABA in a first-order process. The rate of inactivation depended strongly on the concentration of GABA. The concentration of GABA giving a half-maximal rate of inactivation (Ki) was 16 mM. The half-time for inactivation increased from about 8.5 min at saturating GABA concentrations to more than 120 min at GABA concentrations well below Ki (1mM). GABA-dependent inactivation was prevented by the presence of 10 μM PLP and 1 mM Pi, indicating that inactivation occurs by formation of apoenzyme in a manner similar to glutamate-dependent inactivation. The effect of ATP on the inactivation of GAD by GABA was also similar to that previously observed with glutamate-promoted inactivation. In the absence of added PLP, 100 μM ATP slightly accelerated inactivation (half-time = 7 min). In the presence of PLP, ATP strongly accelerated inactivation.

Although the GABA concentration in the GAD compartment is unknown, the GABA concentration in nerve endings is reportedly exceedingly high (>100 mM). Even if the GABA concentration in the GAD compartment were an order of magnitude lower, GABA would still have a strong inactivating influence on GAD.

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86.10 FORMATION, STORAGE AND RELEASE OF GABA FORMED FROM [^{3}H] GLUTAMINE AND [^{14}C] GLUCOSE IN HIPPOCAMPAL SLICES: EFFECT OF DEPOLARIZATION. J. C. Szerb, Dept. of Physiol. and Biophys., Dalhousie U., Halifax, N.S., Canada, B3H 4H7

To study the effect of depolarization on the synthesis, storage and release of GABA, rat hippocampal slices were first incubated in 5 ml Krebs containing 0.25 mM glutamine, 2.5 mM glucose and 3 or 50 mM K^+ followed by 15 min exposure to [^{3}H] glutamine and [U-^{14}C] glucose. Total and labelled glutamine, glutamate and GABA content and GABA release were measured by high-performance liquid chromatography. After incubation with 3 mM K^+ [^{3}H] and [^{14}C] glutamate contents were equal but there was twice as much [^{14}C] than [^{3}H] GABA present. After incubation with 50 mM K^+ labelled glutamate content doubled and labelled GABA content increased 3 fold as compared with incubation in 3 mM K^+ but there was no change in total glutamate and GABA content. When slices were incubated in 0 Ca^{2+} and 10 mM Mg^{2+} 50 mM K^+ no longer increased labelled glutamate content and increased labelled GABA content by only 30%. Slices, which after incubation in 50 mM K^+, were superfused for 40 min with unlabelled 0.25 mM glutamine and 2.5 mM glucose maintained their higher labelled glutamate and GABA contents as compared to those incubated in 3 mM K^+. Furthermore, slices that had been incubated in 50 mM K^+ released twice as much labelled GABA upon a second depolarization than those that were incubated in 3 mM K^+. The specific activity of released [^{14}C] GABA was 50%, that of [^{3}H] GABA 100% higher than of GABA contained in the slices. Superfusion with 1 mM AOAA, after incubation arrested the synthesis of GABA but even in the presence of AOAA slices incubated in 50 mM K^+ released twice as much labelled GABA than those which had been incubated in 3 mM K^+. This indicates that most of the released labelled GABA originated from stores formed during incubation and not during superfusion. Results suggest that depolarization increases in a Ca^{2+}-dependent manner the turnover of releasable GABA stores the same way as it increases the turnover of acetylcholine and norepinephrine in peripheral terminals.

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86.PO ACTIONS OF CLASSICAL AND ATYPICAL NEUROLEPTICS ON DOPAMINE METABOLISM AND RELEASE IN VIVO. P. L. Wood, P. McQuade* and N. P. V. NAIR. Research Lab., Douglas Hospital Research Centre, Verdun, Qué. H4H 1R3.

The neurochemical differentiation of classical and atypical neuroleptics until recently has focussed on possible differential actions in the extrapyramidal and mesolimbic dopaminergic (DA) projections. However, these studies have not revealed consistant preferential action of neuroleptics in either DA system. We have reinvestigated in more detail the actions of these drugs on DA metabolism and release in vivo in the mouse striatum. With classical drugs (haloperidol, perphenezine and chlorpromazine) dramatic increases in both DA metabolism and release were measured with a gas chromatographic-mass fragmentographic assay for dihydroxyphenylacetic acid, homovanillic acid, DA and 3-methoxytyramine. In marked contrast to the classical neuroleptics, the atypical agents (clozapine and thioridazine) increased DA metabolism with no concomitant increase in DA release. Sulpiride had similar actions except at high doses where increases in DA release were also noted. These data indicate the atypical neuroleptics differ from classical agents in their actions on DA release and suggest possible presynaptic actions in addition to their postsynaptic blockade.

STAINING AND TRACING TECHNIQUES

87.1 ULTRASTRUCTURAL LOCALIZATION OF GLYCOGEN AND GLUCOSE-6-PHOSPHATASE (G6Pase) IN CELLS RELATED TO THE CNS. A.M. Cataldo* and R.D. Broadwell. (SPON: G. Samaras) Department of Pathology (Neuropathology), University of Maryland School of Medicine, Baltimore, Maryland 21201

Glycogen and cytochemical activity for G6Pase were identified in neurons, glia, ependyma, choroid plexus epithelia, cerebral endothelia, and cells of the anterior and intermediate lobes of the pituitary gland in mice. Glycogen appeared as electron-dense aggregates scattered throughout the cells, including all portions of the neuron. Glycogen was preserved best by perfusion fixation with 2% glutaraldehyde-2% formaldehyde followed by a 2 hr. post-fixation in 1.5% ferrocyanide-2% aqueous osmium tetroxide. X-ray microanalysis has indicated that the electron-dense aggregates are representative of glycogen (de Bruijn, W.C., J. Histochem. Cytochem. 28:1242, 1980). G6Pase activity was preserved in all cells with a 5 min. perfusion fixation with 2% gluataraldehyde. Tissue sections were incubated in a modified Wachstein-Meisel medium (J. Histochem. Cytochem. 4:592, 1956) using the lead capture technique of Gomori. Glucose-6-phosphate, mannose-6-phosphate, sodium and thiamine pyrophosphates, and B-glycerophosphate served as substrates. Only tissue incubated with glucose-6-phosphate and mannose-6-phosphate exhibited G6Pase activity consistently in the nuclear envelope and rough endoplasmic reticulum (ER) of all cells. G6Pase activity was negligible in axons and terminals. The cytochemical reaction involves the liberation of phosphate ions from the substrate and their subsequent capture by lead ions to form electron-dense lead phosphate. This reaction indicates that G6Pase functions as a phosphohydrolase for converting glucose-6-phosphate to glucose. Absence of reaction product with inorganic pyrophosphate as a substrate suggests that G6Pase does not act as a phosphotransferase to supplement hexokinase activity for converting glucose to glucose-6-phosphate. In neurons and pituitary cells, localization of G6Pase activity was present frequently in the outer (cis) Golgi saccule and less so in the saccule subjacent to it. This latter saccule occasionally exhibited a direct membrane continuity with a reactive profile of ER. Neither the trans Golgi saccules nor GERL (an acid phosphatase reactive, Golgi-associated ER from which Lysosomes arise. Novikoff, A.B., Biol. Bull. (Woods Hole) 127:258, 1964) contained G6Pase activity. Membrane continuity between the ER and the trans Golgi saccules or between the ER and GERL was never observed. The results suggest that in the cells sampled: (1) some glucose-6-phosphate derived from glycogenolysis can be phosphohydrolyzed to glucose by G6Pase; (2) the ER is associated structurally and cytochemically with the forming (cis) face of the Golgi apparatus; therefore, membrane of the Golgi apparatus may be derived, in part, from the ER; and (3) GERL is not related to the ER morphologically or cytochemically; hence, the acronym GERL appears to be a misnomer.

87.2 SELECTIVE DESTRUCTION OF SCIATIC NEURONS USING THE SUICIDE TRANSPORT TECHNIQUE, R.G. Wiley and J. Wall, Lab of Experimental Neurology-VAMC, Vanderbilt University Medical School, Nashville, TN 37203.

Electrophysiological studies of the S-1 cortex after sciatic nerve transection in rats have characterized changes in the cortical representations of the sciatic and saphenous nerves which develop within 1-2 d following sciatic transection. Such transections produce inconsistent and incomplete loss of dorsal root ganglion (DRG) neurons after several days to weeks. In order to study the electrophysiological changes in central representation resulting from destruction of DRG neurons, and presumably their central processes, we sought to develop a technique for selectively destroying the DRG neurons projecting through the sciatic nerve. We chose to employ the suicide transport technique (Wiley, et.al., Science, 216:889, 1982) using the toxic lectin, ricin, by intraneural injection (5 µl of 2-5 mg/ml on 2 separate occasions) of the sciatic nerve at mid-thigh level in adult rats. Additionally, similar injections of wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP: 5 µl of 10 mg/ml) were made either contralateral to the ricin injections or in normal animals for the purpose of mapping the distribution of DRG neurons projecting through the sciatic nerve. WGA-HRP transport was demonstrated using TMB as the chromagen and darkfield optics. WGA-HRP retrogradely labelled 90-92% of neurons in the L5 DRG and a variable, but somewhat smaller proportion of neurons in L4 and L6. Retrogradely transported ricin similarly destroyed 99+% of neurons in the L5 DRG and smaller proportions of the L4 and L6 neuron populations demonstrating that it can be used to remove first-order sensory neurons. At short survival times, ricin produced a complete loss of Nissl substance followed by progressive disintegration of the affected neurons. WGA-HRP also produced robust labelling of sciatic motor neurons in the mid-lateral ventral horn of the spinal cord, of central terminals of sciatic DRG neurons in the dorsal horn of the spinal cord, and of central terminals of sciatic DRG neurons in the nucleus gracilis. Ricin destroyed the appropriate motor neurons but had no detectable effect on dorsal horn or nucleus gracilis neurons. In summary, retrograde ('suicide') transport of ricin can be used to selectively destroy the motor and sensory neurons projecting through the sciatic nerve, but the doses are much larger than those that have been found effective in the vagus, presumably due to the presence of many heavily myelinated fibers in the sciatic nerve. Animals prepared in this fashion should prove useful in studying the CNS response to loss of first-order sensory neurons.

(This work supported by VA Merit Review Award.)

87.3 FURTHER STUDIES ON THE NATURE OF THE CARBONIC ANHYDRASE POSITIVE NEURONS IN THE DORSAL ROOT GANGLION OF THE RAT. V. W. Wong, C. P. Barrett, L. Guth and E. J. Donati* (SPON: F. D. Anderson). Dept. of Anatomy, University of Maryland Sch. of Med., Baltimore, MD 21201.

We have recently demonstrated histochemically that carbonic anhydrase activity is present in the perikaryon as well as the peripheral and central processes of some neurons of the dorsal root ganglion. These neurons are large in diameter (100 um) and have large processes (20 um) suggesting that they might represent muscle spindle afferents. To examine this possibility, we injected horseradish peroxidase (or bisbenzimide fluorescent dye) into the soleus muscle of adult rats. The horseradish peroxidase frozen sections of L4 and L5 spinal ganglia were reacted with fluorescein-conjugated-anti-peroxidase 48 hours later; after this procedure the carbonic anhydrase histochemical reaction was performed on the same sections. Fluorescent labeling was found to be present only in those neurons which reacted positively for carbonic anhydrase We next sought to determine if the carbonic anhydrase positive neurons arose at the same developmental time (viz., the 12th day of gestation) as do Ia neurons. Pregnant rats were injected intraperitoneally with tritiated thymidine (5 uCi/g) at days 10, 11, 12, 13, and 15 of gestation. The L4 and L5 spinal ganglia of the offspring were removed at 33 day postnatally and frozen sections prepared. After being incubated for carbonic anhydrase histochemistry the slides were coated with Ilford emulsion for autoradiography. The amount of radioactive labeling in the carbonic anhydrase positive neurons was greatest after injection at 12th day of gestation, indicating that terminal mitosis occurs in these cells mainly on that day. These results show that carbonic anhydrase positive neurons in the rat dorsal root ganglion have the same anatomical distribution and embryological birthdate as the Ia afferent neurons. We conclude that carbonic anhydrase activity is found in Ia afferent neurons and that the carbonic anhydrase histochemical reaction serves as a useful marker for this neuronal population of the dorsal root ganglion.

Supported by a grant from the NIH (NS 12847).

87.4 IMMUNOCYTOCHEMICAL CHARACTERIZATION OF GROWTH HORMONE, PROLACTIN, AND ACTH CONTAINING CELLS IN THE CETACEAN ANTERIOR PITUITARY. A. Schneyer and D. Odell*. School of Marine and Atmospheric Science, Univ. of Miami, Miami FL 33149.

In order to assess the applicability of various immunochemical techniques to the study of reproductive physiology in the bottle-nosed dolphin, we attempted to identify cells in the anterior pituitary using the PAP immunocytochemical technique. Pituitaries were obtained from several species of stranded cetaceans, fixed in 2% glutaraldehyde for 1.5 hours, rinsed in buffer for 1 hour, dehydrated, and embedded in Epon 812. Sections of 1-2u were affixed to glass slides and exposed to NaOH/EtOH solution to remove the Epon. Antisera to ovine and human pituitary hormones were obtained from the National Pituitary Agency (NIAMDD) and applied at the following dilutions for 48 hours (4°C): prolactin (PRL), 1:5,000; growth hormone (GH), 1:5,000; and adrenocorticotrophic hormone (ACTH), 1:10,000 (human only). Antisera to both human and ovine hormones cross reacted sufficiently with cetacean hormones to produce specific staining in all 3 cell types. Control experiments consisted of pre-incubation of antisera with homologous antigen to eliminate staining, pre-incubation of antisera with other hormones to check for non-specific staining of related cell types, and deletion of various steps of the protocol to eliminate possible non-specific staining in subsequent steps.

PRL cells were less numerous and more variable in shape than GH cells and contained large, pleomorphic granules typical of this cell type. GH cells tended to occur in groups near capillaries and usually contained more granules per cell than lactotrophs. Corticotrophs were much more variable in shape and size, often with thin processes projecting from the cell body. The granules were usually located near the plasma membrane.

The cross reactivity of antisera demonstrated in this study is important in that cetacean pituitaries are relatively scarce, hence purified hormone and cetacean-specific antisera are not available. Furthermore, this is the first report where immunocytochemical techniques have been applied to research on any marine mammal. These results can also be applied to the development of heterologous RIAs for circulating levels of pituitary hormones in cetaceans.

87.5 IS THERE A SPECIES-SPECIFIC MONOCLONAL ANTIBODY WHICH HISTOCHEMICALLY VISUALIZES MOUSE CNS? J.K. Daniloff & M.A. Ariano. Anatomy & Neurobiology, Univ. Vermont College of Medicine, Burlington, VT.

In our search for a species-specific monoclonal antibody (McAb) to mouse CNS, we have utilized two commercially available IgG: 1) species specific rat anti-mouse H-2 (M1/42, Hybridtech), and 2) strain-specific mouse anti-mouse Thy 1.2 (Accurate Chem. Co.). Neither McAb has been characterized immunohistochemically. Our aim was to locate McAb which would a) bind strictly to perfusion-fixed sections of C57Bl mouse brain with no crossreaction to rat brain, and b) permit robust visualization of labeled cells.

Anti H-2 is new to the commercial market and binds to antigens of a variety of haplotypes (J. Immunol. 127: 923, 1981) on C57Bl mice. These cell surface antigens are recognized by T lymphocytes which lyse allogenic virus-infected or chemically-modified cells (Transp. Rev. 29: 89, 1976). Because biochemical assays have revealed H-2 antigens on mouse cerebellum (J. Neuroimmunol. 1: 429, 1981) we attempted to visualize H-2 antigens immunohistochemically. H-2 is best preserved through transcardial perfusion with 4% paraformaldehyde-0.05% glutaraldehyde in cacodylate buffer (pH 7.4). 50 µm vibratome-sections were incubated overnight in the primary McAb, 1:100, using the indirect peroxidase method with DAB as the chromagen. Reaction product was intensified using the Co-Ni meth. (J. Histo. Cyto. 29: 775, 1981). Results indicated robust and widespread labeling of neurons throughout the forebrain of both rat and mouse, including all layers of cortex and hippocampus, thalamus, and striatum. Glial components were rarely visualized.

There are two forms of the allelic Thy-1, 1.1 and 1.2. Only the 1.1 form is found in rat (J. Exp. Med. 136: 1054, 1972) and 1.2 is strain specific to C57Bl mice, among others. Thy-1.2 antigenicity is difficult to maintain (J. Histo. Cyto. 31: 263, 1983). The lightly-fixed brains were friable, and the 50 µm-vibratome sections were incubated overnight with McAb, 1:100. Darkly labeled neurons were apparent throughout comparable neocortical and hippocampal regions of both rat and mouse brain. We found it was necessary to use an affinity purified goat anti-mouse peroxidase conjugate that had been specifically adsorbed against rat (E-Y Lab oratories; American Qualex) to eliminate non-specific staining resulting from the application of the secondary antibody.

The present investigation indicated: 1) both H-2 and Thy-1.2 antigens can be preserved for immunohistochemistry, 2) both McAb recognized determinants in rat CNS, despite claims of mouse species specificity, and 3) anti-mouse and anti-rat peroxidase conjugates crossreact widely. Although the crossreactivity of various antibodies is rarely discussed and less frequently tested, it has widespread implications in the production of false-positive immuno histochemical staining.

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87.6 A PREEMBEDDING COLLOIDAL GOLD STAINING PROCEDURE FOR THE ELECTRON MICROSCOPIC LOCALIZATION OF β-ENDORPHIN/ACTH IN RAT HYPOTHALAMIC NEURONS. R. Lamberts* and P.C. Goldsmith. Dept. Ob/Gyn and Repro. Sci. & Repro. Endo. Ctr., Univ. of Calif., San Francisco, CA 94143.

Colloidal gold has primarily been used as an immunolabel for the electron microscopic demonstration of intracellular antigens in postembedding grid staining, and for the localization of cell surface receptors by preembedding staining. In order to use this versatile marker for both light and electron microscopy, we applied it in the preembedding staining of vibratome sections of brain tissue. Following perfusion fixation with Nakane's PLP, 50-75 µm vibratome sections of rat hypothalamus were processed for immunocytochemistry (ICC). Incubation in primary antisera against β-endorphin or ACTH for 72 hr was followed by goat anti-rabbit IgG coupled to 20 nm colloidal gold (GaRbIgG-Au) for 2 hr. The optimal pH and protein concentrations for coupling were determined according to the method of Frens. In some cases, colour intensification was attained by incubation in a second gold-labeled immunoreagent (RbIgG-Au or protein A-Au). The development of pink to light red cell bodies was monitored under the light microscope. Immunoreactive perikarya exhibited a staining pattern and location in agreement with previous reports using the PAP or immunofluorescence techniques. Positive cell bodies occurred predominantly in the arcuate nucleus, sometimes extending dorsally in the periventricular zone, or laterally, near the ventral hypothalamic surface. Only the proximal portions of neuronal processes were labeled, whether or not the animals were pretreated with colchicine. At the electron microscopic level, positive staining was confined to the periphery of 90-110 nm dense neurosecretory granules. Clusters of colloidal gold particles that seemed to lie free in the cytoplasm in one section were actually associated with secretory granules in adjacent sections. No other cell organelle was consistently labeled; the ergastoplasm and all Golgi elements were routinely negative. Non-specific staining was observed in large blood vessels, along the ventricular surface, and in the contact zone of the median eminence.

This ultrastructural localization of β-endorphin/ACTH in hypothalamic neurons correlates well with biochemical data on the intracellular processing of the peptide precursor in pituitary tumor cells. Our results demonstrate the usefulness of colloidal gold as an ICC label for staining intracellular antigens in vibratome sections. While reliable immunostaining may be restricted to the regions close to section surfaces, the method does not produce the diffuse reaction product obtained with many enzymatic ICC techniques. The specificity and distinctiveness of colloidal gold makes it an important tracer for double staining of neuronal systems in central nervous tissue.

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87.7 DIFFERENTIAL BACKFILLING OF INTERNEURON POPULATIONS IN A LOBSTER ABDOMINAL GANGLION USING RUBEANIC ACID PRECIPITATES OF Co^{++} AND Ni^{++}. K. A. Jones* and C. H. Page. Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08854.

Cobalt or nickel ions when used as intracellular markers form different colored precipitates with rubeanic acid (dithiooxamide - Quicke and Brace, J. Microscopy 115: 161-163, 1979; Delcomyn, J. Neurobiol. 12: 623-627, 1981). Since mixtures of Co^{++} and Ni^{++} form colored precipitates distinct from either ion alone, doubly labelled neurons can be distinguished from those containing only one ion type. We employed this differential staining technique to quantitate and locate the somata of the ascending, descending and bidirectional populations of projecting interneurons in the second abdominal ganglion.

The left ascending and descending hemiconnectives, that carry the axons of interneurons projecting from the ganglion, were simultaneously backfilled with separate solutions of $NiCl_2$ and $CoCl_2$. Two sets of differential backfills were prepared. In the first the ascending hemiconnective was filled with Co^{++} and the posterior with Ni^{++}, while in the second set the two ion solutions were reversed. In the anterior-Ni/posterior-Co set 56 descending interneurons stained yellow (Co^{++} presence), 55 ascending interneurons stained blue (Ni^{++} presence) and 25 bidirectional interneurons stained red (Ni^{++} and Co^{++} presence). Although the other set of backfills (anterior-Co/posterior-Ni) stained a similar number of total interneurons a smaller proportion of these appeared red. More specifically, identifiable groups of somata stained red only in the anterior-Ni/posterior-Co set of backfills. These observations suggest the differential backfilling technique is sensitive to caudal-rostral asymmetries in the structure of the bidirectional interneurons.

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87.8 HISTOCHEMICAL DETECTION OF ADENYLATE CYCLASE ACTIVITY WITH FORSKOLIN. G. Szumanska*, J. Bembry* and M. Spatz (SPON: W. D. Lust). Lab. of Neuropathology and Neuroanatomical Sciences, National Institutes of Health, Bethesda, Maryland 20205.

So far the available histochemical techniques for the visualization of adenylate cyclase (AC) activity in various tissues have been based on hormonal and 5-guanylylimidodiphosphate (GppNp) stimulation of the enzyme activity irrespective of the type of agent (lead or strontium) used as a capturing ion. Recently, forskolin, a known hypotensive and antispasmotic drug, was shown to be a unique stimulator of cAMP-generating systems. This substrate in contrast to isoproterenol and GppNp increases the level of cAMP formation apparently through the direct stimulation of the catalytic unit of the AC without requiring the presence of guanine nucleotide binding subunit (Seamon and Daly, J. Cycl. Nucl. Res. 7: 201, 1981).

In this communication we will demonstrate a histochemical method for the detection of AC activity using various concentrations of forskolin instead of isoproterenol and GppNp as substrate. The essential procedures of the technique were based on those described by Palkama et al. (J. Histochem. Cytochem. 29: 898, 1981) for light and electron microscopy. The AC reaction product formed with each substrate was evaluated in cerebrovascular endothelial cultures derived from brain microvessels and brain sections of rats.

The comparative studies showed that AC reactivity is greater to forskolin than to isoproterenol and GppNp. The histochemical demonstration of AC activity in these tissues was already possible with 0.2 µM of forskolin while 100 µM of isoproterenol and 10 mM of GppNp were needed to visualize this enzyme with the previously reported method.

These findings indicate that forskolin is not only a suitable AC stimulatory agent but it represents a far superior substrate as compared to the other substrates for the demonstration of AC activity histochemically.

87.9 A NEW METHOD FOR STABILIZATION OF THE TETRAMETHYLBENZIDINE (TMB) REACTION PRODUCT FOR USE IN THE VISUALIZATION OF RETROGRADELY TRANSPORTED HORSERADISH PEROXIDASE-WHEAT GERM AGGLUTININ (HRP-WGA). T.M. Perney*[a], D.B. Rye*[a], B.H. Wainer[a], and C.B. Saper[b]. Departments of Pathology and Pediatrics, The University of Chicago, Chicago, IL., 60637[a], and Department of Neurology, Washington University School of Medicine, St. Louis, Mo., 63110[b].

The sensitivity of TMB makes it an ideal chromogen for tracing neural pathways (Mesulam, M.-M., and Rosene, D.L., J. Histochem. Cytochem., 27: 763-773, 1979). The instability of the TMB reaction product in aqueous solutions, however, discourages its use for combined retrograde and immuno- staining methods, and makes EM applications difficult. This study presents a method for stabilizing the TMB reaction product. Following development of TMB by the procedure of DeOlmos et. al.(J. Comp. Neurol., 181: 213-244, 1977), tissue sections were incubated in the presence of DAB and H_2O_2 at 4°C for 10 min. This procedure resulted in a stable brown-colored reaction product at sites of TMB accumulation. Addition of cobalt to the DAB-H_2O_2 solution resulted in a black reaction product. Both procedures were as sensitive as TMB alone. Following cortical injections of HRP-WGA in rats, retrogradely-labeled cells in the basal forebrain were counted, and the following parameters affecting the sensitivity of this stabilization procedure were studied: DAB concentration, temperature, and the requirement for H_2O_2. The reaction was stable at DAB concentrations of 0.1-0.01%, it appeared to require the presence of H_2O_2, and it was more stable when performed at low temperatures. This reaction product was stable when subjected to standard alcohol-xylene dehydration. Finally the color and physical appearance of the reaction product when developed in the presence of cobalt, made it suitable for combinations using PAP immunohistochemistry. This has been confirmed by staining retrogradely labeled cells in the basal forebrain with a monoclonal antibody against choline acetyltransfersase. This work was supported in part by research grants from the Whitehall, McKnight, and American Parkinson Dis. Assoc. foundations, and USPHS NS-17661, HD-04583, NS-18669, NS-0631, 5-T32GM07281, and GM07839.

88.1 PROTEIN PHOSPHORYLATION IN A SINGLE, IDENTIFIABLE MOLLUSCAN NEURON. J.T. Neary, J. Acosta-Urquidi, L.A. Tengelsen*, A.M. Kuzirian*, and D.L. Alkon. Section on Neural Systems, Lab. of Biophysics, NINCDS-NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

Protein phosphorylation may play a role in associative learning[1] and in the modulation[2,3] of a transient, voltage-dependent K^+ current, I_A, which is reduced[4] following associative learning in Hermissenda crassicornis. Previously we found that intracellular injection of Ca^{++}-dependent[5] or cAMP-dependent[2] protein kinase leads to a reduction in I_A. To define the relationship of protein phosphorylation to the modulation of ionic conductance, it is necessary to identify and characterize the proteins which are phosphorylated by the injected kinases. With this goal in mind, we have developed a protocol to study protein phosphorylation in a single, identifiable neuron in Hermissenda.

The cell selected for study, LP-1, is located in the left pedal ganglion[6] and has a diameter of ∿ 150 μ. ^{32}P-phosphoproteins from LP-1 are detected by labeling ganglia in carrier-free ^{32}Pi (125 μCi/200 μL incubation solution), dissecting and homogenizing LP-1 following freeze substitution of the ganglia[7], and separating the proteins by SDS gel electrophoresis and autoradiography[1]. Histological examination of the dissected cell reveals minimal contamination by glial cells or connective tissue. Time course studies indicate that ^{32}Pi uptake, ^{32}P-ATP, ^{32}P-lipids, and ^{32}P-proteins in LP-1 reach a plateau in 8 to 12 hr. ^{32}P-proteins and ^{32}P-lipids from one LP-1 cell can be detected by autoradiography after 2-3 day exposure using intensification techniques. The LP-1 phosphoprotein pattern is similar to that previously observed in Hermissenda circumesophageal ganglia and eyes[1,3].

Based on these studies, it should now be possible to conduct parallel biophysical and biochemical experiments in a single neuron in Hermissenda and to investigate the proteins that are phosphorylated by protein kinases injected into LP-1 and by pharmacological agents that affect Ca^{++} and cAMP levels. LP-1 may also serve as a model cell for investigating biochemical processes which underlie associative learning since LP-1 contains a voltage-dependent I_A current which exhibits Ca^{++}-mediated inactivation[8] and which is similar to that previously observed to change in Hermissenda Type B photoreceptors following associative learning[4].

References: [1]Neary et al., Nature 293, 658 (1981); [2]Alkon et al., Science 219, 303 (1983); [3]Neary and Alkon, J. Biol. Chem., in press (1983); [4]Alkon et al., Science 215, 693 (1982); [5]Acosta-Urquidi et al., Soc. Neurosci. Abstr. 8, 825 (1982); [6]Jerussi and Alkon, J. Neurophysiol. 46, 659 (1981); [7]Bernier et al., J. Neurosci. 2, 1682 (1982); [8]Acosta-Urquidi et al., Soc. Neurosci. Abstr. 9, (1983).

88.2 INCREASED AGE DECREASED RC_1 MODULATION OF R_{15} BURSTING IN APLYSIA. T. L. SKINNER* and B. PERETZ, Dept. of Physiology, Univ. of Ky. Med. Ctr., Lexington, KY 40536

R_{15} is a bursting pacemaker in the abdominal ganglion of Aplysia, whose activity can be modulated by the RC_1 input via the right connective. Repetitive firing of the RC_1-R_{15} pathway for several minutes has been shown to have a prolonged effect on bursting such that the number of spikes per burst increases as well as the interburst interval (Parnas et al., JNP 37:609, 1974). We found that the efficacy of RC_1 modulation of R_{15} activity is decreased with age. Our study examined pre- and post-synaptic characteristics of the RC_1-R_{15} pathway at different ages: mature (MAT) and old (OLD) Aplysia, 121 ± 5 and 214 ± 6 days post-metamorphosis, respectively.

A suction electrode was used to stimulate the right connective. The following parameters were measured, with an intracellular electrode in R_{15}, both before (C) and after (E) a four minute stimulation of RC_1 at rates of 2 and 10/sec: spikes per burst, amplitude of bursting pacemaker potential (BPP), membrane potential (Em, at the trough of the BPP), and membrane input resistance (R_{in}, at the trough of the BPP). Results for the two age groups at a stimulation frequency of 10/sec are given in Table I. With RC_1 stimulation, spikes per burst, Em, and BPP amplitude all increase for MAT R_{15}'s but remain relatively unchanged for OLD R_{15}'s. These data show that RC_1 modulation of bursting pattern was present in MAT animals and was significantly less in OLD animals.

With increased age, decreased modulation by the RC_1 input may result from pre-synaptic and post-synaptic changes. A pre-synaptic change is decreased unitary EPSP size (Table I); also, double pulse facilitation of RC_1 at 100 ms increased, which possibly is related to decreased EPSP size. Post-synaptic changes involved decreased R_{in} which could contribute in part to the decreased EPSP size. These results show that a central synapse in Aplysia is affected by increased age.

TABLE I. X ± SEM - RC_1 STIMULATION AT 10/SEC

	n	Spikes(#)	BPP(mv)	Em(mv)	R_{in}(MΩ)	EPSP(mv)
MAT(C)	7	6.6 ± 2.4	7.8 ± .6	49.0 ± 1.8	9.2** ± .6	15.4** ± 1.2
OLD(C)	6	7.4 ± 1.8	9.0 ± .4	50.5 ± 1.8	4.5 ± .6	4.4 ± 1.6
MAT(E)	7	17.2* ± 5.5	12.5* ± 1.4	55.0 ± 2.5	11.0 ± 1.2	
OLD(E)	6	8.0 ± 2.5	9.2 ± .3	50.6 ± 2.1	4.0 ± .8	

* $p < 0.05$; C vs E; ** $p < 0.05$; MAT vs OLD

(NIMH and NIA)

88.3 DEMONSTRATION OF PHYSIOLOGICAL RELEASE OF THE PUTATIVE NEUROMODULATOR GLYCINE FROM APLYSIA NEURON R14. A.R. Rittenhouse* and C.H. Price (SPON: M.C. Nelson). Department of Biology, Boston University, Boston, MA 02215.

Identified neuron R14, located on the dorsal surface of the parietovisceral ganglion (PVG) of Aplysia contains two possible chemical messengers, the free amino acid glycine and a small peptide. Previous studies indicate that R14 innervates the anterior aorta (AA) where it plays a neuromodulatory role (Sawada et al., Brain Res., 207: 486, 1981). Both bath and iontophoretically applied glycine and stimulation of R14 potentiate serotoninergic-induced contractions of AA longitudinal muscle (AALM) without any apparent changes in muscle membrane electrical parameters. However, the release of free glycine in this preparation has not been demonstrated. In this study, we report that stimulation of the axons or soma of R14 preloaded with ^{3}H-glycine (3H-G) results in a corresponding increase of 3H-G released from R14 axons that terminate in AALM.

The PVG, vulvar nerve (VN), and AA were dissected out and pinned such that the AA occupied one chamber and the PVG another. The VN (which contains R14 axons) projected from the PVG to the AA through a water-tight barrier. The AA chamber was superfused with seawater medium and the PVG was bathed for 8-14 h in medium containing 100 μCi/ml of 3H-G. The incubation period allowed time for R14 to take up the 3H-G and actively transport it to terminal regions in the AA. Both chambers were then repeatedly rinsed to wash off extracellular 3H-G and reincubated in plain medium. A sampling schedule was established so that the medium bathing the AA was removed every 5-10 minutes for scintillation counting.

There is tonic and occasional increases in release (or other efflux) of label from the AA when artificial stimulation is absent. Both bath application of 5 mM histidine (which stimulates R14 to approximately 1 Hz) and direct VN stimulation caused a several-fold increase in 3H-G release above baseline. TCA precipitation of AA medium samples indicates that little of the 3H-label is present in protein. Autoradiographic studies show that only the identifiable axons of R14 in the VN and AA are heavily labelled. Experiments involving intracellular stimulation of R14 above spontaneous levels (0.7 Hz) were difficult (due to membrane accommodation) and were inconclusive. Experiments to test Ca++ dependency of this presumed release are underway but this preliminary data suggest that R14 releases free glycine during prolonged spontaneous and induced electrical activity.

88.4 MOTORNEURON SIGNALING PROPERTIES AND MOTORNEURON-MOTORNEURON SYNAPTIC INTERACTIONS IN THE NEMATODE ASCARIS. Ralph E. Davis* and Antony O.W. Stretton. Neurosciences Training Program and Department of Zoology, University of Wisconsin, Madison, WI 53706.

We have been investigating the physiological properties of Ascaris commissural motorneurons and of their synapses. We reported previously that these cells have high resistance membranes, and that they propogate graded (non-action potential) signals. However, voltage-sensitive channels are present since graded anode-break responses can be elicited (Soc. Neurosci. Abstr. 8, 685). Both electrically evoked depolarizations and anode-break responses are reversibly Co^{2+}-sensitive. No change in commissural input resistance (steady state or early transient) occurs when Ca^{2+} is replaced by Co^{2+}. The lack of all-or-none action potentials in the presence of 50-100 μM veratridine suggests that masked veratridine-sensitive channels are not present in the commissures.

We also previously reported that neuromuscular transmission is graded and there is tonic transmitter release in the unstimulated neuron. We have now demonstrated that the motorneuron to motorneuron synapses share these same properties. The spontaneous EPSPs and IPSPs seen in the five classes of commissural motorneurons are chemically mediated, since they can be reversed with current injection of the appropriate amplitude and polarity. These PSPs persist when the commissure is cut between the recording site (in the commissure) and the dorsal nerve cord, showing that these signals originate in the ventral nerve cord.

Previous anatomical studies have provided a description of the synapses between motorneurons. To examine the physiological properties of these synapses, we have made simultaneous microelectrode penetrations of pairs of motorneurons. Stimulating the presynaptic fiber produces post-synaptic potentials for most of the anatomically-defined synapses. Paradoxically, however, the synapse that is anatomically most common gives the weakest of physiological responses. We are currently investigating whether this is due to the properties of the synapse itself, or due to membrane properties and/or the geometrical relationship of the recording site (in the commissure) and the synapse (in the nerve cord).

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88.5 INTRACELLULAR RECORDINGS AND LUCIFER YELLOW-FILLS OF INTERNEURONS IN THE NEMATODE ASCARIS. James D. Angstadt* and Antony O.W. Stretton (SPON: A. Levin). Neurosciences Training Program and Department of Zoology, University of Wisconsin, Madison, WI 53706.

The motornervous system of the nematode Ascaris lumbricoides contains about 20 interneurons. There are six large interneurons that are identifiable anatomically and about 14 small interneurons that have so far not been individually identified. Anatomical studies have shown that the interneurons make synapses onto motorneurons, as well as among themselves. In order both to investigate the role of the interneurons in controlling the motorneurons, and to develop criteria for identifying the small interneurons, intracellular microelectrode penetrations and Lucifer-yellow dye injections have been performed. We find that like the motorneurons and large interneurons, the morphology of the small interneurons is simple. One bipolar neuron, however, exhibits a somewhat unusual morphology. Its anteriorgoing process abruptly changes direction 180° within the ventral cord and travels posteriorly.

Electrophysiological recordings have shown that the largest ventral cord interneuron (int. A) makes excitatory synapses onto type DE1 motorneurons. In addition, some of the interneurons exhibit rhythmic voltage changes. In some cases the phase of the rhythm can be reset with short current pulses, suggesting that the cell is involved in the generation of the rhythm.

Supported by USPHS Neuroscience Training Grant 5T32 GM 7507 and Grant #AI 15429.

88.6 MONOCLONAL ANTIBODIES TO NEURONS OF THE NEMATODE ASCARIS. Carl D. Johnson* and Antony O.W. Stretton (SPON: Philippa Claude). Department of Zoology, University of Wisconsin, Madison, WI 53706.

With the ultimate goal of generating specific labels that can be used to identify neurons and synapses in light micrographs, we are generating monoclonal antibodies to Ascaris nervous tissue. Using collagenase-treated Ascaris as a source of enriched nervous tissue for immunization, we have generated hybridomas by fusing spleen cells with NS-1 myeloma cells. Screening for interesting antibody-secreting hybridomas was carried out by ELISA using homogenates of nerve cord and collagenase dissociated head pieces (back to, but not including, the retrovesicular ganglion). Specific cellular localization of antibody binding was determined anatomically using the anterior 20-30 mm of collagenase-dissociated worms that had been fixed with formaldehyde, and permeabilized with 1% Triton X-100.

We have found some antibodies that bind to neuronal processes, including commissures, sensory neurons and processes in the nerve cords and nerve ring. A second commonly occurring class of antibodies bind to fibrils in the hypodermis; comparison with electron micrographs suggests that these antibodies react with intermediate filaments. Some of these antibodies selectively stain elements within the sensory system. A third class of antibodies binds to the excretory cell, a subset of the sensory accessory cells, and to subsets of neuronal cell bodies. In particular, the cell bodies of excitatory motorneurons in the ventral nerve cord (Stretton et al., PNAS 75, 3493 [1978]) are differentially stained: type DE2 neurons stain intensely whereas types DE1 and DE3 neurons stain weakly; similarly, among the putative ventral excitatory motorneurons, type V-2 neurons stain intensely and type V-1 neurons stain weakly. It is interesting that types DE2 and V-2 neurons are thought to be involved in posteriorly propagating locomotory waves, while types DE1, DE3 and V-1 neurons are thought to mediate anteriorly-propagating waves. We are currently investigating the chemical basis for these differences.

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88.7 SYNAPSES OF THE THICK ACCESSORY NEURON ON THE STRETCH RECEPTOR NEURON OF THE CRAYFISH, PROCAMBARUS CLARKII. Kazuo Ikeda, Division of Neurosciences, City of Hope Research Institute, Duarte, CA 91010.

The distribution of the synapses on the fast adapting and slowly adapting stretch receptor neurons in the first to the fifth abdominal segments and the eighth thoracic segment was investigated with light- and electronmicroscopy. The thick accessory neuron was found to make synaptic contacts on the soma of the receptor neuron as well as the previously known dendritic contacts.

Whole mount specimens of the receptor neuron and related nerves stained with methylene blue or impregnated with silver revealed that fine axonal branches of the thick accessory neuron forms a basket-like network covering the surface of the soma. The fine branch was accompanied by serially connected bulbs. The fast adapting receptor neurons were more densely covered by this network than the slowly adapting receptor neurons.

Electronmicroscopy revealed that these fine branches on the soma were indeed making en passant type synaptic contacts on the somal membrane. Thus the somal membrane receives numerous synapses. Synapses were also found at the axon hillock region as well as on the axonal membrane as far as 50 μm from the axon hillock. Although the quantitative analysis of the distribution of the synapses on the entire receptor neuron has not been completed, the density of the synapse on the somal membrane appeared to be higher than that on the dendrites. The present result is in accord with the finding of somal synapses reported by Peterson and Pepe (1961). Furthermore, the fine branches on the soma were traced to the thick accessory neuron in the present study. Since the receptor neuron is also known to be innervated by the thin accessory neuron and the x-fiber, the branching of those axons were carefully examined and found to make synapses on the dendrites of the receptor neuron but not on the somal membrane. Thus these somal synapses are identified to be those of the thick accessory neuron. The implication of this distribution for the inhibitory mechanism must be investigated physiologically. (Supported by USPHS NIH Grant NS18858).

88.8 DYE COUPLING AND GAP JUNCTIONS BETWEEN CRUSTACEAN NEUROSECRETORY CELLS. H. Arechiga+, B. Chávez*+ and R. M. Glantz++. +Depts. of Physiology and Cell Biology, CINVESTAV, IPN, Apdo. Postal 14-740, México, D.F. 07000. ++ Dept. of Biology, Rice University, Houston, Texas. 77001.

The X organ in the crustacean eyestalk is a cluster of 100-200 neurosecretory elements mostly located at the medial part of the medulla terminalis. They are known to project to a neurohaemal organ, the sinus gland, and to secrete a host of neurohormones of peptidic nature. There is electrophysiological data suggesting a possible coupling among these elements (Iwasaki & Satow, J. Gen. Physiol., 57:216, 1972). In the present report we describe morphological evidences compatible with such coupling.

The experiments were conducted in adult crayfish Pacifastacus leniusculus or Procambarus clarki, of either sex. The animals were left one hour in advance in cold, oxygenated saline solution once the dorsal carapace was excised and the heart removed. The eyestalk was glued to the carapace and the eyestalk dorsal exoskeleton removed. For the dye-coupling experiments, glass micropipettes filled with 3% lucifer yellow were used. Their tip resistance was 50-100 mΩ. After the impalement and characterization of the electrophysiological properties of the recorded neuron, the dye was introduced with 10 n Amp hyperpolarizing pulses of current for 10-30 min. The eyestalk was removed and treated for wholemount observation. For a number of cells, the dye passed from the injected one to other elements, showing different patterns. Some neurons appear to be coupled only to one or two neighbour cells, others, to several cells. Still, a number of them did not show any evidence of dye-coupling.

The possible presence of gap junctions was explored by subjecting the isolated eyestalks to freeze-fracture. The X organ-sinus gland system was divided in segments. At the level of cell bodies and proximal branches in the neuropil typical formations containing gap-junctions were observed. None were detected in the axons beyond the neuropil, and some again were seen in the nerve-endings at the sinus gland. These observations lend support to the notion of a functional coupling between neurosecretory cells in the X organ-sinus gland system. The physiological significance of these connections remains to be studied.

88.9 IMMUNOHISTOCHEMICAL IDENTIFICATION OF DEVELOPING PURKINJE NEURONS IN CULTURES OF RAT CEREBELLUM. C.F. Franklin* and D.L. Gruol (SPON: R. Anderson). A V. Davis Center for Behavioral Neurobiology, The Salk Institute P O. Box 85800, San Diego, CA 92138.

The well-detailed organization of the cerebellum, the limited number of neuronal types present and known synaptic organization make this brain region ideal for studies of the intrinsic electrical characteristics of identified CNS neuronal types and their function within a known circuitry We have developed a culture preparation of rat cerebellum to facilitate such studies, focusing initially on anatomical methods to identify developing Purkinje neurons in culture. The cultures are prepared from 20 day rat embryos, an age at which the cerebellum is quite small and morphologically immature. By 1 week in culture, extensive development has occured as indicated by the morphological characteristics of the cells and the presence of numerous synapses, identified by immunostaining for Protein I, a protein localized to synapses (Bloom et al, PNAS 76, 1979). Prior to 2 weeks in vitro, morphological identification of cell types in unstained cultures is difficult, but in mature cultures (4 weeks in vitro; Gruol, Brain Res. 263, 1983) three classes of neurons can be identified: Purkinje neurons (PNs) granule cells and inhibitory interneurons. To obtain a more definitive identification of the PNs, immunohistochemical techniques were applied. Mature cultures were examined with an antibody against cGMP-dependent protein kinase, previously shown to stain PNs selectively in histological sections of rat cerebellum (Lohmann et al. PNAS 78, 1981). In the cultures, cells identified as PNs based on their unique morphology were also immunoreactive but all other cells (granule cells, inhibitory interneurons and non-neuronal cells) remained unstained. This antibody was then used to identify developing PNs in 1, 2, and 3 week cultures. The morphological maturation of PNs in the cultures was remarkably similar to that previously described in vivo (Altman, J. Comp. Neur., 145, 1972). As for PNs in vivo, three stages in PN maturation could be identified: (1) fusiform and apical cone stage (1 week cultures), (2) dendritic maturation (2 week cultures) and (3) somatic maturation (3 week cultures). Only minor changes in the morphology of PNs were observed at older culture ages. Electrophysiological studies are now in progress to identify the electrical properties of the PNs during the maturation process in culture. All antibodies were gifts from Dr. Paul Greengard and are gratefully acknowledge. (Supported by NIAAA 03504)

88.10 ISOLATION OF FLUORESCENCE-LABELLED HYPOTHALAMIC NEURONS. D.C.D. Rohrer*, D.M. Gash, M.F.D. Notter*, J.F. Leary* (SPON: F.C. Barone) Departments of Anatomy and Pathology, University of Rochester, Rochester, N.Y. 14642.

A major advance in neuroscience would be to have the capacity to isolate pure populations of viable neuronal types for in vitro and in vivo study. One possible method under current investigation is utilizing retrograde transport of fluorescent tracers followed by fluorescent-activated cell sorting of the dispersed tissue. This model is presently being tested using the hypothalamic neurosecretory system (HNS).

A promising retrograde tracer for these studies is fluorescein isothiocyanate labelled wheat-germ agglutinin (FITC-WGA), a fluorescence-labelled plant lectin which demonstrates limited diffusion from injection sties, fast retrograde transport, packaging into lysosomes, and brilliant fluorescence. Through a parapharyngeal, infrahyoid, trans-sphenoidal approach, the neural lobes of 20 day post-natal and older Long-Evans rats have been exposed and injected with 1.5 ul of 2% FITC-WGA (Sigma).

Time studies show that FITC-WGA is retrogradely transported to magnocellular neurons of the HNS within 10 hours. These cells remain brightly fluorescent for 48 hours in vivo and then gradually lose their fluorescence by 4 to 5 days post-injection.

After a 24 hour survival period, the animals are decapitated, supraoptic and paraventricular nuclei quickly microdissected-out into cold calcium-magnesium-free media with 1% glucose, and then trypsinized. The dispersed cell preparation is run through a fine-grade Sweeney filter, 5 fetal calf serum washes and a bovine serum albumin density gradient to remove debris. Cell viability after this treatment remains greater than 85% in age groups tested. Most importantly, these single cells retain their internal fluorescent marker.

Single-cell preparations containing fluorescence-labelled hypothalamic cells are being examined and sorted by a multiparameter laser flow cytometer-cell sorter (EPICS V, Coulter Electronics). Cells can be sorted viably and sterilely on fluorescence and light scatter at rates of approximately 1000 cells/second, having pre-examined populations at more than 100,000 cells/minute up to 6 parameters/cell.

This presentation will review the properties of labelled and unlabelled cells in dispersed preparations on the parameters of fluorescence and light scatter. In addition, the viability of sorted cells maintained both in vitro and in vivo will be reviewed.

Supported by NS 15109 to DMG.

88.11 CARTWHEEL AND STELLATE NEURONS IN THE DORSAL COCHLEAR NUCLEUS OF THE RAT: ELECTRON MICROSCOPY OF GOLGI IMPREGNATED CELLS. F. G. Wouterlood* and E. Mugnaini. Laboratory of Neuromorphology, University of Connecticut, Storrs, Connecticut 06268.

Of the five types of neuron which can be observed in Golgi sections of the outer two layers of the dorsal cochlear nucleus (DCN), three types, namely bipolar cells, cochlear granule cells and cochlear Golgi cells have been correlated previously with neuronal perikarya and profiles observed in thin sections of standard EM material. Although in the Golgi sections rapid and unequivocal distinction can be made between the two other types of neuron, called "cartwheel" and "stellate" cells, identification in standard EM material of their perikarya, dendritic and axonal profiles has not been resolved satisfactorily. By the present Golgi-EM approach we studied in the electron microscope thin sections of neurons classified by their light microscopic Golgi features as either cartwheel or stellate. Slices of rat brainstem containing the DCN were impregnated according to the Golgi-Del Rio Horteqa procedure. Solitarily impregnated cells of either type were gold toned or treated with either D 19 or Kodalith developers, embedded in epoxy resin, thin sectioned and examined in an electron microscope. Both cartwheel and stellate perikarya have smooth contours and contain an indented nucleus with an eccentric nucleolus. The nuclei of cartwheel cells contain evenly dispersed chromatin, whereas the nuclei of stellate cells display small peripheral clumps of heterochromatin. In contrast to stellate cells, perikarya of cartwheel cells contain small Nissl bodies and large numbers of subsurface cisterns associated with mitochondria. Axo-somatic synapses on cartwheel cells are exclusively of the symmetric type, whereas stellate cells receive axo-somatic synaptic contacts of both the symmetric and asymmetric types. In addition, perikarya of stellate cells are apposed by dendrites of other stellate cells. These appositions include puncta adherentia and gap junctions. Cartwheel cells have thick, smooth primary dendrites and uniformly sized, densely spinous, curved secondary and tertiary dendrites which receive synaptic contacts of the symmetric (predominantly on shafts) and asymmetric (predominantly on spines) types. Stellate cells possess thin smooth dendrites with occasional pleomorphic spines. In addition to synaptic contacts of the same types as on cartwheel dendrites, these dendrites are also engaged in numerous dendro-dendritic and dendro-somatic appositions, featuring puncta adherentia and gap junctions, with profiles of stellate cells. Both the axons of cartwheel cells and of stellate cells were observed to acquire a thin myelin sheath.

(FGW supported by the Niels Stensen Foundation of Amsterdam, The Netherlands; this study was supported by USPHS grant 09904-13.)

90 SYMPOSIUM. LESIONS OF THE VISUAL SYSTEM DURING INFANCY OR ADULTHOOD: EFFECTS ON MORPHOLOGY, PHYSIOLOGY AND BEHAVIOR. R.E. Kalil, Univ. of Wisconsin (Chairman); J. Dineen*, Univ. of Washington; D.E. Mitchell, Dalhousie Univ.; E.H. Murphy, Medical College of Pennsylvania; V.H. Perry*, Univ. of Oxford; P.D. Spear, Univ. of Wisconsin.

It is well known that the infant brain may respond to damage differently from the adult. For example, the developing brain often appears to compensate for damage, using mechanisms that are apparently not available to the adult. As a result, brain damage during infancy frequently, but not always, leads to milder deficits than comparable damage to the mature brain.

This symposium will focus on the consequences of damage to the visual system during development and adulthood. The effects of early versus late damage of the visual cortex or superior colliculus in several species will be reviewed. When possible, behavioral results will be correlated with recent information concerning changes in the underlying anatomy and physiology.

V. Hugh Perry will describe the effects of superior colliculus lesions in the neonatal rat on ganglion cells of the retina and their central projections. Visual acuity deficits will be related to the anatomical consequences of lesions made at different stages of development. E. Hazel Murphy will compare the morphological consequences of early cortical ablation in cats and rabbits, especially with respect to retinal ganglion cells and the retino-thalamic projection. Differences between these species will be discussed in relation to the timing of central visual system development and the role of sustaining collaterals in ganglion cell survival. Ronald Kalil will review the morphological changes that occur in the retina, thalamus, and cortex following removal of visual cortex in newborn and adult cats. Peter Spear will discuss the neurophysiological effects of visual cortex damage in neonatal or adult cats. Particular attention will be paid to the functional compensation that occurs in the lateral suprasylvian visual area following removal of visual cortex in young cats but not in adults. John Dineen will contrast the effects of striate cortex ablation in infant and adult macaque monkeys. Anatomical results in retina, thalamus and cortex will be described, and related to behavioral measurements of visual acuity. Donald Mitchell will report behavioral evidence regarding the visual acuity and depth perception of cats with lesions of visual cortex made during infancy or adulthood. The superior performance of infant operated animals will be discussed in relation to the anatomy and physiology of extrastriate visual areas.

The session will conclude with an open discussion of questions from the audience.

91 WORKSHOP. NEW APPROACHES TO THE STUDY OF THE MECHANISM OF FAST AXONAL TRANSPORT. D. S. Forman, Uniformed Services Univ. of the Health Sciences; T. Reese, NINCDS, NIH and Marine Biological Lab.; D. J. Goldberg, Columbia Univ.; M. B. Willard, Washington Univ.; S. T. Brady, Case Western Reserve Univ.

Ever since the discovery of fast axonal transport there has been speculation about the molecular mechanisms that drive fast transport. However, until recently the experimental evidence about the molecular mechanism has been rather limited, and has been mainly pharmacological. In the last few years new technical approaches have been used to provide new information about the biochemical and morphological substrates of fast transport. In this workshop, some of these new methods and new findings will be presented. Tom Reese will describe the new picture of the ultrastructure of axoplasm that is emerging from studies of specimens prepared by ultra-rapid freezing, followed by freeze-substitution or freeze-etching. Daniel Goldberg will present the results of experiments in which experimental probes were microinjected into single identified axons of Aplysia. By using the microinjection technique it has been possible to study the effects on fast transport of probes that would not normally enter intact axons. Another method for introducing impermeant probes into cells is to permeabilize the plasma membrane with detergents and reactivate movement with exogenous ATP. David Forman will describe studies using lobster axons permeabilized by treatment with saponin. Mark Willard will present immunocytochemical and radiolabeling studies of the structure of the axonal cytoskeleton. Scott Brady will describe fast transport in isolated squid axoplasm studied with the AVEC-DIC microscopic technique. The speakers will discuss their current views of the molecular mechanism of fast axonal transport.

DEVELOPMENT: ENDOCRINE CONTROL AND TRANSMITTER PLASTICITY

92.1 CONDITIONED MEDIUM ALTERS DISTRIBUTION OF PEPTIDES IN ENTERIC NEURONS IN CELL CULTURE, R. Nishi and A. Willard, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We have previously shown that cultures of neurons dissociated from small intestines of newborn rats express many of the differentiated properties of enteric neurons in vivo. We are interested in how these differentiated properties can be altered by changing the environment in which the neurons are growing. We have tested the effect of medium conditioned by a monolayer of heart cells. Previous work by our collaborators has shown that such conditioned medium (CM) contains a factor that induces cholinergic properties in cultured rat sympathetic neurons (see P. Patterson, 1982, Mol. Genetic Neurosci, pp 437-443).

CM concentrated 10x by ammonium sulfate precipitation was resuspended in standard growth medium for enteric neurons (MEM with 25mM K^+ + 5% rat serum). All enteric neurons were initially plated in standard growth medium. Three days after plating half the cultures were switched to CM-containing medium. Cultures were assayed 3-4 weeks after plating.

CM caused little change in the number of neurons surviving. Cell bodies in CM were larger overall, with a range in diameter of 10-50um, versus 10-25um in control medium. Synthesis and storage of ^{3}H-ACh from ^{3}H-choline in a 3 hr. period was 2-4x higher in CM neurons. This might be accounted for by the larger size of the somata. Neurons grown under either condition could not be distinguished electrophysiologically on the basis of electrical excitability or the frequency of occurrence of spontaneous or evoked synaptic potentials.

The most dramatic effect of CM was on the levels of peptides in the enteric neurons. Processes of cells grown in CM lacked the usual high levels of peptide-like immunoreactivity as demonstrated by PAP immunohistochemistry. Antisera tested were those specific for met-enkephalin, somatostatin, substance P, and VIP. No apparent differences in immunoreactivity of the somata + CM were detected. In contrast, 5-HT-like immunoreactivity in both processes and somata were enhanced by CM. The decrease in peptide-like immunoreactivity was correlated with a change at the ultrastructural level. Enteric neurons grown under control conditions normally contain a heterogeneous population of synaptic vesicles, of which 100-150nm opaque core vesicles predominate. Processes of CM neurons contained many clusters of 200-400nm vesicles that either lacked the opaque core or contained one that was considerably reduced in size. These were very rarely seen in control cultures.

We are currently investigating the mechanism(s) of this effect of CM on the levels of peptides.

This study was funded by NINCDS, the Muscular Dystrophy Association, and the American Heart Association.

92.2 EXPRESSION OF CATECHOLAMINE ENZYMES IN CHICK EMBRYO CILIARY GANGLIA IN VIVO AND IN VITRO. L. Iacovitti, G. Teitelman, L. Grayson*, T.H. Joh and D.J. Reis. Laboratory of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neurons of the cholinergic ciliary ganglia (CG) of chick embryo are able to express the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) when grown in vitro in the presence of notochord (36 out of 43, 83%) (Teitelman et al., Neurosci. Abstr. 8:257, 1983). To further evaluate the capacity of CG neurons to express a CA phenotype, we examined CG neurons for the appearance of CA enzymes during development in vivo. CGs were removed from chick at embryonic day E 5 to E 14, fixed, sectioned, and processed for immunocytochemical localization of the CA enzymes TH and phenylethanolamine N-methyltransferase (PNMT) according to the PAP technique. A few cells containing TH were found in each 16 um section of CG from E 5 to E 14. These cells ranged in size from 18 to 22 um and had long stained processes. Fine varicose terminals containing TH were also throughout the ganglion. At no time were cells containing PNMT found in the CG. This finding raised the possibility that CG neurons may also express CA enzymes in vitro in the absence of notochord. To test this, CG's from stage 30 to 35 chick embryos (E 7-E 9) were dissociated and plated onto collagen-coated dishes at a density of 1 CG/dish. After 5 to 8 days in vitro, cultures were processed for immunocytochemical detection of TH or PNMT. To test for the presence of aromatic L-amino acid decarboxylase (AADC), another CA enzyme, cultures were incubated with L-DOPA (60 min at 37°) and processed for detection of CA histofluorescence. We found that in 50% of the experiments (n=135 cultures) all CG cultures stained for TH while in the remaining experiments only an occassional culture was stained. In those experiments where staining was observed, it was seen in the majority of neurons in the dish. In general, stained cultures contained widely distributed neurons with numerous branching processes and few non-neuronal cells. Cultures of this morphology also expressed PNMT. Similar results were obtained when the neuronal density was increased up to 5 CG/dish. In contrast, dopamine histofluorescence was present in all cultures preincubated with L-DOPA irrespective of morphology, suggesting that the expression of AADC and that of TH and PNMT are differentially regulated.

We conclude that at least a small population of CG neurons have the intrinsic ability to express CA traits during development in vivo and that this number increases when CGs are grown under certain tissue culture conditions. These findings suggest that the expression of CA traits is elicited in CG neurons by a number of environmental cues including, possibly, notochord. (Supported by Grant NS03346.)

92.3 TRANSMITTER PHENOTYPIC PLASTICITY IN HUMAN PHEOCHROMOCYTOMA CELL CULTURES. A.S. Tischler[1]*, Y.C. Lee[2]*, R.L. Perlman[3]*, J.E. Jumblatt[1], and S.R. Bloom[2]. [1]Dept. of Pathol., Tufts Univ. Sch. of Med., Boston, MA 02111; [2]Dept. of Med., Hammersmith Hospital, London W120HS, England; [3]Dept. of Physiol. and Biophys., Univ. of Illinois Coll. of Med., Chicago, IL 60680.

It has been known for some time that chromaffin cells from human pheochromocytomas exhibit spontaneous and nerve growth factor (NGF)-induced neurite outgrowth in culture (A.S. Tischler et al, Science 192:902, 1976). In the present investigation transmitter phenotype was studied in freshly dissociated cells and in 21-day old monolayer cultures from human pheochromocytomas to determine whether neurite outgrowth is accompanied by functional alterations. In 6 tumors studied, production of vasoactive intestinal peptide (VIP)-like immunoreactivity, whether measured per culture dish or per mg cell protein, was consistently enhanced by culturing in McCoy's 5A medium with 20% fetal bovine serum, rising from levels undetectable in four of the cases at day 0 to as much as 3.5 pmol/mg protein at day 21. This effect was consistently magnified by 2.5S NGF(100 ng/ml), resulting in levels as high as 9 pmol/mg protein. In one of the 6 tumors, neurotensin-like immunoreactivity was detected at day 0 (0.2 pmol/mg protein) and rose along with VIP (0.9 pmol/mg protein at day 21 in control medium, >1 pmol/mg with NGF). In one of 3 tumors studied, somatostatin-like immunoreactivity rose from undetectable levels at day 0 to as much as 0.8 pmol/mg protein at day 21, but this increase was not affected by NGF. Choline acetyltransferase (CAT) activity was studied in one of the six tumors and was also found to increase markedly in culture (16±13 pmol/min/mg protein at day 0, 188±7 pmol/min/mg at day 21 in control medium, 295±24 pmol/min/mg with NGF).

In contrast to the large increases in immunoreactive peptides and in CAT activity, catecholamine content in the same culture dishes decreased to approximately 1/8 to 1/80 of initial levels per mg of cell protein and to 1/150 to 1/250 per dish, and was either unaffected or slightly decreased by NGF. We conclude that capacity for VIP production is a characteristic which is latent in many human pheochromocytomas, and is expressed in response to environmental signals which also favor neurite outgrowth in monolayer cultures. At least some human pheochromocytomas can also express cholinergic traits. Studies of human pheochromocytoma cultures may provide insights into molecular and cellular mechanisms in the regulation of a number of human neuroendocrine genes.

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92.4 NEUROPEPTIDE DEVELOPMENT IN CULTURED EMBRYONIC RAT DORSAL ROOT GANGLIA. J.E. Adler, J.A. Kessler, I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., NY, NY 10021.

Substance P (SP) and somatostatin (SS), putative peptide neurotransmitters, are localized to non-overlapping populations of small sensory neurons in the dorsal root ganglion (DRG). We have previously reported that the SP content of neonatal DRG sensory neurons increases markedly in culture. To further investigate the expression and ontogeny of neuropeptides, we have extended our observations to embryonic ganglia.

Cervical dorsal root ganglia from rat embryos were explanted onto reconstituted collagen and grown in medium supplemented with 10% fetal calf serum and nerve growth factor (10u/ml). To characterize developmental potentials at different ages, ganglia were explanted at different gestational stages. At embryonic day 13 (E13) ganglia contained low but detectable amounts of SP, which remained constant for up to 7 days in culture. In contrast, explanted E14 ganglia initially contained similar quantities of SP, but exhibited a 3-fold rise after a 3 day lag. Further, E15 ganglia exhibited a 10-fold increase of SP from the same baseline levels. Consequently, from E13 to E15 there is a progressive increase in the apparent potential for SP development in vitro, suggesting that critical regulatory events occur during this period.

To determine whether development of other sensory peptides paralleled that of SP, we examined SS. The E15 DRG contained detectable levels of SS. However, in contrast to SP, SS decreased significantly during the 7-day culture period. Since SS and SP appear to be localized to different DRG neuronal populations, these peptides may be useful markers for differential developmental requirements of different neurons in the same ganglion.

In summary, our observations suggest that (a) there is a critical period in the development of SP in the DRG, and (b) SP and SS development are differentially regulated.

(Supported by Fellowship from Committee to Combat Huntington's Disease and NIH Grants NS10259 and HD12108).

92.5 TARGET REGULATION OF ADULT SENSORY NEUROTRANSMITTER PLASTICITY. D.M. Katz, K.A. Markey, J.E. Adler and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., NY, NY 10021.

Studies in this laboratory have recently documented expression of functional catecholaminergic (CA) characteristics by primary sensory neurons in the nodose (NG) and petrosal (PG) ganglia of the normal adult rat, in vivo (Katz et al, PNAS, in press; Katz et al, 1982, Soc. Neurosci. Abs. 8:8). These characteristics include catalytically active tyrosine hydroxylase (TOH), the rate-limiting enzyme in CA biosynthesis, formaldehyde-induced catecholamine fluorescence, and sensitivity of intraneuronal catecholamine levels to pargyline, a specific inhibitor of monoamine oxidase. These findings demonstrated that adult expression of CA phenotypic characteristics, in the periphery, is not restricted to cells of the sympatho-adrenal axis, and indicated that catecholamines may serve autonomic sensory, as well as motor, functions.

To define the mechanisms regulating CA metabolism in sensory neurons, we examined the acute effects of axotomy on petrosal TOH. Peripheral axotomy dramatically reduced TOH catalytic activity and immunoreactivity in adult PG neurons within one week after surgery. However, enzyme activity and immunocytochemical staining returned to control levels within one month. These data suggest that PG neurons do not die following peripheral axotomy, but rather remain viable, expressing reduced levels of TOH activity. Consequently, target factors may regulate TOH levels in the innervating PG neurons. Moreover, colchicine blockade of axonal transport in PG axons appears to reproduce the effects of axotomy, indicating that transport mechanisms may mediate target influences on sensory TOH.

To further characterize target effects on sensory CA characteristics, we have begun to examine TOH expression by PG neurons in tissue culture. Preliminary studies indicate that PG neurons, grown for one week in the presence of their normal carotid body target, exhibit TOH immunocytochemical staining. Definition of factors regulating sensory TOH in vitro is currently in progress.

Supported by NIH post-doctoral fellowship NS 06623 to D.M.K. and NIH grants NS 10259 and HD 12108.

92.6 NOT ALL TRANSIENTLY CATECHOLAMINERGIC CELLS OF THE RAT EMBRYO POSSESS NOREPINEPHRINE UPTAKE. G. M. Jonakait, K. Markey, M. Goldstein, and I.B. Black. Div. Develop. Neurol., Cornell Univ. Med. Coll., New York, N.Y., and Dept. of Psychiatry, NYU Med. Center, New York, N.Y.

We have been working with several cellular populations in the rat embryo which have in common the transient expression of tyrosine hydroxylase (T-OH), the rate-limiting enzyme in catecholamine (CA) biosynthesis. We wondered whether these populations, located in gut mesenchyme (Cochard et al., 1978; Teitelman, et al., 1979) and in several cranial sensory and dorsal root ganglia (Jonakait, et al., 1983) possessed, in addition to their other CA traits, the specific, high-affinity uptake of ^{3}H-norepinephrine (^{3}H-NE). While high-affinity uptake of NE has already been demonstrated for a population of cells in embryonic rat gut (Jonakait et al., 1979), the coincidence of uptake and T-OH in the same cells has not been established (Jonakait, et al., 1979; Gershon et al., 1982).

Utilizing simultaneous immunocytochemical and radioautographic localization of T-OH and ^{3}H-NE respectively, we found no T-OH-positive gut cells at 11.5 days of gestation (E11.5) which also exhibited uptake of ^{3}H-NE. However, by E12.5 20% of T-OH-positive gut cell profiles had overlying silver grains, and by E13.5, this percentage had increased to almost 60% for T-OH-positive cells throughout the gut, and attained almost 95% for T-OH-positive cells oral to the umbilical flexure. A few cells, negative for T-OH, exhibited silver grains, but these were sparse. By E14.5 T-OH immunoreactivity was fading, but even faintly stained cells continued to exhibit NE-uptake, though increasing numbers of cells negative for T-OH took up ^{3}H-NE. By E15.5, though only a few cells were positive for T-OH, radioautography revealed that gut cells continued to take up ^{3}H-NE. At all ages examined, uptake was blocked by desmethylimipramine.

In contrast to cells of the gut, uptake of ^{3}H-NE was never exhibited by cells in the trigeminal, nodose, or petrosal ganglia, even though these cells, too, exhibit transient expression of CA traits (Jonakait, et al., 1983).

We conclude, therefore, 1) that embryonic cells which transiently express aspects of the CA phenotype do not necessarily exhibit specific, high-affinity uptake of NE; and 2) that the population of cells responsible for uptake in the embryonic gut is coincident with the population of transiently CA cells previously described, and that uptake remains a feature of these cells after their loss of other CA traits.

This research has been supported by NIH grants NS17814, NS10259 and HD12108.

92.7 TRANS-SYNAPTIC REGULATION OF ADRENAL LEU-ENKEPHALIN BY IMPULSE ACTIVITY. E.F. LaGamma*, J.E. Adler and I.B. Black (SPON: E.M. Bloom). Division of Developmental Neurology & Perinatology Center, N.Y. Hospital-Cornell Medical Center, N.Y. 10021.

Trans-synaptic impulse activity has long been known to increase catecholamine (CA) biosynthesis and CA synthetic enzymes in the adrenal medulla. Since opiate peptides are co-stored and co-released with CA's in medullary cells, and since medullary opiate peptides may be important modulators of the sympatho-adrenal stress response, we examined trans-synaptic regulation of the putative peptide neurohumour, leucine-enkephalin (L-enk). Adrenals were unilaterally denervated in adult rats and L-enk was determined using a sensitive and specific radioimmunoassay. Four days postoperatively L-enk increased by 250% compared to sham-operated or contralateral control adrenals. Further, treatment with chlorisondamine, a long-acting nicotinic receptor antagonist, also significantly increased L-enk, suggesting that impulse activity and medullary depolarization per se decreased L-enk. To define underlying molecular mechanisms, medullary explants were grown in culture. 4 days after explantation the (now denervated) medullae exhibited a 100-fold increase in L-enk. Exposure of explants to veratridine (V), which causes membrane depolarization by increasing Na^+ influx, blocked the increase in L-enk. Tetrodotoxin (Ttx) which blocks the Na^+ flux effects of V, prevented the effect of V on L-enk. Moreover, depolarization with 50mM KCL also prevented the rise in L-enk. Neither Ttx alone or 50mM NaCL controls affected the increase of L-enk. We conclude that trans-synaptic stimulation decreases medullary L-enk through postsynaptic depolarization and increased Na^+ influx. Thus, trans-synaptic impulses simultaneously regulate opiate peptides and biogenic amines in the adrenal medulla.

(Supported by NIH Grants HL00756, NS10259 and HD12108).

92.8 ORIGIN OF ENTERIC SEROTONERGIC NEURONS FROM THE NEURAL CREST AND DETERMINATION OF THE SEROTONERGIC PHENOTYPE BY THE ENTERIC MICRO-ENVIRONMENT: A STUDY USING QUAIL-CHICK INTERSPECIES CHIMERAS. M.D. Gershon, P. Cochard*, D. Sherman* and T.P. Rothman, Dept. Anatomy and Cell Biol., Columbia Univ., P&S, New York, NY and Institut d'Embryologie du CNRS et du Collège de France, Nogent-sur-Marne, France.

The enteric nervous system (ENS) differs structurally, neurochemically, and physiologically from other regions of the PNS. In particular, the phenotypic diversity of its component neurons exceeds that of any other group of peripheral ganglia. Amongst the neurons intrinsic to the bowel are enteric serotonergic neurons. These neurons constitute about 2-3% of the enteric neuronal population and project widely as interneurons throughout the enteric plexuses. Through the use of quail-chick interspecies chimeras, LeDouarin and co-workers have established that enteric ganglia arise from the vagal (somites 1-7) and sacral (caudal to somite 28) levels of the neural crest and have proposed that phenotypic expression by enteric neuroblasts may be determined by the microenvironment of the bowel rather than by the level of origin of these neuroblasts in the neural crest. The current experiments were done: (1) to determine whether enteric serotonergic neurons are derivatives of the neural crest and (2) to test the hypothesis that the serotonergic phenotype is dependent upon a microenvironmental-neural precursor interaction within the bowel. Interspecies chimeras were constructed in which neural tube and crest were grafted from donor quail embryos into chick recipients. The vagal neuraxis was removed from the hosts prior to stage 10, before the onset of neural crest migration. Either the vagal or the truncal neuraxis was grafted from the quail to the vagal region of the chick. Grafts were allowed to survive until day 8 to 15 of incubation, after which the gut was removed and exposed to ^{3}H-serotonin (^{3}H-5-HT; 0.5 uM) in the presence of pargyline (0.1mM). The developing ENS in the resulting chimeras was examined by light (LM) and electron microscopic (EM) radioautography. The quail nuclear marker was identified for LM by specific DNA staining and for EM by selective extraction of stain from DNA with EDTA. Enteric serotonergic neurons of quail origin were identified as doubly marked with the quail nucleus and ^{3}H-5-HT. Grafts of vagal and even truncal neural crest gave rise to these neurons. It is concluded that enteric serotonergic neurons are of neural crest origin. Since truncal neural crest (somites 8-15) does not ordinarily give rise to serotonergic neurons, the observation that it will do so when grafted so as to migrate to the gut, supports the view that the enteric microenvironment, through induction or selection, plays a role in serotonergic phenotypic expression.

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92.9 DEVELOPMENTAL PLASTICITY OF SYMPATHETIC NEURONS INDUCED BY GONADAL STEROIDS. L. L. Wright, P. Beaston-Wimmer* and A. J. Smolen. Dept. of Anatomy, Med. Coll. of Penn., Philadelphia, PA 19129.

We have found that adult male rats have 20% more neurons in the superior cervical ganglion (SCG) than females. This difference is not present at birth, and the number of SCG neurons in adult males can be significantly reduced by neonatal castration. These results show that the normal low levels of endogenous testosterone in neonatal male rats alter the final number of SCG neurons.

To determine whether the gonadal steroids increased the numbers of neurons by enhancing proliferation or reducing neuron degeneration, pregnant females were injected with (^{3}H)-thymidine on gestational day 16. The pups were then treated with 17-beta-estradiol (E2) or vehicle on alternate days from the day of birth until postnatal day 15 (P15), when they were sacrificed. Significantly more labeled neurons were present on P15 in the E2 treated animals. Other animals were injected with (^{3}H)-thymidine on P3 and sacrificed 2 hours later. Although many non-neuronal cells were labeled, there was no evidence of postnatal neuron proliferation in SCGs of either vehicle or E2 treated animals. There is a temporal correspondence between developmental neuron death in the rat SCG and the effects seen with neonatal gonadal steroid treatment. No differences between animals treated with E2 or vehicle from birth were found at 3 days of age, prior to the onset of developmental neuron death. However, after the period of cell death, at P15, the number of neurons had not declined in animals treated with E2 from birth, as it had in vehicle treated littermates. These studies indicate that the increase in neuron numbers seen after neonatal gonadal steroid treatment is the result of enhanced survival of sympathetic neurons through the period of developmental neuron death.

Treating neonatal rats with testosterone or E2 almost doubles the number of synapses found in the SCG. This treatment difference seen on day 15 is eliminated by cutting the preganglionic cervical sympathetic trunk (CST) on postnatal day 13 and allowing the extrinsically derived synapses to degenerate. This indicates that the additional synapses formed in the SCG as a result of gonadal steroid treatment arise from the axons of the CST, and not from the intrinsic postganglionic neurons or SIF cells.

The modification of gonadal steroids of neuron and synapse numbers in the SCG is an example of neuronal plasticity during development. It is of particular developmental interest because physiological levels of these hormones initiate a sequence of highly specific developmental alterations that are likely to be relevant to the function of sympathetic target tissues.

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92.10 DEVELOPMENT OF THE ADRENERGIC PHENOTYPE IN RAT BRAIN: CHARACTERIZATION OF A GLUCOCORTICOID RESPONSIVE PERIOD. M.C. Bohn and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., New York, N.Y. 10021.

The adrenergic phenotypic character, PNMT, (phenylethanolamine N-methyltransferase), the epinephrine-forming enzyme, is expressed in both the peripheral and central nervous systems. To compare factors involved in phenotypic expression in brain and periphery, we have studied the initial expression, development and hormonal regulation of PNMT in adrenal medulla, superior cervical ganglion (SCG) and medulla oblongata MO). We have previously reported that PNMT is initially observed in brain on embryonic day 14 (E14), whereas PNMT does not appear in the periphery until E17. These observations suggest that the initial expression of PNMT is differentially regulated in brain and periphery. In the periphery, PNMT development and regulation during adulthood are glucocorticoid-dependent. To determine whether central PNMT is similarly regulated, we have now studied the effects of perturbations of the pituitary-adrenal axis and various steriod treatments on PNMT in MO.

Prenatally, PNMT was increased in fetal adrenal and SCG following dexamethasone (DEX) treatment of late gestation pregnant rats. As in the periphery, treatment of pregnant rats from E18 to E21 at doses of 1 or 4 mg/kg increased PNMT activity in MO at E22 45% and 60%, respectively. In both the periphery and brain, DEX treatment prior to E18 was without effect.

Postnatally, we have previously observed that an increase in glucocorticoid levels on days 0-6 increases PNMT levels in SCG. In contrast, treatment of rats on postnatal days 0-2, 6-9, or 7-13 with DEX (0.1μg/g) or cortisol (200μg/day) did not affect PNMT activity in MO at 3, 10 or 14 days. Adrenalectomy (ADX) on postnatal day 7 also did not affect development of PNMT in MO.

In adult rat, decreased levels of glucocorticoids following ADX or hypophysectomy (HPX) had no effect on PNMT activity in MO during the postoperative month. In contrast, PNMT activity in the adrenal fell following HPX as observed previously (R. Wurtman & J. Axelrod, J. Biol. Chem. 241, 2301, 1966).

Our experiments demonstrate striking differences in glucocorticoid regulation of PNMT in brain and periphery. In adrenal, the developmental increase in PNMT as well as the maintenance of PNMT in adult are steroid-dependent. In contrast, brain PNMT is not affected during development or adulthood by glucocorticoid deficiency. Nevertheless, prenatal DEX treatment during late gestation increases PNMT in both periphery and brain. Consequently, all adrenergic cells may be responsive to glucocorticoids during specific developmental phases.

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92.11 TESTOSTERONE-INDUCED ENHANCEMENT OF MALE MEDIAL PREOPTIC TISSUE TRANSPLANT VOLUMES IN FEMALE RECIPIENTS: A "NEURONOTROPHIC" ACTION OF TESTOSTERONE. G. W. Arendash and R. A. Gorski. Dept. of Biology, College of Natural Sciences, University of South Florida, Tampa, Florida 33620 and Lab. of Neuroendocrinology, UCLA Brain Research Institute, Los Angeles, CA 90024.

The critical importance of the medial preoptic area (MPOA) for the expression of masculine sexual behavior in the rat has been well established. Through the use of a new intraparenchymal transplantation technique, we have recently reported that immature brain tissue from male neonates not only survives transplantation into the MPOA of female neonates, but that male MPOA tissue transplants can apparently develop functional connectivity with the female recipient's brain to enhance dramatically her display of masculine sexual behavior during adulthood (Science 217: 1276-78, 1982). To gain some insight into the mechanisms(s) by which gonadal hormones "organize" or alter the development of the mammalian brain to bring about its sexual and neuroanatomical differentiation, the present study tested the ability of testosterone treatment to modify the volume of male brain tissue transplants in female recipients. 1-day old females, in addition to having either male MPOA or Caudate nucleus (CN) tissue bilaterally implanted into their MPOA's, also received either 200 ug testosterone propionate (TP) or oil (sc) concurrently, and 200 ug TP or oil for the following four days. All recipients were sacrificed at 30 days of age, their brains serially sectioned at 50 um and stained with Thionine. An analysis of male transplant volumes indicated that MPOA transplants in oil-treated recipients were substantially reduced in size (0.06 ± .01 mm^3) compared with the initial transplant volume of 0.19 mm^3. However, MPOA transplants in recipients treated with TP showed a 79% increase above the initial transplant volume (to 0.34 ± .05 mm^3). This 5- to 6-fold enhancement in MPOA transplant volume induced by TP treatment was highly significant ($p < .001$). In sharp contrast, the same TP treatment to recipients receiving male CN transplants resulted in no such enhancement. Therefore, transplants involving a brain area known to concentrate ^{3}H-labeled testosterone neonatally (i.e., the MPOA) responded to TP treatment with an enhancement of volume which was not observed for transplants consisting of brain tissue not known to concentrate ^{3}H-labeled testosterone (i.e., the CN). The above results suggest that testosterone is a "neuronotrophic" agent during development that acts specifically on cells within steroid-sensitive brain areas to prevent neuronal death within these areas. Such a CNS mechanism of steroidal action may be involved with the development of sexual dimorphisms in behavior, hormonal secretory patterns, and CNS neuroanatomical structure. (Supported by NIH Grant HD-01182 and USF FRCS Grant #19-2702-005).

92.12 SEXUAL DIMORPHISM AND PERSISTENCE OF THE SYNAPTOGENIC EFFECT OF ESTROGEN IN PREPUBERAL RATS

Richard W. Clough and Jorge F. Rodriguez-Sierra, Department of Anatomy, Univ. of Nebr. Medical Center, Omaha, NE 68105.

We have previously reported that treatment of 25-day-old female Sprague-Dawley female rats with estradiol benzoate (EB) results in an acute induction of synaptogenesis in the hypothalamic arcuate (ARC) region two days later (Clough and Rodriguez-Sierra, Am. J. Anat., in press). The present studies were conducted to determine if (1) this acute induction of synapse formation in prepuberal female rats is also manifest in EB-treated male rats and (2) if the increased number of ARC synapse after EB in female rats on day 27 persists beyond this 2 day period. Twenty-five day old Sprague Dawley female and male rats were administered 10 µg of EB or oil vehicle subcutaneously at 1200 hours. Male rats were bled on day 27 and female rats were bled on day 27 or 31 of age by jugular venipuncture at 1600 hours. Plasma was assayed for LH by radioimmunoassay. After bleeding, all rats were perfused transcardially with a paraformaldehyde-glutaraldehyde fixative in phosphate buffer. The ARC, preoptic area (POA) and medial septal area (MSA) were dissected out and processed for electron microscopic analysis of synaptic area density.

	ARC Area Density	
Group (N)	27 day	31 day
oil-male (4)	10.06 ± .46	--
EB-male (4)	10.05 ± .48	--
oil-female (6)	10.75 ± .42	14.35 ± .44
EB-female (6)	18.51 ± .46*	17.47 ± .66*

(*$p<0.05$; $\bar{X}$ ± S.E.M./100 μ^2, compared to oil controls)

Results (table above) indicated that EB treatment does not induce an increase in all synaptic area density in prepuberal male rats. In contrast, prepuberal female rats show a significant increase in synaptic area density in the ARC on day 27. The increase in synaptic area density of the ARC, induced by EB, was found to persist in 31 day old female rats. We found no significant effect of EB on synaptogenesis in the POA or MS in either sex.

We conclude from these studies that there is a dramatic sexually dimorphic neural, as well as endocrine, response to EB administration in prepuberal rats. Females respond with a precocious surge of plasma LH and associated induction of ARC synaptogenesis; while males show neither an LH surge or increased synapse formation following EB administration. Furthermore, the acute induction of synaptogenesis seen on day 27 remains through at least day 31 in the prepuberal period suggesting the relative permanency of this maturational effect of EB in prepuberal female rats (Supported by NIH grant, HD 13219).

92.13 THE SPECIFICITY OF MOTONEURON TO MUSCLE INNERVATION CAN BE MANIPULATED HORMONALLY IN THE RAT. S. Marc Breedlove, Department of Psychology, University of California, Berkeley, CA 94720.

Motoneurons of the spinal nucleus of the bulbocavernosus (SNB) innervate the perineal muscles bulbocavernosus (BC) and levator ani in male rats. These muscles are present in female rats at birth, but disappear shortly thereafter. Thus adult females possess neither the SNB nor its target muscles. Neonatal treatment of female rats with either testosterone propionate (TP) or dihydrotestosterone propionate (DHTP) results in adult females which, like males, have an SNB and its target muscles. However, these two androgens exert disparate effects on this neuromuscular system when they are administered just before birth. Prenatal TP causes the muscles and the SNB to persist into adulthood, but prenatal DHTP treatment paradoxically causes the muscles to persist without increasing the number of SNB cells. This condition is the result of either: a) muscles without innervation, b) muscles innervated by cells in the SNB region which do not morphologically resemble motoneurons, c) muscles innervated by a very few SNB cells, each innervating many muscle fibers, or d) muscles innervated by motoneurons with a normal morphology, but which are outside their normal anatomical locus in the SNB. The present results of horseradish peroxidase (HRP) injections into the muscles of androgenized females indicates that the last alternative is correct.

Pregnant Sprague-Dawley rats were injected with 2 mg/day of either TP or DHTP from the 17th - 22nd days of gestation. Upon delivery (day 23), the female pups were toe-marked and cross-fostered to untreated dams. The androgenized females were implanted with silastic capsules of testosterone in adulthood in order to increase the size of the perineal muscles. At least 2 weeks after capsule implantation the androgenized females were anesthetized and their BC muscles were injected with either 0.5 or 1.0 µl of 30% HRP in saline via a microsyringe guided under a dissection microscope. The rats were sacrificed 24-48 hrs later and the spinal cords were horizontally sectioned and stained for HRP by a tetramethylbenzidine method. The experiments were done in five yoked pairs consisting of 1 prenatal DHTP and 1 prenatal TP female injected, stained and analyzed together.

In females given TP prenatally, most motoneurons innervating the BC were found in the same location as in normal males: the medial motoneuronal column normally occupied by the SNB. However, the BC of females prenatally treated with DHTP was innervated by motoneurons in an anomalous location: the lateral motoneuronal column more than 600 µm away. In prenatal TP females 71% of the labeled motoneurons were in the (normal) medial column, while in prenatal DHTP females only 18% of the motoneurons were in this medial position. These results represent a unique example of an alteration in the pattern of motoneuron to muscle innervation using normally relevant hormonal cues. An understanding of how this anomalous pattern comes about may elucidate mechanisms by which "proper" innervation patterns are normally achieved.

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92.14 THE MORPHOLOGIC DEVELOPMENT OF SEROTONERGIC NEURONS IN THE CHICK EMBRYO: NEW FINDINGS OF LATE DEVELOPING NEURONAL SYSTEMS. J.A. Wallace, Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.

As a continuation of earlier morphologic studies of the development of serotonergic systems in the chick embryo (Wallace et al. Neurosci. Abs. #255.2, 1981) the present investigation extends the findings of immunocytochemical staining patterns for serotonin (5-HT) in embryos from 3 to 12 days of incubation (E3 to E12). Because of the larger number, as well as the later ages of embryos examined, the results reported here concern the observation of 5-HT-containing cells not mentioned in the earlier study.

As noted previously, the majority of central 5-HT neurons in the chick embryo begin to stain immunocytochemically for 5-HT as early as E4 and E5. However, with further investigation it was found that a number of cells do not begin to stain for 5-HT until considerably later in development. The most striking examples consist of neurons that were first detected immunocytochemically at E12 in the hypothalamus and the optic lobes of the mesencephalon. 5-HT neurons in the hypothalamus were located immediately adjacent to ependymal cells lining the III ventricle in the roof and dorsolateral walls of the mammillary recess. In the optic lobes, spindle shaped cells stained for 5-HT comprised a significant proportion of cells (approximately 25%) in the innermost lamina of the three layers present in the optic tectum at this age. This latter finding of cells containing 5-HT (or a related indoleamine) in the optic lobes is particularly novel since monoaminergic neurons have not previously been reported at this location in adult or developing chickens. 5-HT neurons were also initially detected at E7 to E9 in the lateral portion of the medulla at the point of entrance of cranial nerves VII and VIII. These spindle shaped cells were oriented with the cranial nerve fascicles as they entered the medulla. The possibility that these 5-HT neurons project axons peripherally into these sensory nerve roots is currently under study. Cells immunocytochemically stained for 5-HT were also noted for the first time outside the CNS in sympathetic chain ganglia and in the anterior pituitary. Large numbers of cells in the sympathetic ganglia were stained as early as E5, however the proportion of these cells in the ganglia at later stages is unknown. The immunostaining of cells at all the locations mentioned above was blocked by the preincubation of the antiserum with 10µM 6-hydroxy-tetrahydro-ß-carboline, a condensation product of 5-HT formed during tissue fixation with formaldehyde (Schipper and Tilders, J. Histochem. Cytochem. 31: 12, 1983).

The possibility that these later formed 5-HT cells occur transiently during development or are derived from cells that begin their differentiation expressing another neurotransmitter phenotype is currently under investigation. Supported by NSF BNS 82-08433.

93.1 NEW OBSERVATIONS ON THE TOPOGRAPHIC ORGANIZATION OF THE THALAMO-CORTICAL PROJECTIONS TO THE MOTOR CORTEX IN THE CAT. A. Morán* and F. Reinoso-Suárez. Departamento de Morfologia, Fac. de Medicina, Univ. Autónoma, Madrid 34 (Spain).

The absence of systematic anatomical studies of the thalamo-cortical connections of areas 4 and 6 of the cat motor cortex (MC), including those which end in its hidden parts situated in both banks and the bottom of the cruciate sulcus, suggested the value of an HRP retrograde technique study in which all levels of the thalamus could be analyzed serially. For this purpose, systematic small injections of HRP aqueous solution (sigma type VI) were made in 24 adult cats with careful avoidance of enzyme contamination of the surrounding cortical areas. Our results contribute greater detail to the description of the sites of origin in thalamic nuclei of projections to the MC (rostral part of ventral anterior, ventral anterior-ventral lateral complex, principal and basal ventral medial, rostral dorsolateral part of ventral posterolateral, medial subdivision of the posterior nuclear group, ventral posteroinferior, central lateral-paracentral, centromedian-parafascicular and midline nuclei). In addition we describe new thalamic regions which also project to MC, such as the lateral subdivision of the posterior nuclear group (to area 4), medial dorsal (not only medially to area 6 in the proximity of area 8 but also in the most lateral part of area 6), lateral intermediate oral, lateral anterior, lateral intermediate caudal and lateral medial nuclei (to areas 4 and 6) and the suprageniculate nucleus (also to both areas 4 and 6). We suggest an overall topography of these thalamo-cortical connections, which in spite of their overlapping show a sequential arrangement proceeding from zones which are partially aligned in a band-like fashion in the thalamus and send projections which are also aligned in bands in the cortex. This thalamocortical relationship generally extends beyond the borders of the thalamic nuclei and the cortical subareas and is arranged along latero-medial coordinates. This topographic organization also shows that the arrangement in the thalamus from ventral to dorsal may be reflected in the MC from rostral to caudal.

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93.2 MODIFICATION OF CORTICO-CORTICAL PROJECTION AFTER ELIMINATION OF THALAMOCORTICAL PROJECTION IN THE CAT. H. Asanuma, E. Kosar, N. Tsukahara and H. Robinson* The Rockefeller University, New York New York, 10021

We have reported (Kosar et al., 1982, Neurosci. Abstr.) that in normal cats association fibers arising from area 2 of the sensory cortex (S-Cx) terminate in the superficial layers (II and III) of the motor cortex (M-Cx), area 4δ. The neurons receiving this input were primarily stellate interneurons. In the present study, we have examined the changes of projection from area 2 to area 4δ after ablation of neurons in the ventro-lateral (VL) nucleus of the thalamus.

Cats were anesthetized with Nembutal and 2.5 µg of kainic acid was injected into VL nculeus stereotaxically. After one month survival time the cats were re-anesthetized (Nembutal 35 mg/kg) and a double chamber was installed over the motor and sensory cortices. 3-5 stimulating electrodes were implanted in area 2 of S-Cx and a stimulating pulse of 0.2 ms duration, 30 µA intensity was delivered at 2/sec through each electrode. Intracellular recordings were made from motor cortical neurons through a pipette electrode filled with 10% HRP in 0.5 M KCl. In several animals, depth reversal of evoked potentials in the motor cortex elicited by stimulation of S-Cx was examined. At the end of the experiments, animals were deeply anesthetized, perfused, and sections were cut and reacted with TMB method.

A total of 49 M-Cx cells was successfully impaled and their EPSPs were compared to those in the control animals. The major changes were 1) EPSPs beared multiple notches whereas in the control animals the majority was monophasic. 2) The time from the beginning to the peak of EPSP became longer. 3) Some of the neurons receiving S-Cx input were located in layer V. 4) The surface positive evoked potential showed a negative focus in the depth whereas in the control group no such focus was present. 5) Some of the neurons stained with HRP were not typical stellate cells.

It is concluded that elimination of one input produced significant changes to the other input which might explain the recovery of motor function after deprivation of thalamic input to the motor cortex.

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93.3 MOTOR PROGRAMMING AND THE PREMOTOR CORTEX OF THE RHESUS MONKEY Karl-Heinz Mauritz and Steven P. Wise. Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

The premotor cortex has been suggested to subserve motor programming, and class of directionally specific and "set-related" neurons could be involved in such programming (Weinrich and Wise, J. Neurosci. 2: 1329). These set-related neurons are activated or inhibited during a delay period between the presentation of a visual or auditory instruction stimulus (IS) and a subsequent trigger stimulus. Most set-related cells show qualitatively different changes in activity for impending limb movements in opposite directions, e.g. an increase in neuronal discharge before flexions and a decrease before extensions. In order to see how a change in the visuospatial instruction influences neuronal activity, several visuomotor tasks were presented randomly to an operantly conditioned rhesus monkey.

Target-On Condition: When the monkey pressed a center key for 1 s, either a left or a right target key was illuminated to serve as the IS. After a randomly varied delay period (1.5, 2.25, or 3 s) a light above the target was turned on to serve as a trigger signal for a reaction time movement. Target-Off Condition: The IS was turned off (after 1 s) during the delay period and the monkey had to remember the direction of the next required movement for the remainder of the randomly varied delay period. Target-Change Condition: The IS was switched, after 1 s, from one side to the opposite side.

Directional set-related unit activity was profoundly affected by the target-change condition. After a change of the IS from one side to the other, the sustained activity changed from activation to inhibition or vice versa with a mean latency of 143 ± 37 ms to reflect the new impending movement direction. All 31 directionally specific set-cells that have been observed to date have displayed this behavior. In the target-off condition, the sustained activity pattern is comparable to that shown when the target remains on. Additionally, about 24% of the set-related neurons showed either transient bursts or inhibitions 169 ± 42 ms after the target light was turned off. There was almost no modulation in the neuronal activity during repetitive, self-paced, and slower movements between the left and right targets.

The results are consistent with the hypothesis that directionally specific set-related premotor cortex neurons reflect the motor preparations of the animal. Furthermore, these results indicate that the directionally specific set-related cells are activated or inhibited when changed targeting necessitates a rapid reprogramming of the next motor act.

93.4 MICROSTIMULATION MAPS OF THE ARM AREA OF THE MONKEY'S MOTOR CORTEX BEFORE, IMMEDIATELY AND TWO MONTHS AFTER MEDULLARY PYRAMIDOTOMY. A.R. Mitz* and D.R. Humphrey. Lab. Neurophysiology, Emory Sch. Med., Atlanta, GA 30322.

Recent mapping studies of the motor cortex of the cat have shown that two major outputs emerge from the zone assumed previously to control only distal forelimb muscles: (i) a low-threshold pyramidal tract outflow which affects principally distal forelimb muscles, and (ii) a higher-threshold, extrapyamidal outflow which affects proximal limb and trunk muscles (Asanuma et al, J. Neurophysiol., 46: 694-703). Similar spatial overlap has been observed in the motor cortex zones which control wrist, elbow and shoulder muscles in the monkey, but it is not known whether distal and proximal arm muscles are effected principally by separate corticofugal systems. To obtain such information, we used intracortical microstimulation methods (2-60 µA, 0.2 msec biphasic pulses, 10-30 pulse trains, 300 pps) to map the precentral arm area in two lightly tranquilized monkeys before, 3-7 days after, and 42-60 days after unilateral medullary pyramidotomy. Evoked movements were carefully recorded, and chronically implanted EMG leads were used to continuously monitor evoked activity in five major muscle groups of the arm. Our major results were as follows. (1) Before pyramidotomy, low-threshold (3-20 µA) stimulation at various cortical loci evoked any one of the major forms of fundamental movements observed about finger, wrist, elbow or shoulder joints in the alert animal. In particular, isolated movements of the thumb, the index finger (digit 2), and of digits 3-5 were easily evoked. (2) 3-7 days after medullary pyramidotomy, the cortical map was drastically reduced in spatial extent, with only 'in-concert' movements of the fingers and of the thumb, often in a grip-like movement, remaining. No evoked activity was observed in biceps or triceps EMGs, and only questionable responses were seen from two cortical loci in shoulder girdle muscles, even with the most intense stimuli and the longest cortical trains used. (3) 42-60 days after pyramidotomy, however, the map returned partially toward the pre-lesion configuration, with shoulder, wrist and, to a lesser extent, elbow movements now being evoked at higher thresholds (20-60 µA) and with longer stimulus trains. Movements of digits 2-5, independent of thumb movements, also returned.

Thus, movements of proximal limb muscles evoked by motor cortex stimulation in the monkey appear to depend upon the integrity of pyramidal tract fibers, a situation unlike that in the cat. Moreover, the only functional outflow from the arm area immediately after pyramidotomy seems to be one for controlling crude movements of the hand. Those movements of more proximal muscles that are recovered later appear to depend upon adaptive changes in other extrapyramidal systems. (Supported by NIH Grant NS 10183)

93.5 THE RELATION OF CALLOSAL CONNECTIONS TO MICROSTIMULATION MAPS OF PRECENTRAL MOTOR CORTEX IN OWL MONKEYS. H. J. Gould, III, C. G. Cusick, T. P. Pons and J. H. Kaas. Dept. of Anatomy, LSU Medical Center, New Orleans, LA 70112 and Dept. of Psychology, Vanderbilt Univ., Nashville, TN 37240

Microstimulation mapping procedures were combined with injections of horseradish peroxidase (HRP) in the same animals to relate the functional organization of motor cortex to patterns of corpus callosum connections in adult owl monkeys. A typical procedure was to label callosal pathways in anesthetized animals by making multiple injections of HRP into the entire somatomotor cortex of one cerebral hemisphere, and then explore motor cortex of the uninjected hemisphere for a remaining 24-48 hour survival period. In order to obtain extensive and detailed maps of motor cortex, 200-600 sites were stimulated in each animal. At the end of mapping, reference lesions were made at known sites, and the brains were removed, flattened, and cut parallel to the cortical surface so that the areal pattern of callosal connections could be readily appreciated in sections reacted using tetramethyl benzidine or benzidine dihydrochloride. Movements to 0.5-10 μA levels of current were obtained over a 6 mm wide strip of cortex bordering Area 3a and including Area 4 and parts of Area 6. Stimulation sites near the medial wall related to tail movements, and hindlimb, foot, trunk, forelimb, hand, face, and finally tongue movements were elicited by successively more lateral sites. In cortex rostral to the tail motor representation, there was a representation of the head and forelimb in what appears to be the supplementary motor region. Rostral to the motor representation of the shoulder, face and eyelids, stimulation of a restricted region of cortex resulted in eye movements (frontal eye fields). Within the low threshold motor field, some body movements appeared to be represented both rostrally and caudally. Zones of callosally projecting cells and zones of callosal terminations within the motor field were quite similar. Densities of connections varied considerably. In particular, two 2-3 mm wide patches of dense callosal connections, one rostral to the other, were noted. The caudal patch included motor representation of parts of the trunk, shoulder, and neck, and it also extended somewhat out of motor cortex into Area 3a. Stimulation of the rostral patch also resulted in movements of the trunk, hips, and shoulder. Cortex devoted to movements of the hand and foot was relatively free of callosal connections.

Supported by NIH Grant NS-16446.

93.6 **MOVEMENT DEPENDENT MODULATION OF FORELIMB PERTURBATION RESPONSES IN MONKEY PARIETAL CORTEX.** D.J.Crammond*, W.A.MacKay and J.T.Murphy. Department of Physiology, University of Toronto, Toronto. Ontario. Canada. M5S 1A8.

Late myotatic reflex responses have been shown to be modifiable under many conditions and are known to be modulated during a movement cycle(W.A.MacKay et al; Electroenceph. Clin. Neurophysiol.,1983. In press). The aim of the present research was to similarly examine the proprioceptive input to somatosensory cortex at different times during a movement cycle, to see if at the cortical level the responses are constant and if not investigate the relationship between the motor program and the responsiveness of individual neurones. Single unit recordings were undertaken in the parietal cortex of awake monkey (Macaca arctoides). The animal was highly trained to sequentially flex and extend his elbow in order to track a line on a video monitor with a cursor corresponding to the elbow position. Movement amplitude was 15°. At 5 phases during both movement directions a torque perturbation (70 msec ramp) was applied to either flex or extend the elbow. Brachialis and triceps EMG was rectified and integrated to record active and reflex activity. Standard transdural unit recording techniques were used. Both isolated single units (bandpass 450 to 3K Hz) and evoked field potentials (DC to 100Hz) were simultaneously recorded from a single glass insulated platinum/iridium microelectrode. The peripheral input to all cells was examined by passive manipulation of joints and touch to the skin to localise receptive fields and to classify modality as either cutaneous or 'deep'. Single unit responses to the perturbation were collected at each phase of both movements for comparison of raster histograms. Analysis of responsive cells indicated that there was clear modulation at the cortical level. Generally,for units with proprioceptive input from an elbow muscle, torque responses were maximal just prior to or at the onset of stretch in the muscle from which the proprioceptive information arose and could be totally absent when the same muscle was actively contracting. Although for some units a 'positional dependence' may be the reason for sensory gating, others showed a true phasic modulation. Measurement of the consistancy of the imposed perturbation makes a mechanical basis for modulation unlikely. Units with cutaneous receptive fields on the forearm were also stimulated by the perturbation. Although also modulated, responses were not consistantly related to any phase of movement.The field responses also showed clear modulation but unlike single unit data they were maximal prior to movement, diminished during and recovered after movement ended. Standardised comparisons were made by recording fields only in large cell layer V and on this basis modulated responses are localised to small foci. It is postulated that proprioceptive sensory information originating from muscles involved in a motor program is utilised particularly when required most (ie at movement onset and termination) and may be gated out when less relevant. Supported by the MRC of Canada.

93.7 DORSAL COLUMN PROJECTION TO THE THALAMIC RELAY TO THE MOTOR CORTEX IN CAT: AN ANATOMICAL AND PHYSIOLOGICAL DEMONSTRATION. R. S. Waters, Y. Tamai* and H. Asanuma. The Rockefeller University, New York, N.Y. 10021.

The motor cortex of the cat receives a direct projection from a border region in the thalamus between n. ventralis lateralis and n. ventralis posteriolateralis. Neurons in this thalamic region are responsive to cutaneous and deep structure stimulation and project to cells in the motor cortex having similar receptive fields. However, there is still an ambiguity about the input to this thalamic region. Therefore, we set out to determine whether this region receives an input from the dorsal column nuclei, using a combination of anatomical and physiological techniques.

Under inhalation anesthesia the motor cortex was exposed and a recording chamber was installed over the skull. Through the chamber lid 7 tungsten-in-glass stimulating electrodes were inserted to a depth of 1.2 mm around the cruciate sulcus. A second chamber was stereotaxically placed to provide access to the thalamic border region and a microelectrode, filled with 0.2M KCl and a 20% solution of HRP, was driven into the thalamus while stimulating pulses (0.2ms duration, 30μA) were delivered simultaneously through the 7 electrodes in motor cortex. When an activated neuron was encountered the responsible motor cortex electrode was identified, the latency and threshold were measured and the collision test employed. If the antidromically activated neuron responded to somatosensory stimulation, then HRP was iontophoretically injected (5μA x 10min) through the same electrode. After a 48 hr survival time, the subject was sacrificed, and the tissue processed using the TMB method. In the second part of the study the procedures were identical to the above with the exception that the recording electrode was replaced by a tungsten microelectrode. When an antidromically activated thalamic neuron was identified the receptive field was measured. The recording electrode was then used to deliver single stimulating pulses, while a third microelectrode was inserted in the contralateral cuneate nucleus to record antidromically activated neurons. The receptive field of the activated neuron was examined and compared to the receptive field(s) of neurons around the thalamic stimulating electrode.

Using these procedures the following results were obtained: (1) Short latency antidromically activated neurons having clear receptive fields were located in the thalamic border region, (2) HRP delivered into the physiologically identified thalamic site labelled neurons in the ventro-caudal parts of the contralateral caudal cuneate nucleus, and (3) This result was corroborated in the second part of the study, i.e., antidromically activated neurons were found in a narrow region of the ventro-caudal cuneate nucleus. Supported by NIH # NS-10705

93.8 CORTICAL EFFERENTS IN THE MACAQUE: THE DENSITY OF PONTINE PROJECTIONS FROM SUBDIVISIONS OF THE CEREBRAL CORTEX. M. Glickstein and B. Mercier* M.R.C. Unit on Neural Mechanisms of Behaviour, 3 Malet Place, London WC1E7JG, U.K.

The pontine nuclei are one of the main targets of efferent fibers from the cerebral cortex. Orthograde tracing methods clarify the spatial organization of this pathway but they are less appropriate for determining the number of corticopontine fibers contributed by different cortical areas. We injected horseradish peroxidase into the pontine nuclei of eleven monkeys. In cases in which the pontine nuclei were filled on one or both sides, we counted the number of labeled cortical neurons. The linear extent of each cortical area was measured planimetrically, and the number of filled cells was divided by the length to yield the number of labelled cells per millimeter of cortex. We describe here the results of these counts for the dorsal aspects of cortex including all or part of Brodmann's Areas 17,18,19,7,5,2,1,3,4,6,8 and 9. Note that these are linear measurements; the density per unit area would be approximately equal to the square of these number. Area 4 had the highest density of filled cells (Mdn = 18.8/mm). Surprisingly, areas 6 (Mdn = 14.1/mm) and 8 (Mdn = 16.7/mm) contribute nearly as many corticopontine fibers per unit length of cortex. Areas 5 (Mdn = 14.3/mm) and 7 (Mdn = 11.1/mm are also well represented. The primary somatosensory areas contribute rather fewer fibers than previous studies had suggested (areas 3, 1 and 2 combined Mdn = 8.1/mm). In addition to area 7 the principal visual contribution is from Area 19 (Mdn = 4.9/mm). There was an occasional filled cell in area 18 (Mdn =.4/mm) and no filled cells in dorsolateral area 17. The dorsal aspect of Area 9 had a small (Mdn = 2.2/mm) number of filled cells adjacent to area 8.These data emhasize the contribution to the cortico-ponto-cerebellar system from cortical regions outside the primary motor and sensory areas.

93.9 SENSORY PROPERTIES OF MOTOR CORTEX NEURONS IN THE AWAKE RAT. C.F. Sievert* and E.J. Neafsey. Dept. of Anatomy, Loyola Univ. Med. Cntr., Maywood, IL 60153.

Although the sensory properties of neurons in rat somatosensory cortex have been described by several laboratories, there is little information available on how neurons in rat motor cortex respond to peripheral sensory stimulation. One reason for this lack of information is that in the anesthetized rat it is very difficult to find any motor cortical neurons that respond to sensory stimulation. The present experiments have, therefore, investigated this question in awake, head-restrained rats. To prepare the animal for recording, he was first habituated to handling and mild restraint, and then, under anesthesia, a head-restraint-recording chamber device was implanted on the skull using screws and dental acrylic. When the animal had recovered from surgery he required several days to become accustomed to having his head rigidly fixed for several hours; recording sessions were then begun. (The animals showed no signs of pain during this procedure). During each recording session a single transdural penetration was made using a glass-insulated tungsten microelectrode. The depth of each cell from the surface (defined by encountering neuronal activity) was noted as was the depth where the white matter appeared to begin. On most tracks intracortical microstimulation was also done and the movements evoked were noted. Small marking lesions (10 μamps DC for 10 sec) were made at the end of a number of penetrations. After 10-15 recording sessions the animal was sacrificed by perfusion and the brain sectioned. The electrode tracks were reconstructed.

Thus far 2 rats have been studied. From 26 penetrations histologically located in motor cortex, 138 neurons were recorded; 27 (20%) of these had peripheral receptive fields. Of these 27 cells, 15 (55%) responded to "deep" inputs such as passive joint movement or pressure around a joint while 12 (45%) responded to cutaneous inputs such as hair deflection or light touch. Special attention was devoted to the sensory responsiveness of neurons located in the caudal and rostral forelimb areas we have previously described (BR 232,1982). In the caudal forelimb area 51 neurons were recorded and 18 (35%) had peripheral sensory input, primarily (72%) deep. In contrast, in the rostral forelimb area 24 neurons have been recorded and none responded to peripheral sensory input. This lack of sensory inputs to the rostral forelimb area is consistent with the notion that this region is part of the rat's supplementary motor cortex.

(Supported by NIH grant NS16146 and BRSG grant RR05368 from Loyola University).

93.10 A MICROELECTRODE STUDY OF THE TOPOGRAPHICAL PROJECTION OF THE SENSORI-MOTOR CORTEX TO THE ROSTRAL TRIGEMINAL SENSORY NUCLEI OF THE RABBIT. K.Å. Olsson*, K. Sasamoto* and J.P. Lund. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal (Québec), Canada.

As a part of studies on the modulation of sensory transmission during jaw movements, we have examined in detail the effects of electrical stimulation of a wide area of the anterior and lateral cerebral cortex on the trigeminal sensory nuclei. The experiments were carried out on rabbits anesthetized with urethane. The uncut inferior alveolar, infraorbital and lingual nerves trunks were directly stimulated. Nerves in the upper lips, fore- and hind-paws were stimulated by electrodes inserted through the skin. The electromyographic activity of jaw opening and closing muscles, and jaw movements were recorded.

In the first part of the study, evoked potentials and/or single unit activity were recorded on the surface or within the cerebral cortex. After sensory maps had been drawn, bipolar stimulating electrodes were inserted and fixed in the centers of the receiving areas of the various nerves and also into sites known to give jaw movements at lowest threshold. The focal potentials and unit activity were recorded in the main sensory trigeminal nucleus, the bordering dorsal, medial and ventral areas and the rostral parts of the nucleus of the spinal trigeminal tract, while stimulating peripheral nerves and cortical sites.

The distribution of peripheral receptive fields in the nucleus agrees with previous observations in the cat: the mandibular dermatome is represented dorsally, with the maxilla deeper and more laterally situated. The ophthalmic division is ventrolateral. In general, stimulation of the corresponding cortical Sensori-motor area evoked potentials in the same regions of the main sensory nucleus and rostral parts of the nucleus of the spinal trigeminal tract as the peripheral input. However, the maximum amplitude of the cortically-evoked potentials occurred ventral to that of the corresponding peripheral potential. The maximum effects of stimulation of the region of the Motor-sensory cortex that causes short latency effects on jaw muscles were found in more medial parts of the sensory nuclei and in the medio-ventral bordering areas that are known to contain interneurons projecting to the trigeminal motor nucleus.

The majority of neurons that were excited by sensory inputs were also excited by stimulation of more than one cortical electrode. However, the point with lowest threshold was usually in a topographically-related region of the sensory or motor cortex.

(Supported by the Canadian Medical Research Council).

93.11 CONTRALATERAL, MID-LINE AND WHITE MATTER MOTONEURONS OF DORSAL NECK MUSCLES. V.C. Abrahams and J. Keane*, Department of Physiology, Queen's University, Kingston, Ontario, Canada. K7L 3N6

Older anatomical descriptions have suggested that neck motoneurons are not confined to lamina IX, but are also found in more dorsomedial regions of the ventral horn. Previous HRP experiments using DAB as a chromogen confirmed this observation and also showed that some motoneurons may be located in commissural structures. Using the more sensitive TMB method for the demonstration of retrogradely transported HRP, it has now been found that neck motoneurons are not only present in the ipsilateral lamina IX, the ipsilateral dorsomedial and commissural nuclei, but they are also present at some contralateral ventral horn sites. Up to 19% of retrogradely filled cells in cats whose C2 and C3 biventer cervicis and complexus muscle nerves were dipped in HRP were found in laminae VII and VIII of the contralateral ventral horn and up to 12% of retrogradely filled cells were present in the commissural nuclei close to the mid-line.

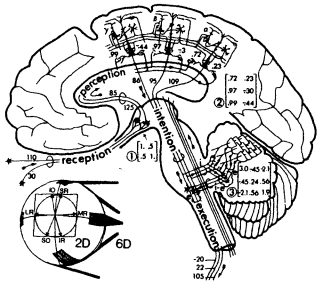
Measurements of equivalent cell soma diameter have shown that neck motoneuron somas are generally somewhat smaller than those supplying hind legs. All the contralateral and mid-line motoneurons were small, with equivalent soma diameters less than 40 μm. Thus the possibility is raised that mid-line and contralateral motoneurons are fusimotor.

Supported by the Medical Research Council of Canada.

93.12 GENERATION & MODIFICATION OF NEURONAL NETWORKS ACTING AS METRIC TENSORS: A COMPUTER DEMONSTRATION OF THE PROCESS OF ORGANIZING SENSORIMOTOR TRANSFORMATIONS. A. Pellionisz, G. Ostriker and R. Llinas. Department of Physiology and Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Tensor network theory assumes that, globally, the function of the CNS is that of matching relationships of the external invariants with internal multidimensional geometries, comprised in neuronal networks acting as metric tensors. Sensorimotor coordination, described by other theories as transformations of vectors via matrices expressed in external Cartesian frames, can be generalized via tensor network theory. Since tensorial models can use generalized natural coordinates intrinsic to sensorimotor systems rather than being restricted to Cartesian description, the problem of coordination may be solved in a reference-frame independent manner as a covariant embedding followed by covariant-contravariant metric-type transformation.

The question of how such CNS networks may be generated and modified then remains the core of the problem. Here results, offered by tensor network theory (MS, submitted for publication) are demonstrated, where metric networks are organized and tailored by the process called in the MS metaorganization. Results of the procedure, based on the well-known biological phenomena of oscillations set up by re-entry to the system, are demonstrated here both in a general sensorimotor scheme (Fig), modeling the emergence of covariant embedding and cerebellar metric tensor-type matrices (1-3) and in a tensorial computer model of gaze-control, involving vestibulo-ocular reflex and neck muscle coordination. Here, the procedure of generating a network acting as an oculomotor metric tensor, necessary for transforming a vestibular vector to a higher dimensional gaze vector, accounts for Listings' law, which confines movements of the 6D eye muscle system into 2D. The general concept of metric network metaorganization leads to experimentally testable paradigms of the genesis and functional maturation of such networks, eg. in the emergence of the cerebellar metric via the climbing fiber system. --- Supported by USPHS grant NS13742.



94.1 ACTIVATION OF A PEPTIDE-ACTIVATED NEURON BY CYCLIC AMP RELEVANT AGENTS. Jeffrey L. Ram and Kurt A. Haller.* Dept. of Physiology, Wayne State University, Detroit, MI 48201.

In Aplysia, the neuropeptide egg laying hormone (ELH) activates buccal ganglion motoneuron B16, which innervates buccal muscle I5 (Ram, Soc. Neurosci. Abs. 8:285; Br. Res., in press, 1983). Since peptide actions are often mediated by cyclic AMP, experiments were initiated to test the involvement of cyclic AMP in this electrophysiological response. We tested whether the peptide response could be mimicked by 8-bromo cyclic AMP, an analog of cyclic AMP; isobutylmethylxanthine and theophylline, inhibitors of cyclic AMP degradation; and forskolin, an activator of adenylate cyclase which reversibly activates the enzyme in vivo independently of specific agonist receptors. In most experiments, these agents were applied in artificial sea water containing 150 mM Mg++ and 1 mM Ca++, which blocks chemical synapses in the ganglion but does not block activation of B16 by ELH. Under these conditions all four agents produced a response similar to ELH: a slow depolarization, leading to tonic activation of action potentials. Depending on the agent and concentration used, latency until the first action potential was generally 2-10 min.

Threshold concentrations to activate action potentials were 10^{-4} to 6 x 10^{-4} M 8-bromo cyclic AMP (4 preparations), 2 x 10^{-4} to 10^{-3} M isobutylmethylxanthine (4 preparations), 10^{-3} and 3 x 10^{-3} M theophylline (2 preparations), and 3 x 10^{-5} to 10^{-4} M forskolin (4 preparations). At lower concentrations, these agents produced subthreshold depolarization from a resting potential averaging 10 mV below threshold in this medium.

The ability of forskolin to raise cyclic AMP content of buccal ganglia was tested. Cyclic AMP in buccal ganglia, extracted by 6% TCA and washed with ethyl ether, was measured by radioreceptor assay. Cyclic AMP content in the presence of various concentrations of forskolin (FSK) was 24 + 10 pmole cAMP/mg protein, 0 FSK; 318 + 152 pmole/mg, 5 x 10^{-5} M FSK; and 473 + 285 pmole/mg, 10^{-4} M FSK; n=3 ganglia each.

These data are consistent with the possible mediation of the response to ELH by cyclic AMP. Further experiments to test this hypothesis would include measurement of cyclic AMP changes in B16 in response to ELH and testing the effect of injection of protein kinase inhibitor into the cell. The present data do not exclude the possibility that cyclic AMP may be mediating the response to other physiological depolarizing substances that affect the cell.

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94.2 ALL THE BAG CELL NEURONS OF APLYSIA PRODUCE BOTH ELH AND AP. G. Whitney* and S. Arch (SPON: G. F. Gwilliam). Biological Laboratories, Reed College, Portland, OR 97202.

The bag cell neurons of Aplysia californica are organized in two clusters of about 400 cells each. The cell population appears to be homogeneous upon morphologic, cytochemical, and physiologic investigation. It is known that the principal secretory products of the cells are synthesized as parts of a common precursor molecule. Thus, it could be anticipated that each cell produces the same proportions of these products. Alternatively, individual cells could process the common precursor differently as has been seen to be the case for POMC in the rat pituitary. Moreover, molecular studies have disclosed the possibility that there may be several genes coding for closely similar precursor sequences. We therefore attempted to determine if differential precursor processing and/or multiple precursor expression is occurring in individual bag cells by assaying single cells for their relative contents of newly synthesized egg-laying hormone (ELH) and acidic peptide (AP). From kinetic studies on cell clusters it is known that these peptides appear with a fixed stoichiometry. Since the sequence for each appears in tandem on the best characterized cloned gene, the relative amounts of these species provides an index for differential expression in the cells. Bag cell clusters were, therefore, labeled in vitro by exposure to ^{3}H-leucine and then rinsed in a low-Ca^{++} saline. After the rinse they were transferred to a low-Ca^{++} solution containing collagenase. The collagenase treatment was terminated with an ice-cold low-Ca^{++} saline and the clusters were dissected with glass needles into individual cells. These were taken up in a minimum of medium and frozen on dry ice. Isoelectric focusing electrophoresis was performed on the freeze-thaw disrupted cells and scintillation counting of sequential gel slices performed to determine radiolabel content. In 64 cells assayed from 10 clusters both ELH and AP were present at their native pI's. Thus bag cells are not specialized for production of either of these peptides to the exclusion of the other. There is, on the other hand, evidence of variability in relative quantities of the two species present in the gels. While this would appear to suggest variability in the expression or processing of precursor species, variation in the recovery of such small amounts of protein cannot be excluded at present.

94.3 Analysis of inhibition produced by the candidate neurotransmitter, α-bag cell peptide, in identified neurons of Aplysia. Karen Sigvardt†, Barry S. Rothman†, and Earl Mayeri††. †Department of Physiology, Univ. of Calif., San Francisco, CA 94143, and ‡Department of Basic Sciences, California College of Podiatric Medicine, San Francisco, CA 94115.

A burst discharge of the neuroendocrine bag cells produces several types of excitatory and inhibitory responses in specific identified neurons of the abdominal ganglion. Two neuropeptides, egg-laying hormone and α-bag cell peptide (α-BCP), are candidate neurotransmitters for mediating different subsets of these responses (Branton et al., PNAS 75: 5732; Rothman et al., PNAS, in press). The two peptides as well as others are represented on a single gene, which encodes the ELH/α-BCP precursor molecule (Scheller et al., Cell 32:7). In the present study we focused on the role of α-BCP as a possible transmitter mediating bag-cell-induced inhibition of left upper quadrant (LUQ) cells, L_3 and L_6.

α-BCP(1-7) acts directly on LUQs. The presence of protease inhibitors in the perfusion medium increased the potency of the peptide by 3-10 fold, suggesting that the peptide is rapidly degraded in vascular and interstitial spaces of the ganglion. LUQ cells were unaffected by arterial perfusion of 4 other other peptides present on the ELH/α-BCP precursor, acidic peptide, ind β-, γ-, & δ-BCP. The inhibition caused by α-BCP was unaffected by coapplication of α-BCP and the other peptides at equimolar concentration. When α-BCP was perfused at high concentration (1 mM) for prolonged periods (30 min), the response desensitized and a subsequent electrically triggered discharge of the bag cells produced little or no inhibition, suggesting that cross desensitization had occurred. Membrane conductance was measured by periodic injection of a 3-step current pulse. Arterial perfusion of α-BCP produced a conductance increase that appeared to be voltage-sensitive. A similar conductance change occurred during inhibition produced by a bag cell discharge, suggesting that the two ionic mechanisms of inhibition are identical. The combined data provide further evidence that α-BCP is an inhibitory neurotransmitter.

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94.4 APLYSIA EGG-LAYING HORMONE: PRECURSOR PROCESSING ACTIVITY IN BAG CELL EXTRACTS. M.E. Yates and R.W. Berry. Dept. Cell Biol. and Anat. Northwestern Univ. Sch. of Med. Chicago, IL 60611.

The bag cells of Aplysia produce a peptide egg-laying hormone, ELH, along with other neurosecretory products, by proteolytic cleavage of ca. 29,000 dalton precursor. Since the primary structure of this precursor is known, and since the processing sequence leading to the secretory products has been established, it is of interest to identify and characterize the enzyme or enzymes responsible for processing. We have begun to do so by studying processing in bag cell homogenates. Bag cells homogenized in low ionic strength buffer retain the capacity to cleave previously-synthesized precursor, and to produce products which are indistinguishable from those produced by intact cells, as judged by sequential SDS-PAGE and isofocusing. There is no evidence of non-specific degradation. The processing activity is recovered in the supernatant of homogenates which have been frozen and thawed and centrifuged at 1000xg for 30 min. The pH optimum for processing is 5.5-6.5, similar to that of the proopiomelanocortin and proinsulin converting enzymes (P.N.A.S. 79:108-112, 1982: P.N.A.S. 79: 4613-4617, 1982). Processing activity is not inhibited by monensin or chloroquine, nor by inhibitors of trypsin or cathepsin B, and its inhibitor spectrum is distinct from that of either of the converting enzymes cited above. This suggests that, even though each precursor is cleaved at sites consisting of pairs of basic amino acids, the cleavage enzymes are not identical.

Supported by NIH NS-16756.

94.5 FURTHER CHARACTERIZATION OF BIOLOGICALLY ACTIVE ATRIAL GLAND PEPTIDES IN APLYSIA. G. T. Nagle, S. D. Painter and J. E. Blankenship. Marine Biomedical Institute, Univ. Tx. Med. Br., Galveston, TX 77550.

When the neuroendocrine bag cells of Aplysia are triggered to fire, they release an egg laying hormone (ELH) into the blood; ELH directly causes egg release from the ovotestis and results in egg laying. The atrial gland, an exocrine organ in the reproductive tract, contains two peptides, A and B, structurally similar to each other but unrelated to ELH. These peptides cause a bag cell discharge which leads to egg laying; they do not cause egg laying in bag cell-less animals. The atrial gland also contains another peptide, egg releasing hormone (ERH), that has strong sequence homologies to the N-terminal half of ELH and to the C-terminal half of peptides A and B. In keeping with these sequence homologies, ERH is able to directly induce egg laying in intact or bag cell-less animals (like ELH), as well as trigger bag cell discharges in vitro (like peptides A and B). By using two bioassays, we can distinguish between ELH-like peptides (egg laying in bag cell-less animals) and A/B-like peptides (induction of bag cell discharge in vitro). We have used these bioassays in conjunction with gel filtration, isoelectric focusing (IEF) and HPLC purification procedures to further investigate and characterize peptides from the atrial gland.

Atrial glands were homogenized in an acid medium and fractionated by Sephadex G-50 chromatography. Three fractions (C,D and E) in the 3-15 k daltons MW range induced egg laying in intact and bag cell-less animals. Two of these, D and E, were subjected to IEF (pH 3.5 to 10) on gels containing 6M urea and 1% Triton. Peptides were eluted from gel slices and bioassayed in bag cell-less animals and in an in vitro neuroassay.

Several basic peptides with ELH-like activity were found in both D and E, but these were not further characterized. Peptides with A/B-like activity were found at pI 7.9-8.1 and 9.0-9.2 in peak E; presumably these correspond to peptides A and B, respectively. Two hybrid peptides were found at pI 5.9-6.1 and 7.8-8.1 in fraction D. The pI of the more basic species presumably corresponds to that of ERH. Traces of the more acidic species were detected in fraction E, but only on the more sensitive neuroassay. This molecule is a hybrid, like ERH, but is presumably not ERH. These biologically active peptides are being further purified by HPLC. Supported by NIH NS 07025 (G.T.N.), NS 07010 (S.D.P.), NS 11255 and NSF PCM 82-15185 (J.E.B.).

94.6 A NEWLY SEQUENCED NEUROPEPTIDE (SCP_B) IS A PHYSIOLOGICAL MODULATOR OF SOMATIC MUSCLE IN APLYSIA. Philip E. Lloyd*, Irving Kupfermann, Klaudiusz R. Weiss. Center for Neurobiology and Behavior, New York State Psychiatric Institute, and Departments of Physiology, Anatomy and Cell Biology, and Psychiatry, Columbia P&S, and School of Dental and Oral Surgery, New York, N.Y. 10032.

Small cardioactive peptide B (SCP_B) has recently been purified from Aplysia neural tissue and sequenced (Morris et al., Nature 1982). SCP_B levels in tissues were determined by means of reverse phase HPLC and bioassay on an isolated snail heart (Lloyd, J. Comp. Physiol., 1978). SCP_B was found in a variety of tissues, including the accessory radula closer (ARC), a somatic muscle involved in feeding. SCP_B is also present in the nerve and ganglion which innervates the ARC, and preliminary immunocytochemical analysis suggests that the peptide is localized to axonal fibers and varicosities in the muscle.

Low doses of SCP_B (threshold: 10^{-9}M) applied to the ARC do not directly cause contractions but do enhance the amplitude of contractions produced by stimulating identified cholinergic motorneurons. SCP_B appears to function in a manner similar to that previously shown for serotonin (Weiss et al., J. Neurophysiol. 1978). It enhances contractions produced by pulsed applications of ACh suggesting that its action is post-synaptic. Furthermore it was as effective as serotonin in increasing cAMP levels in the muscle. High Mg^{++}, low Ca^{++} solutions did not inhibit this action of SCP_B indicating that it does not increase cAMP levels indirectly by releasing serotonin from nerve terminals in the muscle. SCP_B (10^{-5}M) causes a significant additional elevation of cAMP in the ARC when the serotonin dependent increase in cAMP is saturated by 10^{-3}M serotonin. This suggests that SCP_B acts via a different receptor than serotonin. These results suggest that SCP_B is a physiological modulator of the ARC. Because serotonin acts in this muscle to mediate an aspect of food induced behavioral arousal, it is possble that SCP_B is also involved in this arousal. (1 RO1 MH36730, 5 RO1 MH 35564).

94.7 THE PRESENCE, DISTRIBUTION, AND EFFECTS OF FMRFamide-LIKE PEPTIDES IN THE HORSESHOE CRAB, LIMULUS POLYPHEMUS. W.H. Watson, III, J. Groome*, B. Cromwell*, J. Bishop*, and T. O'Donohue. Dept. of Zoology, UNH, Durham, NH 03824 and Exp. Ther. Branch, NINCDS, Bethesda, MD 20205.

The tetrapeptide FMRFamide was first discovered in the sunray venus clam, Macrocallista nimbosa (Greenberg, M., Price, D., Science, 197:670, 1977). Subsequent immunocytochemical and radioimmunological studies have demonstrated that FMRFamide-like peptides are present in a wide variety of animals including humans (reviewed in Greenberg, M., Price, D., Ann. Rev. Physiol., 45:271, 1983). We now present evidence that a FMRFamide-like peptide is also present in the nervous system of the horseshoe crab and that it has direct actions on the neurogenic Limulus heart.

Regions of the Limulus nervous system were assayed for FMRFamide using radioimmunoassay (RIA). The antibody used in these studies did not cross-react with any known FMRFamide-like peptides (CCK 1-4, Met-enkephalin, BPP) or proctolin. The highest concentration of FMRFamide immunoreactivity was found in the brain, but substantial quantities were also detected in abdominal and cardiac ganglia. These observations were confirmed with immunocytochemical studies. FMRFamide-like immunoreactivity was observed throughout the CNS and cardiac ganglion. It was located primarily in the neuropile, and cell bodies were stained in abdominal and circumesophageal ganglia. A few immunoreactive axons were observed on the surface of cardiac muscle. No fluorescence was observed in sections after preabsorbing the antisera with synthetic FMRFamide.

In order to determine if the peptide recognized by our antibody was authentic clam FMRFamide, we fractionated Limulus brain homogenates using reversed phase high pressure liquid chromatography on a μBondapak C18 column. We then measured FMRFamide levels in each fraction using RIA and a FMRFamide bioassay (the Busycon radula protractor muscle). Some of the immunoreactive and bioactive material coeluted with authentic FMRFamide. However, the major immunoreactive peaks of activity did not coelute with FMRFamide. One of the major immunoreactive peaks corresponded with fractions containing most of the bioactivity. Thus, it appears as if there are several FMRFamide-like peptides in Limulus.

In order to examine the physiological role of FMRFamide in Limulus we examined the effects of synthetic FMRFamide on the Limulus heart. At a concentration of 5×10^{-5}M it caused a 100% increase in both the rate and strength of heart contractions. These effects were due to a direct action of FMRFamide on both the cardiac ganglion neurons and the heart muscle.

94.8 NEUROPEPTIDE PROCTOLIN IN THREE IDENTIFIED POSTURAL MOTONEURONS OF THE CRAYFISH. C.A. Bishop, J.J. Wine and M. O'Shea. Dept. of Psychol., Stanford Univ., CA 94305 and Dept of Pharm. Physiol. Sci., Univ. of Chicago, Chicago, Il 60637.

Using reverse phase liquid chromatography and a sensitive bioassay, we have purified, from extracts of the crayfish central nervous system (CNS), a component indistinguishable from proctolin (H-Arg-Try-Leu-Pro-Thr-OH). The distribution of proctolin in the nervous system of the crayfish was investigated using a specific polyclonal rabbit antiserum. Proctolin-like immunoreactivity (PLI) in the crayfish CNS was widely distributed; immunoreactive cell bodies were stained in every ganglion.

Among the cell bodies that stain in the crayfish, we have found that three are identified excitatory motoneurons of the tonic flexor muscles which help control abdominal posture. PLI containing cell bodies appropriate in size and position to motoneurons f1, f3 and f^4 were seen in the abdominal ganglia. Prominent PLI was visualized in up to three axons in the tonic flexor motor root, which contains just six axons distinguishable on the basis of relative diameters. Stained axon diameters were consistent with these being f1, f3 and f^4. We could not trace stained axons to the cell bodies, so we verified that PLI was located in the cell body of f3 by double labelling with Lucifer yellow and the proctolin antiserum, using the PAP procedure. PLI was seen in the axons as they branch onto the muscle and terminals were stained over the entire muscle. Cutting the nerve two months prior to immunohistochemical processing eliminated all PLI from the muscle.

A sensitive and specific bioassay (O'Shea, M. and Bishop, C., J. Neurosci., 2:1242-1251, 1982) detected bioactivity equivalent to about 370 fmole proctolin per tonic flexor muscle. All bioactivity was eliminated in muscles denervated for two months and in muscle homogenates pre-incubated with proctolin antiserum.

These results indicate that proctolin is present in three excitatory motoneurons that are thought to use either L-glutamate or acetylcholine as their transmitter. A precedent for our findings is the proctolin-containing postural motoneuron of the cockroach (Adams, M. and O'Shea, M., Sci., in press, 1983). We are currently investigating the role of proctolin in modulating tension generation in the tonic flexor muscles.

Supported by NIH grant NS-06684 (C.A.B.), NSF grants BNS 82-02515 (M.O.) and BNS 81-12431 (J.J.W.).

94.9 PROCTOLIN IN LOBSTERS: GENERAL DISTRIBUTION AND CO-LOCALIZATION WITH SEROTONIN. K. King Siwicki* and E. A. Kravitz. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Radioimmunoassay with an antiserum raised against the pentapeptide proctolin (Arg-Tyr-Leu-Pro-Thr) has demonstrated proctolin-like material to be widespread in the lobster nervous system. The highest concentrations were found in the pericardial organs and proximal portions of the second roots of thoracic ganglia: both of these are neurosecretory regions that also contain high concentrations of monoamines.

To bring the analysis of the role of proctolin in lobsters to a cellular level we have used immunohistochemical techniques to map the distribution of proctolin-like immunoreactivity. The specificity of our antibody preparation was demonstrated by (1) a complete block of the staining by pre-absorbing the antiserum with a proctolin-BSA conjugate, or with free proctolin, (2) the failure to eliminate the staining by pre-absorbing with FMRF-amide, Substance P, leu-enkephalin, serotonin, or octopamine, and (3) the failure to detect, by radioimmunoassay, cross-reactivity of the antiserum with these substances or 7 other peptides.

The pericardial organs are covered with a dense network of fine varicose processes that stain with the antiserum. We have not seen immunoreactive cell bodies in pericardial organs, but 1-2 stained axons are found in each of the thoracic roots that project to pericardial organs. Every central ganglion contains stained fibers and varicosities. After colchicine treatment, many small immunoreactive cell bodies are seen in the nerve cord: the greatest numbers are in the subesophogeal and fifth thoracic ganglia. In addition, the fifth thoracic and first abdominal ganglia each contain a pair of large immunoreactive cell bodies. These are identical to the paired cells in these ganglia that show serotonin-like immunoreactivity (Beltz & Kravitz, J. Neurosci. 3:585, 1983). In 25 µm alternate serial sections of the ganglia, the same cells showed staining with an anti-serotonin antiserum and an anti-proctolin antiserum. These two pairs of cells project anteriorly and are thought to branch into the thoracic second roots, where the neurosecretory regions described above are found. Thus it is possible that some neurosecretory endings release both proctolin and serotonin. In addition, in each of the paired circumesophogeal ganglia, a single, large anterior cell shows proctolin-like immunoreactivity. These cells may correspond to the dopamine cells in these ganglia described by Barker, et.al. (Br. Res. 161:99, 1979). At present we are exploring the chemical and physiological properties of these large cells to define their roles in the lobster nervous system. (Supported by NIH)

94.10 CO-LOCALIZATION OF BURSICON BIOACTIVITY AND PROCTOLIN IN IDENTIFIED NEURONS. Michael E. Adams and Mary N. Phelps. Research Laboratories, Zoecon Corp., Palo Alto, CA 94304.

The proctolin-containing lateral white (LW) neurons occur as individual pairs in cockroach (Periplaneta americana) abdominal ganglia (O'Shea and Adams, Science 213:567-569, 1981). Axon projections from these neurons extend to the heart and to the perivisceral organs, which are specialized neurohaemal release sites. We now report that both proctolin and bursicon, a neuropeptide involved in cuticular tanning, are localized in cell bodies of LW neurons.

LW somata were isolated from adult cockroach ganglia by dissection in ice-cold physiological saline, disrupted by sonication and freeze-thaw treatments, and centrifuged at 12,000 x g. An equal number of octopaminergic dorsal unpaired median (DUM) somata were collected from metathoracic ganglia as control cells. Supernatants were assayed for bursicon bioactivity on isolated wings from pharate adult Manduca sexta (Reynolds, J. Exp. Biol 70:17-39, 1977). Bursicon levels were quantified on a relative basis by serial dilution of samples until tanning activity disappeared.

Bursicon bioactivity was detected in LW somata extracts at threshold doses of 0.6 cell equivalents. A single LW cell body pair accounted for about one-fifth the bioactivity measured in an entire abdominal ganglion. Control cells showed no bursicon bioactivity at doses up to 2.5 cell equivalents. The coexistence of proctolin and bursicon in LW neurons may indicate a common precursor relationship as well as co-release from nerve endings at neurohaemal release sites. These hypotheses are currently under investigation.

The distribution patterns of bursicon and proctolin were compared in the central nervous system and gut. Substantial bursicon bioactivity was detected in all ganglia of the CNS. Thoracic ganglia and terminal abdominal ganglia consistantly showed the highest levels, which were approximately twice the amounts found in brain, subesophageal and abdominal ganglia. In contrast, proctolin levels in the terminal abdominal ganglion as measured by RIA (Bishop, et al., PNAS, 78:5899-5902, 1981) were 4 to 20 times those found in all other ganglia. Therefore, the relative amounts of the two peptides depend on their location in the CNS. Both proctolin and bursicon occur in the fore- and hindgut, where proctolin has been suggested to function as a neurotransmitter. Although the function of bursicon in the gut is not known, local release from nerve endings may play a role in the dynamics of the inner cuticular lining which is not directly exposed to hemolymph.

94.11 STRUCTURE AND BIOLOGY OF TWO NEW RELATED NEUROPEPTIDES FROM INSECT: FURTHER EVIDENCE FOR A PEPTIDE FAMILY. J. Witten, M. Schaffer* and M. O'Shea. Dept. Pharmac. and Physiol. Sci., The University of Chicago, IL 60637.

We have isolated two neuropeptides from the corpora cardiaca or cc (a major neurosecretory structure in insects) and CNS of the American cockroach Periplaneta americana. Three biological actions are identified: contraction of skeletal muscle, acceleration of an accessory heart and elevation of blood lipid levels. We call these peptides MI and MII.

The cc contains about 50pmol MI and 20pmol MII. They have been purified to homogeneity by reverse-phase HPLC and subjected to amino acid and FAB-Mass Spec. analysis. Compositions are - MI: GLX-1, ASX-2, SER-1, VAL-1, PHE-1, PRO-1; MII: GLX-1, ASX-1, THR-2, LEU-1, PHE-1, PRO-1. Fluorescence at 280nm indicated the presence of TRP or TYR but TYR was excluded by amino acid analysis. We confirmed the presence of TRP by the incorporation of ^{3}H-TRP by in vitro synthesis. Molecular weights are 972 (MI) and 987 (MII), consistent with one TRP, GLX = pGLU, ASX = ASN, and carboxy terminal amides for both peptides. Incorporation of ^{3}H-TRP established the cc as the site of synthesis as well as storage for both. MI and MII are released into the blood from the cc by a calcium-dependent mechanism. During a ten minute exposure to 50mM-K^+ saline we have purified about 10% of the total store of MI and MII from the superfusate. Reverse-phase HPLC confirms the presence of both peptides in the CNS and gut. Hormonal and transmitter functions are therefore indicated in the cockroach. A survey in other species suggests MI and MII are present in the moth Manduca sexta, both may be present in crayfish and neither were found in the rat brain.

The grasshopper cc does not contain MI or MII in abundance, but does contain a related peptide - locust adipokinetic hormone or AKH (Stone, et al. Nature, 1976). MI and MII produce AKH-like activity in the locust as does another arthropod peptide called red pigment concentrating hormone or RPCH (Carlson et al. Insect Biochem., 1979). The sequences of AKH and RPCH are homologous and both are pGLU and -NH_2 terminally blocked. This data plus our structure and activity studies suggest MI, MII, AKH, and RPCH are members of a family of homologous peptides. We therefore suggest the following tentative sequences; MI: pGLU-VAL-ASN-PHE-SER-PRO-ASN-TRP-NH_2 and MII: pGLU-LEU-THR-PHE-THR-PRO-ASN-TRP-NH_2.

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94.12 REGULATION OF PEPTIDE RELEASE BY NEUROTRANSMITTERS IN THE ISOLATED EYESTALK OF THE FIDDLER CRAB, UCA PUGILATOR. L. S. Quackenbush* AND M. Fingerman. Dept. of Biology, Tulane Univ., New Orleans, LA 70118.

The neuroendocrine complex within the eyestalk of crustaceans releases at least two pairs of antagonistic peptidergic neurohormones which regulate pigment migration in integumentary chromatophores in the fiddler crab, Uca pugilator. The pigment within the chromatophores disperses or concentrates in response to a specific peptide. Melanophores, containing black pigment are regulated by black pigment dispersing hormone (BPDH), and black pigment concentrating hormone (BPCH). Erythrophores, containing red pigment, are regulated by red pigment dispersing hormone (RPDH) and red pigment concentrating hormone (RPCH). The isolated eyestalk remains viable when perfused with saline, and releases these four peptides (BPDH, BPCH, RPDH and RPCH) in a voltage dependent manner upon stimulation. They can be separated from each other subsequently on a Sephadex G-25 column. Previous in vivo experiments demonstrated that norepinephrine (NE) induces BPDH release, and dopamine (DA) induces RPCH release, and 5-hydroxytryptamine (5-HT) induces RPDH release. In vitro preparations supported the previous findings with NE and 5-HT, and also revealed that DA releases BPCH as well as RPCH. The crustacean eyestalk contains 0.6 µg/g octopamine with no previously demonstrated role in the crustacean color change system. We now report octopamine (10^{-8}M) inhibits the release of BPDH, but not RPCH or BPCH, in vitro, in a dose dependent manner. Octopamine has no direct activity in the bioassay used. A preliminary report (Kirk and Glantz, 1982, Neurosci. Abstracts, 8:532) demonstrated GABA produces IPSP's in neuroendocrine cells in the eyestalk of a crayfish. We found that GABA, inhibits release of BPCH and RPCH release in vitro (10^{-7}M) and in vivo (10^{-5}M), in a dose dependent manner. This inhibition of endocrine output is consistent with the role of GABA controlling neurohormone release in the crayfish proposed by Kirk and Glantz.

Recent preliminary immunocytochemical studies demonstrated the presence of Met-enkephalin-like and Leu-enkephalin-like immunoreactivity in the eyestalk complex of Uca pugilator (Fingerman et al., 1983, Neurosci. Abstracts). Met-enkephalin (10^{-8}M), but not Leu-enkephalin, induces RPCH and BPCH release in a dose dependent manner in vitro. Naloxone (10^{-6}M) blocks both this response to Met-Enkephalin and the voltage stimulated release of RPCH and BPCH. The release of the chromatophorotropic hormones from the crustacean eyestalk is controlled not only by stimulatory neurotransmitters but by inhibitory ones as well.

94.13 MONOCLONAL ANTIBODIES TO INSECT PEPTIDERGIC NEURONS. PH Taghert, NJ Tublitz, JW Truman and CS Goodman Dept. of Biol. Sciences, Stanford Univ., Stanford CA 98305 and Dept. Zoology, Univ. of WA Seattle, WA 98195.

We are interested in the expression and regulation of insect neuropeptides. There are to date only two insect peptides - proctolin and the adipokinetic hormone - that have been purified, sequenced and for which immunological probes are available. In an effort to create probes to as yet unpurified neuropeptides, we have made monoclonal antibodies against a neurohaemal organ, the transverse nerve, from the moth Manduca sexta. The transverse nerve is a segmentally repeated structure that contains 2 motor axons and the axons and terminals of 18 identified neuroendocrine neurons (including 8 bursicon-containing and at least 2 cardioactive peptide-containing neurons). 3000 nerves were used to hyperimmunize a single mouse. Fusion of the spleen cells with NS-1 myeloma cells produced 382 hybrid-containing wells, each of which was screened histologically on serial frozen sections of single abdominal ganglia. 31 positive wells were identified and and hybrids cloned according to the criterion that subsets of abdominal ganglion neurons were stained.

29/31 monoclonal antibodies (mabs) appear to stain cytoplasmic antigens. 10 mabs appear to stain a similar pattern: some to many granular inclusions in approximately 5% of the 700 neurons in the ganglion. 5 mabs specifically stain two pairs of neurons that extend throughout the entire CNS. 1 mab intensely stains two of the four bursicon-containing neurons, 1 mab stains all four and 3 mabs stain all four plus four unpaired neurons at the mid-line of the ganglion. 3 other mabs intensely stain unpaired neurons exclusively.

Thus we have generated a set of monoclonal antibodies that exhibit diverse patterns of specific staining among the 18 neuroendocrine neurons in the moth abdominal ganglion. We are currently testing the ability of these antibodies to recognize known insect neurohormones. These antibodies and this technique may prove useful in the localization and purification of new biologically active neuropeptides.

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VESTIBULAR REFLEXES: FUNCTIONAL ORGANIZATION

95.1 FUNCTIONAL SEMICIRCULAR CANAL (SCC) GEOMETRY REFLECTED IN VESTIBULAR NYSTAGMUS AND SINGLE UNIT ACTIVITY IN THE ALERT RHESUS MONKEY. H. Reisine, V. Henn*, A. Böhmer*, and J.I. Suzuki*. Dept. of Neurology, University Hospital, 8091 Zürich, Switz. and Dept. of Otolaryngology, Teikio University, Tokyo, Japan.

The general question how an applied angular acceleration (AAA) to the head in any plane results in the vestibulo-ocular reflex (VOR) is addressed. Specifically, Rhesus monkeys were chronically prepared with Ag-AgCl electrodes for EOG monitoring of eye position, bolts for head fixation, and a device for recording single unit activity with varnished tungsten microelectrodes. Turntable and gimbal equipment permitted application of angular acceleration in any desired head plane. Finally, results from normal monkeys were compared with those of animals with various combinations of SCCs plugged.

In normal monkeys horizontal slow phase eye velocity (HSPEV) is maximal when the AAA is in the plane of the HSCCs (17° nose down) and decreases as a cosine function of the angle between the AAA and HSCC planes. The plane of the HSCCs does not coincide with the functional null plane of the vertical canals (33° nose down). VSPEV is maximal when the AAA is in the sagittal plane and also decreases as a cosine function of the angle between the AAA and sagittal planes. In canal plugged animals HSPEV can be generated without HSCC function and almost normal VSPEV can be generated in animals with different combinations of VSCCs plugged.

Single unit activity in the vestibular nuclei modulated by AAA can be nulled in two rotational planes and is maximal in a third, defining a functional SCC plane. As the AAA plane is rotated out of the functional SCC plane the modulation of the single unit activity decreases as a cosine function. In an animal with all SCCs plugged on one side single unit responses did not grossly differ from those of the normal situation. In general, most units sensitive to the AAA did not respond to static head tilt. Units sensitive to HAAA responded to horizontal optokinetic stimulation, whereas VAAA units did not. Vertical optokinetic responses could not be tested. Unit activity also reflected various strenghts of an eye position signal.

Thus, AAA to the head in a specific plane can be resolved into components lying within each SCC plane. Neural coding of these components has been found in the peripheral nerve and, in this report, in the vestibular nuclei. Central generation of patterns of vestibular nystagmus, induced in any rotational plane, in normal and canal plugged animals can thereby be analyzed in terms of the distribution of a head velocity signal in the vestibular nuclei that is organized in head coordinates. Supported by Swiss National Foundation for Scientific Research 3.718.80.

95.2 THE VALIDITY OF MATRIX ANALYSIS FOR THE DESCRIPTION OF SPATIAL VESTIBULO-OCULAR COORDINATION IN CAT AND RABBIT. W. Graf and K. Ezure*. The Rockefeller University, New York, NY 10021

The spatial coordination of the vestibulo-ocular reflexes in cat and rabbit was evaluated by taking into account the geometrical arrangement of semicircular canals and extraocular muscles (from our own measurements and those of Blanks, Curthoys & Markham, 1972) in the form of sensitivity and action vectors.The canal (C) and extraocular muscle matrices (M) then read:

$$C_{Rabbit} = \begin{array}{c} \\ ARPL \\ PRAL \\ HRHL \end{array} \begin{pmatrix} X & Y & Z \\ .748 & .615 & -.221 \\ .748 & -.615 & -.221 \\ .000 & .000 & -.977 \end{pmatrix} \quad M_{Rabbit} = \begin{array}{c} \\ X \\ Y \\ Z \end{array} \begin{pmatrix} IOSO & IRSR & LRMR \\ -.657 & -.825 & .188 \\ -.670 & .482 & -.208 \\ .303 & .294 & .959 \end{pmatrix}$$

$$C_{Cat} = \begin{array}{c} \\ ARPL \\ PRAL \\ HRHL \end{array} \begin{pmatrix} X & Y & Z \\ .711 & .687 & .079 \\ .711 & -.687 & .079 \\ .000 & .000 & -.990 \end{pmatrix} \quad M_{Cat} = \begin{array}{c} \\ X \\ Y \\ Z \end{array} \begin{pmatrix} IOSO & IRSR & LRMR \\ -.813 & -.470 & .129 \\ -.539 & .872 & -.059 \\ .210 & .133 & .989 \end{pmatrix}$$

IOSO,IRSR,LRMR of the left eye are obtained by averaging the antagonists IO and SO (IO:inferior oblique, SO:superior oblique etc.),similarly ARPL,PRAL,HRHL stand for averages of two coplanar canals (AR:right anterior,PL:left posterior,etc.)in Cartesian coordinates.From these matrices the brain stem matrix(T)of the underlying neuronal network was calculated for a symmetric or an asymmetric VOR gain(in the latter, the torsional gain was set to one half of the horizontal or vertical gains).

$$\text{Rabbit:}\quad T = \begin{array}{c} \\ IOSO \\ IRSR \\ LRMR \end{array} \begin{pmatrix} ARPL & PRAL & HRHL \\ .704* & -.018 & -.231 \\ .112 & .829* & .080 \\ -.245 & -.258 & 1.093* \end{pmatrix} \quad \text{(asym.)}$$

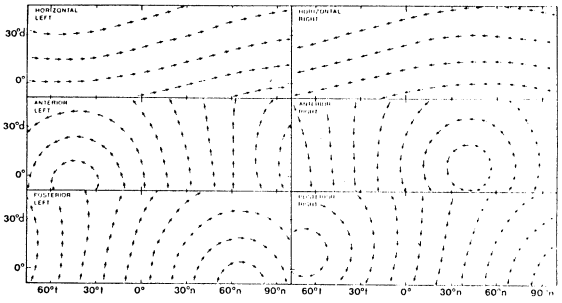
$$\text{Cat:}\quad T = \begin{array}{c} \\ IOSO \\ IRSR \\ LRMR \end{array} \begin{pmatrix} ARPL & PRAL & HRHL \\ .977* & .265 & .186 \\ -.242 & .986* & .180 \\ -.175 & -.189 & .958* \end{pmatrix} \quad \text{(sym.)}$$

Principal VOR connections are indicated by an asterisk(*).From the above matrices we observe that e.g. the averaged muscle IOSO not only is linked to ARPL, the principal reflex connectivity,but also receives accessory input from PRAL and even the horizontal canals,HRHL. Comparing calculated pathways to experimentally obtained data we find good agreement between the two in the cat, but not in the rabbit. For an asymmetric gain,the situation is improved in the rabbit. The functional role of the accessory (non-principal)VOR connections is interpreted to equalize the incongruences between semicircular canal and extraocular muscle planes. This statement is certainly justified for the cat. From the comparison between theoretical and experimental data,we also conclude that largely three-neuron-arc connections are responsible for the spatial coordination of the vestibulo-ocular reflexes. Supported by NIH grants EY04613 and NS02619.

95.3 VISUAL FIELD MOTION RELEVANT TO OPTOKINETIC EYE MOVEMENTS PREDICTED FROM THE PLANES OF EACH OF THE SEMICIRCULAR CANALS IN RABBIT. Barbara J. Winterson, Department of Physiology, New England College of Osteopathic Medicine, Biddeford, Maine 04005.

Optokinetic eye movements and the vestibulo-ocular reflex work in a synergistic manner to produce compensatory eye movements. Recently, it has been proposed that the preferred directions of visual neurons in the retina, accessory optic system, and flocculus are aligned with the visual field motion induced by rotation of the head such that a particular semicircular canal is maximally stimulated (Simpson, et al, 1979, Prog. Brain Res. 50, 715-724).

In order to assess this hypothesis, semicircular canals (surgically exposed) had points along them measured. These points were used to compute best-fit planes using a least square method. Mean planes were computed for 7 rabbits (4 Dutch and 3 New Zealand White). The mean planes were used to predict mathematically the visual field motion paths at infinity for each of the mean semicircular canals and plotted on a Mercator projection of that portion of the left visual field that corresponds to the optokinetic sensitive area (Dubois & Collewijn, 1979, Vis. Res. 9-17). These motions are shown in the figure below.



(d=dorsal, t=temporal, n=nasal)

Comparison of the predicted motion to preferred directions of visual neurons presumed to be in optokinetic pathways showed moderate to good correspondence to selected semicircular canals. Open-loop optokinetic slow phase direction (Dubois & Collewin, 1979) corresponded excellently to motion induced by the left horizontal canal and quite well to that of the left posterior canal and the right posterior canal.

95.4 TRANSFORMATIONS OF COORDINATES INTRINSIC TO THE VESTIBULO-OCULAR REFLEX. John I. Simpson, Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016

Intrinsic coordinate systems of sensorimotor integration for the vestibulo-ocular reflex can be identified in the periphery and the transformations properly interpreted (Pellionisz & Llinas, 1980; Simpson & Graf, 1981; Robinson, 1982). The principal response axes of the semicircular canals constitute a sensory coordinate system while the eye rotation axes of the extraocular muscles constitute a motor coordinate system. From in situ measurements of plastic casts of the rabbit labyrinth, the direction cosines of the canal axes are expressed relative to a right handed Cartesian reference frame fixed to the head after elevating the nasal bone 57° from earth horizontal, as in the freeze position (Hughes, 1971). The z = 0 plane is horizontal; the y = 0 plane is the midsagittal plane. Anterior, left and dorsal are positive. The average (n=4) direction cosines (x,y,z coordinates) of the rotation axes for best exciting the left canals are: horizontal, -.122, .018, .992; anterior, -.712, .702, .026; posterior, -.794, -.605, -.062. This set of unit vectors, along with the mirror symmetric set, establishes an intrinsic coordinate system for detection of rotational head movement. With the eye in primary position (assumed to be its position in the freeze state), the orientation of the eye rotation axis for each muscle is computed from measurements of the location of the insertion and origin and the eye rotation center, under the assumption that the sclera is part of a spherical surface and that the muscle courses over the globe along a great circle route to its point of departure from the globe. From preliminary measurements in two rabbits, the direction cosines (x,y,z coordinates) of the rotation axis for contraction of each of the muscles of the left eye are as follows: medial rectus, .006, .155, -.988; lateral rectus, -.412, .050, .910; superior rectus; 802, -.579, .147; inferior rectus, -.883, .470, -.013, superior oblique, .702, .563, .435, inferior oblique, -.656, -.717, .236. This set of unit vectors establishes an intrinsic coordinate system for execution of eye movements. With both the peripheral sensory and motor coordinate systems known, the central transformations of intrinsic coordinates can be explored theoretically using matrix and tensor operations. From among the possible theoretical predictions, those relating to the sign of the net connectivity between canals and muscles are presently amenable to comparison with existing anatomical and physiological observations. Supported by USPHS Grant 13742.

95.5 VESTIBULAR NUCLEI ACTIVITY IN THE ALERT MONKEY DURING CONSTANT VELOCITY AND SINUSOIDAL OPTOKINETIC STIMULATION. U. Büttner*, R. Boyle* and U.Schreiter* (SPON: V. Matsuo).
Dept. of Neurology, Univ. of Düsseldorf, D-4000 Düsseldorf (Germany)

During sinusoidal rotation of large field optokinetic stimuli the sensation of circularvection can be induced in humans up to frequencies of 2 Hz (Büttner and Henn, Ann.N.Y.Acad.Sci., 374:274-283, 1981). Furthermore such stimuli also lead to compensatory eye movements. The aim of this study was to determine a possible involvement of the vestibular nuclei in these responses.

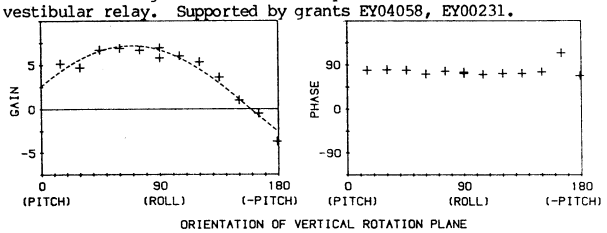
Prior to experiments monkeys (Macaca fascicularis) were chronically prepared for single unit and eye position recordings. During the experiment the monkey sat upright in a primate chair with his head fixed on a turntable. For vestibular stimulation the monkey was rotated about a vertical axis in complete darkness at 0.1-0.2 Hz. During optokinetic stimulation a large striped cylinder completely surrounding the monkey was rotated at constant velocity (5-100 deg/s) or sinusoidally (0,02-2 Hz, 5-100 deg/s) around the stationary monkey. For each neuron initially the responses to vestibular and constant velocity optokinetic stimuli were obtained. For the responses during sinusoidal optokinetic stimulation gain and phase relative to cylinder velocity was determined. Results indicate, that most vestibular nuclei neurons do not respond to optokinetic stimuli above 0,2 Hz. However, a few neurons still responded strongly at 1 Hz. These results will discussed in relation to the psychophysical and oculomotor effects observed at higher frequencies of optokinetic stimulation.

(Supported by Deutsche Forschungsgemeinschaft SFB 200, A 2, and Alexander v. Humboldt Stiftung (R.B.))

95.6 CONVERGENCE OF CANAL INPUTS TO SECONDARY NEURONS IN CAT VESTIBULAR NUCLEI. J. Baker, J. Goldberg, G. Hermann*, and B. Peterson. Northwestern Univ. Med. School, Chicago, IL 60611

The organization of vestibular reflexes requires convergence of vestibular inputs to muscles. For example, spatial orientations of semicircular canals and eye muscles dictate that to produce proper compensatory vestibulo- ocular movements each eye muscle must receive input from all three pairs of canals. We studied input convergence to secondary vestibular neurons in awake cats. Secondary neurons were identified by short latency responses (<1.3 msec.) to labyrinth stimulation via electrodes on round and oval windows. We applied sinusoidal rotations successively in 12 or more vertical and horizontal planes to determine spatial response properties of each neuron. Rotation with sum of sinusoid waveforms determined a cell's frequency response; rotation of the body with the head fixed in space tested for neck afferent inputs; and many cells were tested for eye position sensitivity.

We subdivided our sample of 76 sufficiently well studied neurons into two groups, 50 cells that we believe received vestibular input primarily from semicircular canals, and 26 "STC" cells with complex response properties which suggest they received canal and otolith inputs. An accompanying abstract describes STC cells. Canal cells had responses related to head velocity during sum of sine stimuli at frequencies above 0.2 Hz, and exhibited clearly defined planes of rotation in which their response was maximal or null. The figure below shows a canal cell's responses to rotation in several vertical planes at 0.5 Hz. Canal cell data were well fitted by sine functions based on the assumption that responses were the result of a linear sum of inputs from the three canal pairs. We calculated the angles between a cell's maximum response plane and the canal planes. Assuming an angle <75 deg. indicated significant input, 22 cells had input primarily from a single canal pair, 20 cells significant input from two canal pairs, and 8 cells input from all six canals. In addition, we found that many secondary neurons received neck and/or eye position input. Thus, extensive convergence occurs as early as the first central vestibular relay. Supported by grants EY04058, EY00231.

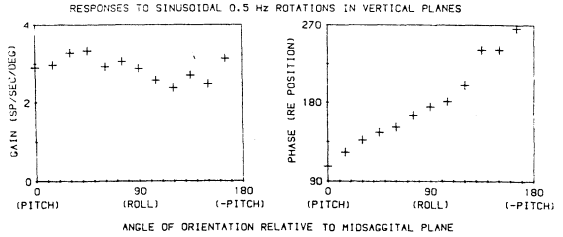


95.7 SPATIO-TEMPORAL CONVERGENCE ONTO SECOND ORDER VESTIBULAR NEURONS. J. Goldberg, J. Baker, G. Hermann*, B. Peterson. Department of Physiology, Northwestern Univ. Med. Sch., Chicago, Il 60611.

This abstract continues the description of identified second order neurons in the alert cat studied by the methods described in the preceding abstract by J. Baker et al.

The figures below show responses of a second order neuron to vertical rotation as the animal is positioned at different angles around its yaw axis. Note that the gain does not have a null and the phase is a continuous function of the orientation angle, unlike the responses of 'canal cells' described in the preceding abstract. Of the 76 neurons studied, 26 exhibited the above properties during sequences of rotations in vertical planes. These spatio-temporal response patterns cannot be produced by summation of inputs differing only in their spatial orientation and not in the phase of their response during sinusoidal rotation. Instead, inputs differing both in spatial and temporal properties must converge on these second order vestibular neurons, which we therefore call spatio-temporal convergent (STC) neurons. Comparisons of responses to horizontal and vertical plane rotations and the presence of responses to low-frequency (0.01-0.05 Hz) and static vertical rotations suggest that many of these cells receive convergent canal and otolith inputs. In some cases STC behavior may also be produced by convergent canal inputs with different dynamic (temporal) properties.

STC neurons, with rotational response phase dependent on the plane of head rotation, may be suitable for controlling neck muscles during vestibulocollic reflex, since moving the head in different planes requires inputs with different dynamics to these muscles. Convergent canal neurons that receive inputs with different spatial but the same temporal characteristics may be hypothesized to drive muscles that encounter relatively constant dynamic loads regardless of the plane of motion, such as the extraocular muscles. Supported by grants EY04058 and EY00231.



95.8 EYE MOVEMENTS OF THE ALERT CAT DURING OTOLITH AND OPTOKINETIC STIMULATION. J. H. Anderson and M. Le Taillanter*. Depts. of Otolaryngology and Physiology, University of Minnesota Medical School, Minneapolis, MN 55455.

When an animal is in his normal upright position and is subjected to a disturbance such as pitch rotation, otolith and semicircular canal inputs interact to produce a symmetrical vestibulo-ocular reflex response (cf., Barmack, J. Physiol., 314:547-564, 1981). In contrast, when an animal is on its side, which causes a change in the gravity bias on otolith organs, the vertical VOR and vertical optokinetic reflex are asymmetric (cf., Anderson, Dev. Neuro., 12:395-401, 1981; Matsuo et al., Brain Res., 176: 159-164, 1979; Darlot et al., Exp. Br. Res., 41: 420-426, 1981; Matsuo, Ph.D. Thesis, 1981). The present work focused on some characteristics of this asymmetry and the responses due to otolith stimulation alone or in combination with optokinetic stimulation. Alert, restrained cats were subjected to sinusoidal linear accelerations in the horizontal plane or they were stationary and viewed a rotating optokinetic pattern. For linear motion, the frequencies used covered the range 0.05 to 0.25 Hz and the peak accelerations were 0.029 to 0.75 m/s^2. Eye movements were recorded with the magnetic search coil technique. When the animal was positioned in a normal upright manner and subjected to accelerations along its longitudinal (X) axis, there were no significant vertical eye movement responses in the dark. Also, when the animal was positioned laterally on its side and subjected to accelerations along its X- or vertical (Z) axis, there were no responses in the dark. However, when viewing an optokinetic pattern during movement along the Z-axis, there were responses. The vertical eye movements were very asymmetric. The velocity of the downward slow eye movements was small, compared to that of upward eye movements, and the ratio of downward to upward eye velocities was less than 1/4. These results indicate that small amplitude linear accelerations (less than 0.75 m/s^2) do not cause a measurable otolith-ocular response in the cat. However, when there is an optokinetic input in addition to the otolith input, there is a vertical eye movement response which is asymmetric. This may be due to the static change in head orientation relative to gravity (Matsuo, Ph.D. Thesis, 1981) and the modulated otolith input interacting with the optokinetic system (Buizza et al., Exp. Br. Res., 39:165-176, 1980).

95.9 DIRECTIONAL SELECTIVITY OF TILT-SENSITIVE CENTRAL VESTIBULAR NEURONS IN THE CAT. R. H. Schor, A.D. Miller and D.L. Tomko. Lab. of Neurophysiology, Rockefeller Univ., New York, NY 10021 and Dept. Physiology, Univ. of Pittsburgh, Pittsburgh,PA 15261.

Responses to head tilt were recorded from vestibular neurons in and around the lateral vestibular nucleus (LVN) of the decerebrate cat. Each animal had all six semicircular canals rendered nonfunctional by a plugging procedure. Each cell was studied by slowly tilting the cat using one or both of two paradigms. The first method employed sinusoidal tilts (0.05 or 0.1 Hz, up to 20 degrees) about earth-horizontal axes, oriented in up to 12 directions including longitudinal (roll) and transverse (pitch) tilt. The second method imposed a constant 10-degree tilt; the direction of the tilt was rotated with a 10 or 20 second period around the animal by an appropriate combination of roll and pitch motions.

Neurons responded by maximally increasing their discharge frequency in a particular direction of head tilt from the horizontal. Each cell's response could be described by a vector in the animal's horizontal plane whose orientation was given by the direction of the most effective stimulus, and whose length represented the neuron's sensitivity to tilt. The two methods of stimulation yielded equivalent response vectors.

Response vectors have been obtained for more than 100 neurons. The distribution of vector directions for these vestibular neurons was not uniform; there was a conspicuous absence of neurons with fore/aft-directed vectors. The median sensitivity of these cells (length of the response vector) was 50 impulses/sec/g. Neurons whose vectors lay in the ipsilateral half-plane (which would be excited by ear down tilt) tended to be less sensitive than those with contralateral vectors. Neurons excited by ear up tilt tended to be located ventrally in the LVN while those excited by ear down tilt were more evenly distributed. There was no other obvious correlation of vector orientation with the anatomical locus of the cell in the brainstem.

A subset of these neurons was also tested with a broad frequency bandwidth of tilt stimuli. The response vectors determined at higher frequencies (e.g. 0.5Hz) often pointed in the opposite direction, since responses which developed a phase lag of more than 90 degrees (Schor and Miller, EBR 47: 137-144, (1982) reversed the apparent excitatory and inhibitory directions of tilt. (Partially supported by NASA grants NSG-2380 and NAG2-155, and PHS grants NS17808, NS02619 and RR07065).

95.10 GRAVITATIONAL EFFECTS ON VISUAL-VESTIBULO-OCULOMOTOR COORDINATE TRANSFORMATIONS GENERATING COMPENSATORY EYE MOVEMENTS. T. Raphan and B. Cohen. Departments of Computer and Information Science, Brooklyn College and Neurology, Mount Sinai School of Medicine, New York, N.Y.

Rotation of the visual surround that induces horizontal optokinetic nystagmus (OKN) excites the velocity storage mechanism and induces horizontal optokinetic after-nystagmus (OKAN). If horizontal OKN is induced while monkeys are tilted with regard to gravity, then the OKAN which follows has a vertical component. The direction of the vertical component is gravity specific. When the slow phases of OKN are directed away from gravity, the OKAN is oblique with the vertical component of the slow phases in the downward direction. If the slow phases of OKN are toward the direction of gravity, the OKAN has an upward slow phase component. The vertical component of the nystagmus does not appear immediately but builds up and decays to zero as if excited by an exponential function. This suggests that under appropriate circumstances stored information associated with horizontal nystagmus can excite a mode of the storage mechanism that generates vertical nystagmus. Such cross coupling is also demonstrated in other ways. Pure vertical OKN with the animal upright is associated with little vertical OKAN. Consistent with this, if monkeys are upright when they receive an oblique optokinetic stimulus, only horizontal OKAN is induced and the vertical component is absent. However, if animals are tilted with regard to gravity and are given an oblique optokinetic stimulus, oblique OKAN is now induced with a strong vertical component. The vertical component is symmetrical regardless of whether the slow phases are up or down. This is in contrast to vertical OKAN induced in a 90° roll position which is stronger when the slow phases are up. To incorporate the effects of gravity, we posit that velocity storage is best represented by a multidimensional system, with specific states of that system establishing a coordinate basis for interpreting head movement and movements of the visual surround. Gravity appears to reorient the basis vectors for the state space of the system by modifying the coupling from the visual and vestibular systems to the storage mechanism. This has the effect of generating cross coupled eye velocity.

Supported by NIH Grants EY 04148, NS 00294 and Core Center Grant EY 01867

95.11 EFFECT OF ABNORMAL VESTIBULAR-VISUAL INTERACTIONS UPON POSTURE CONTROL IN TWO DIFFERENT TYPES OF VESTIBULAR DEFICIT PATIENTS. L.M. Nashner and F.O. Black. Neurological Sciences Institute and Department of Neuro-otology, Portland, OR 97209.

A group of 48 vestibular deficit patients was assessed on the moving platform and independently by standard clinical means. Patients were selected based upon two clinical criteria: complaints of postural instability not adequately categorized by standard clinical tests, and/or benign paroxysmal positional nystagmus (BPPN). Platform tests exposed patients to six sensory conditions: normal support surface and visual surrounds (S_NV_N), normal support with eyes closed (S_NV_C) and with visual surrounds rotated to precisely follow the patient's A-P sway motions (S_NV_S), then repeating in the same order the three visual conditions but not with the support rotated to precisely follow A-P sway motions (S_SV_N, S_SV_C, S_SV_S) (see Nashner, et al, J. Neuroscience 2, 1982).

Based upon the configuration of normal and abnormal performance under the six conditions, 38 of the 48 fell into one of three categories (5 tested normal and 5 too impaired to characterize).

Category 1: 18 showed a configuration of abnormality identical to that described previously for symmetric and/or asymmetric reduced vestibular function; i.e., within normal range (<1 S.D.) in all S_N and the S_SV_N tests (Nashner, et al, ibid), abnormal in the S_SV_S test (>2 S.D.), and inconsistent in the S_SV_C test.

Category 2: 6 patients were distinguished from the previous 18 by abnormal performance in the S_NV_S test (>2 S.D.) and consistently normal performance on the S_SV_C (<1 S.D.) (i.e., a relatively normal vestibular inertial gravitational reference to posture).

Category 3: 14 patients were significantly abnormal (>2 S.D.) under those test conditions representing an overlap of categories 1 and 2.

Category 2 and 3 patients presented symptoms consistent with one or both posterior canals being sensitive to linear as well as angular accelerations (i.e., BPPN). Category 2 patients experienced motion sickness symptoms under the V_S conditions. Apparently, a perceptually undetectable "distortion" of vestibular space (in contrast to reduced or asymmetrical vestibular output) leads to very different visual-vestibular interactions. Those with distorted vestibular posterior canal function could not ignore the inappropriate visual reference, even when provided correct somatosensory information and what appeared to be a relatively normal vestibular upright reference to posture.

95.12 GEOMETRICAL ANALYSIS OF POSTURAL INSTABILITY IN BPPN (VESTIBULAR) PATIENTS. G. McCollum and L.M. Nashner. Neurological Sciences Inst., 1120 N.W. 20th Avenue, Portland, Oregon 97209.

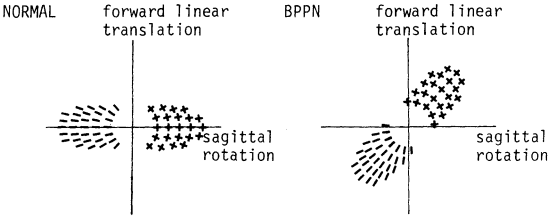
The postural behavior of the BPPN patients described in the previous abstract varied with sensory modality and with mix of sensory modalities. Stabilized ankle angle input was appropriately ignored when the eyes were closed. But stabilized visual input inappropriately overrode even correct ankle angle information in the presence of the distorted BPPN vestibular signal.

Since the BPPN patients were able to use ankle angle information appropriately in the absence of vision, the purest measure of their direct vestibular input to posture was stance upon the stabilized support surface with eyes closed. In that case, they oscillated forward and backward with a characteristic frequency, but about an upright of normal accuracy.

This finding is consistent with BPPN canals sensing not pure rotation but a combination of rotational and linear motions. In the diagrams below, radius gives response amplitude for motions of constant instantaneous acceleration of the canal.

Postural motions, about axes outside the canals, include movements of the head both rotational and linear to the canals. Also, visual sensation of head movement includes both rotation and translation. Sensation of linear motion, indistinguishable for the canals from rotational, gives the axis of canal sensation the freedom to wander in both postural and visual spaces. When normal visual response superimposes on distorted BPPN canal response to leave an area of overlapping responses of opposite sign, there is sensory conflict. That is, canal response to postural motion is inappropriate and disagrees with visual response.

This sensory conflict, rather than lack of vestibular input, causes BPPN patients to follow visual cues even when they are inappropriate.



ENDOCRINE CONTROL: CENTRAL PATHWAYS I

96.1 FUNCTIONAL INTERRELATIONSHIPS BETWEEN ANDROGEN RECEPTOR OCCUPATION IN BRAIN AND PITUITARY AND SERUM TESTOSTERONE (T) AND LUTEINIZING HORMONE (LH) LEVELS IN MALE RATS. M.Y.McGinnis and L.C.Krey (SPON:P. Anderson). Dept. Anatomy, Mount Sinai School of Medicine, New York,N.Y. 10029 and Rockefeller University, New York, N.Y. 10021.

We have characterized the dynamics of cell nuclear androgen receptor binding, and serum T and LH levels associated with the insertion and removal of two x 10 mm T-filled Silastic capsules implanted sc into preformed, open flank pouches of awake, castrated (2-3 wks), male rats. Rats were sacrificed at 1/2,2,4,8 or 24h after capsule insertion. Additional groups of rats were sacrificed prior to T insertion, or 2,4, or 8h after capsule removal (24h T exposure). Cell nuclear androgen receptor binding was measured in combined hypothalamus, preoptic area, amygdala and septum (HPAS) and pituitary (PIT) using an exchange assay (McGinnis et al.Brain Res.1983). In no-T castrates serum T and cell nuclear androgen receptor levels were barely detectable, while serum LH levels were elevated. Serum T rose to 2-4 ng/ml during the 1/2 to 24h of T exposure. Nuclear androgen receptor levels during the first 1/2 to 8h increased dramatically in the HPAS (12-23 fold) and PIT (18-24 fold). Further slight increases occured at 24h. Serum LH levels began to decline 4h after capsule insertion, and reached undetectable levels only after 24h T exposure. Following capsule removal, serum T and nuclear androgen receptor levels in HPAS and PIT fell precipitously. Serum T was undetectable at 2h; androgen receptor levels fell by one half at 2h and approached castrate values at 4h. Serum LH levels began to rise at 4h and approached castrate levels by 8h. These results suggest relatively rapid association and dissociation rates of androgens to their receptors in brain and pituitary. Results further indicate that T must be present for >2h for suppression of LH secretion, and that complete suppression of LH requires >8h of T exposure. Moreover, feedback actions of T are retained following androgen receptor clearance from the cell nucleus. Presently we are examining the influences of these T treatments on frequency:amplitude characteristics of episodic LH release in male rats.

Supported by grants from The Mount Sinai Medical Center and The Rockefeller Foundation.

96.2 PATTERN OF SYNAPSES ON VENTROMEDIAL HYPOTHALAMIC NEURONS IN THE FEMALE RAT: AN ELECTRON MICROSCOPIC STUDY. M. Nishizuka* and D. Pfaff (SPON: L.-M. Kow). Laboratory of Neurobiology & Behavior, Rockefeller University, New York. NY 10021.

Ultrastructural observations of synaptic types in limbic and hypothalamic structures have been useful in neuroendocrine studies, for example in the demonstration of sex differences (Nishizuka & Arai, Brain Res. 212:31, 1981). Synapses impinging on ventromedial hypothalamic (VMH) neurons must be described in the analysis of inputs controlling cell bodies important for lordosis and other behaviors. In this analysis, the existence of intrinsic synapses can be tested by looking for those which remain after transections that circumscribe VMH.

Ovariectomized female rats were used. Some were given 2 weeks exposure to systemic estrogen. Also, some rats were subjected to surgery in which the VMH was isolated by a circumscribing knife cut; these were perfused 5 days after surgery. Perfusion with 2% paraformaldehyde-2% glutaraldehyde, osmification, embedding in epon, sectioning and staining followed standard electron microscopic procedures.

In unoperated animals, normal axodendritic synapses were seen, with a predominance of synapses on dendritic shafts as opposed to spines. Axosomatic synapses were also detected. Dense-cored vesicles could be seen in some synaptic terminals. Notable were large clear-centered vesicles (maximum diameter 130 nm) in a small percent of terminals. Also, in a few terminals in individual sections multiple contact zones could be visualized. In these respects, estrogen-treated rats were qualitatively similar to ovariectomized controls.

Synapses on VMH neurons survived total VMH isolation, proving that some VMH somas give rise to axons with intrinsic synapses. Axodendritic intrinsic synapses were detected on both shafts and spines, and intrinsic axosomatic synapses were also seen. These intrinsic synapses comprised a substantial proportion of the total synapses on VMH neurons. Overall, the synaptic patterns observed allow for a variety of types of physiological controls over VMH neurons, including possible effects of "local circuit neurons".

Supported by NIH grant HD-05751.

96.3 LOCALIZATION OF LH-RH-LIKE IMMUNOREACTIVITY IN THE OVINE BRAIN. Juan Pablo Advis* and Rodrigo O. Kuljis. Dept. of Animal Sciences, Cook College, Rutgers University, New Brunswick, N.J. 08907 and Depts. of Neurology and Neurobiology and Behavior, S.U.N.Y. at Stony Brook, N.Y. 11794.

Immunocytochemical techniques for the localization of LH-RH have been employed in a variety of mammalian species, including the rat (Baker et al., '74), the guinea pig (Krey and Silverman, '78) and primates (Barry and Carette, '75). The present study analyzes the distribution of LH-RH-like immunoreactivity in the brain of the sheep.

Two 10-month old female sheep were anesthetized and decapitated The heads were perfused with 0.9% saline followed by periodate-lysine-paraformaldehyde fixative (McLean & Nakane, '74) via the carotid arteries. The brains were freeze-sectioned coronally at 30 μm in a sliding microtome, and the sections processed for LH-RH-like immunoreactivity by means of the peroxidase-anti-peroxidase (Sternberger, '74) and indirect fluorescence (Coons, '58) methods. The primary antibody was raised in rabbits and generously supplied by Dr. V.D. Ramirez from the University of Illinois.

LH-RH-like immunoreactivity is present in cell bodies located in the medial preoptic area, lamina terminalis, dorsal aspect of the preoptic portion of the supraoptic nucleus, arcuate nucleus, retrochiasmatic area, medial septal nucleus, triangular septal nucleus, diagonal band of Broca and anterior perforated substance. LH-RH-like immunoreactivity is present in fibers located in the paraventricular portion of the hypothalamus, forming rather loose bundles between LH-RH-containing cell groups in the preoptic area and the ventro-basal hypothalamus. A dense fiber system descends from the arcuate nucelus into the median eminence. Loose fiber bundles appear to extend from the preoptic area and adjacent limbic structures into the ventral tegmental and basal area of the midbrain, after traversing through the hypothalamus in the medial forebrain bundle.

These observations suggest that LH-RH is present in discrete cell groups and fiber systems in the ovine basal forebrain, in agreement with LH-RH determinations by radioimmunoassay (Advis & Kuljis, in progress).

Supported by HATCH 08616 (J.P.A.) and PHS 1-F05-TW02947 (R.O.K.).

96.4 IMMUNOCYTOCHEMICAL LOCALIZATION OF LHRH IN THE INFUNDIBULAR STALK AND NEUROHYPOPHYSIS: A COMPARATIVE STUDY. E.L.P. Anthony* and J.C. King (SPON: D.A. Damassa). Dept. of Anatomy and Cellular Biology, Tufts University Schools of Medicine, Boston, MA 02111.

Complex vascular relationships have recently been demonstrated between the anterior and posterior pituitary lobes, as well as between these regions and the basal hypothalamus. These interactions suggest re-examination of the routes whereby hypothalamic hormones may reach the anterior pituitary. The distribution of the hypothalamic hormone LHRH was studied using light microscopic immunocytochemistry in the hypothalamic-pituitary complex of several mammals. Primary antisera employed included 1) IJ29 (I.M.D. Jackson), 2) R422 (A. Arimura), and 3) CRR11B73 (V. Ramirez); each of these antisera binds a different portion of the LHRH molecule. In humans, rhesus monkeys, cynomolgos monkeys, little brown bats and ferrets, LHRH-immunoreactive fibers were observed in both the internal and external zones of the median eminence. Additionally, substantial numbers of immunoreactive fibers traversed the infundibular stalk and entered the neural lobe of the pituitary. In all of these species, the majority of fibers entering the neural lobe coursed along the posterior border of the adenohypophysis, although individual fibers commonly diverged from this region and entered deeper portions of the neural lobe. In addition to immunoreactive fibers, LHRH-immunoreactive neuronal perikarya were observed within the median eminence in bats and within the infundibular stalk in rhesus monkeys and ferrets. This distribution of LHRH cells and fibers bears little resemblance to that demonstrated in the rat by equivalent immunocytochemical procedures. In rats, immunoreactive fibers were numerous in the external, but not the internal, zone of the median eminence. Only occasional fibers and no perikarya were associated with the infundibular stalk and neural lobe in this species. These striking interspecific variations suggest that multiple routes may exist in the tuberoinfundibular region for the delivery of LHRH to the pituitary. In addition, some neurovascular endings of LHRH neurons in this region may distribute the peptide to distant sites via systemic circulation or return it to the hypothalamus via retrograde flow. This study was supported by grants HD00352 from NIH and PCM 8103243 from NSF to JCK.

96.5 THE INFLUENCE OF DIFFERENT LIGHT WAVE LENGTHS ON THE SHORT PHOTOPERIOD-INDUCED COLLAPSE OF THE MALE SYRIAN HAMSTER REPRODUCTIVE SYSTEM. George C. Brainard, Mary K. Vaughan, and Russel J. Reiter. Department of Anatomy, The University of Texas Health Science Center, San Antonio, Texas 78284 and Department of Neurology, Thomas Jefferson Medical College, Philadelphia, Pennsylvania 19107.

Environmental light perceived by the retina is responsible for entraining circadian rhythms, regulating reproductive cycles, and influencing a variety of endocrine and metabolic functions. Specifically, hamsters exposed to short photoperiods (12.5 hours of light or less) undergo an inhibition of the neuroendocrine-reproductive axis. The following study was designed to determine which light wavelengths are capable of blocking this suppression of the reproductive system. Groups of 10 hamsters each were exposed to short photoperiods (11 hours) of bright white light at 400 $\mu W/cm^2$, extended by 3 hours of either white light, near-ultraviolet light, blue light, green light, yellow light, red light or darkness. The white light was produced by cool white fluorescent light sources. The restricted wavelength sources had half-peak bandwidths of 330-371 nm (near-ultraviolet), 435-500 nm (blue), 515-550 nm (green), 558-636 nm (yellow), 635-770 nm (red). The lights used in the additional 3 hour period were adjusted to produce an irradiance of 0.2 $\mu W/cm^2$. After 12 weeks of exposure to these different photoperiodic conditions, paired testes, accessory sex organs, and pituitary glands were weighed. Blood samples and pituitary glands were assayed for prolactin by radioimmunoassay. Animals exposed to short photoperiods extended by white, near-ultraviolet, blue or green light had testicular weights which were typical of reproductively competent hamsters. Animals exposed to short photoperiods extended by 3 hours of yellow light, red light, or darkness had significantly suppressed testicular weights ($P<0.001$). This reproductive suppression was also observed in the accessory sex organs. Interestingly, hamsters exposed to near-ultraviolet, blue, and green lights had significantly higher pituitary PRL levels than all other groups ($P<0.01$). These data demonstrate that the neuroendocrine reproductive axis is differentially responsive to different wavelengths of visible light. However, it appears that not all aspects of the reproductive axis respond in a parallel fashion to all wavelengths.
(Supported by NSF Grant No. PCM 8003441. The experimental light sources were generously supplied by the Durotest Corporation, North Bergen, New Jersey.)

96.6 LESIONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS BLOCK THE INFLUENCE OF PHOTOPERIOD ON GONADAL REGRESSION AND PINEAL MELATONIN IN MALE HAMSTERS. E.L. Bittman, M.N. Lehman, and S.S. Winans. Reproductive Endocrinology Program and Dept. Anatomy, Univ. Mich., Ann Arbor, MI 48109.

The pineal gland mediates photoperiodic control of hamster neuroendocrine function. Retinal input to the suprachiasmatic nucleus (SCN) and sympathetic innervation of the pineal are critical to the inhibition of reproduction by short days. Intervening structures are unidentified. In the rat, the hypothalamic paraventricular nucleus (PVN) receives extensive input from the SCN (Berk & Finkelstein, Brain Res., 226:1), and may influence autonomic function via its brainstem and spinal cord projections (Swanson & Sawchenko, Neuroendo., 31:410). We therefore studied the role of the PVN in photoperiodically-induced testicular regression in the golden hamster. Initially, 11 males housed in long days (14L:10D) received either bilateral PVN lesions (n=7) or sham lesions (n=4). After 3 weeks all males were blinded by bilateral enucleation. When inspected 11 weeks later the gonads of sham-lesioned males were uniformly regressed. Six hamsters with complete destruction of the PVN had large testes similar to those of sighted males in long days. One lesioned male exhibited substantial but incomplete testicular regression; in this animal the lesion spared a large dorsocaudal portion of the PVN.

In a second experiment, 25 males housed in long days either received bilateral PVN lesions (n=7), SCN lesions (n=9), sham lesions (n=7), or served as normal controls (n=5). Three weeks later all males were transferred to short days (10L:14D). After 11 weeks animals were decapitated under dim red illumination 3-5 h prior to lights on, and pineal melatonin content was determined by radioimmunoassay (Tamarkin et al., Endo., 104:385). Sham-lesioned and normal control hamsters all had regressed, spermatogenically inactive testes (combined weight, x=0.53) while hamsters bearing complete bilateral lesions of the PVN or SCN had large, spermatogenically active testes (x=3.81). As expected, control animals exhibited high nocturnal levels of pineal melatonin, while the pineals of PVN-lesioned males contained significantly lower levels. Effective PVN lesions were restricted to the caudal portion of this nucleus and completely spared the SCN. The PVN may therefore be an important relay for photoperiodic information from the SCN to spinal structures responsible for rhythmic autonomic control of pineal melatonin synthesis and gonadal function.

(Supported by NIH grants NS-14071 and HD-11311 to S.S.W. and HD-07048 to E.L.B. and M.N.L.)

96.7 EFFECTS OF SMALL DOSE OF ESTROGEN AND PROGESTERONE ON SINGLE UNIT ACTIVITY IN THE MEDIAL BASAL HYPOTHALAMUS AND LH RELEASE IN FEMALE RHESUS MONKEYS. R.R. Yeoman & E. Terasawa. Wis. Reg. Primate Res. Ctr. University of Wisconsin, Madison, WI 53715-1299.

The hypothalamus is involved in the progesterone (P) induced LH surge in ovariectomized and estrogen primed rhesus monkeys since pentobarbital blocks this release (J. Endocrinol. 92: 327, 1982). Single unit activity (SUA) in the ventral part of the medial basal hypothalamus (MBH) increased during LH release after P injection (Endocrinol. 112: Suppl. 156, 1983). However, the extent of neural activity change after the priming dose of estrogen alone remains to be investigated. The present studies compare P and estrogen effects on SUA of the MBH in relation to the time of release of LH. Five ovariectomized female rhesus monkeys were chronically implanted with a pedestal on their crania using X-ray ventriculography under halothane anesthesia. Small capsules containing estradiol-17β (EB) were implanted subcutaneously 2 wks before the experiments. SUA was recorded during 2 different steroid treatments: 1) 22-36h and 40-50h after 15-25μg EB injection, and 2) 1-12h after 2.5 mg P injection which was administered 24h following 15-25μg EB. The animals were chaired up to 14h only during the recording periods under minimal sedation (3mg ketamine/h). For SUA recording, a sheathed 50μm dia. stainless steel electrode with a 2-5μm tip was used and the position of electrode was verified by X-ray each time. The units sampled were within a stereotaxically defined area of 2.0mm lateral, 1.5mm anterior and 2.0mm posterior to the infundibular recess of the 3rd ventricle. The firing frequency was calculated by a programmed data analyzer. Serum samples were obtained periodically for LH analysis. The frequency of unit firing of the ventral and dorsal MBH before EB administration was 1.6 ± 0.4 Hz, (n=28),(mean ± SE) and 3.8 ± 0.9 Hz, (n=24), respectively. Twenty to 30h after the priming dose of EB neither firing rate of SUA (ventral: 1.5 ± 0.1 Hz, n=65; dorsal: 5.9 ± 0.7 Hz, n=96) nor LH level changed. Similarly 40 to 50h after EB the SUA (ventral: 1.7 ± 0.3 Hz, n=15; dorsal: 5.5 ± 1.6 Hz, n=18) did not change although LH increased slightly at this time. In contrast, 2h after P the SUA of the ventral MBH started to increase and 6-10h after P it reached maximum firing rate (5.7 ± 1.4 Hz, n=24). An increase in LH release occurred in a similar time course significant at 3h and peaking at 9h after P. Mean firing rate 2-12h post P was 4.7 ± 0.5 Hz, n=101 ($p < 0.001$). Dorsal MBH activity was not changed significantly (5.2 ± 0.7 Hz, n=61). In summary a small dose of EB alone did not change hypothalamic SUA nor circulating LH, while additional P increased the SUA of the ventral MBH coincident with the LH surge. Therefore, P but not the priming dose of EB, activates the neural substrate influencing LH release. (Supported by NIH grants RR-00167, HD-15433 and HD-11355).

96.8 EFFECTS OF PHOTOPERIOD ON ESTROGEN RECEPTOR IN HAMSTER BRAIN. P. Mak* and G.V. Callard*. (SPON: A. Miller). Biology Dept., Boston Univ., Boston, MA 02215

Exposure of hamsters to short days (8:16 LD) in contrast to those maintained in long days (16:8 LD) leads to increased sensitivity to the negative feedback effects of androgen (A) but decreased responsiveness to the behavioral effects of the hormone. Aromatization of A to estrogen (E) in situ is known to mediate many central A actions, and we previously observed decreased aromatase in brain tissues of short day (Solomon and Callard, Biol. Reprod. 22:85A, 1980) and blinded (Callard et al., Biol Reprod. 21:33, 1979) hamsters. In order to determine whether changes in the quantity of E formed locally might be reflected in the uptake, binding, and translocation of receptors (ER), hamster brain was examined for specific E binding activity. Using a protocol similar to that for measuring ER in hamster uterus (Evans et al., Endocrinology 107:383, 1980) and LH 20 column chromatography an E binding macromolecule having the characteristics of a receptor was detected in crude cytosolic and nuclear extracts of brain tissues. These binding proteins exhibited high affinity (K_d = 10.8 x 10^{-10}M for cytosol; 11.5 x 10^{-10}M for nuclear) and limited binding capacity (20 - 50 fmol/g tissue). Binding was specific for E (estradiol > estrone > estriol) but not for A (testosterone, 5α -dihydrotestosterone) or progesterone. Cytosolic ER had a sedimentation coefficient of 7-8S in a 5-20% linear sucrose gradient (no salt), whereas nuclear ER sedimented in the 5S region of a high salt gradient. Intact and castrated hamsters showed a similar neuroanatomic distribution of ER (limbic > remaining forebrain ≈ mid/hindbrain), and 2 h after a single injection of E, ER was depleted in limbic regions with a concomitant increase in nuclear ER. ER was measurable in nuclear extracts from brain of intact males, but values were lower or non-detectable following castration or when testes were regressed as a consequence of short day exposure. This suggests that the hamster gonad normally secretes estrogen or aromatizable androgen which is responsible for ER translocation. When nuclear ER was measured in limbic tissues after E injection, values were similar in animals exposed to long days or short days whether or not the testes were present. Thus, photoperiod does not affect ER or ER translocation per se. By contrast, after injection of androstenedione, an aromatizable androgen, nuclear ER was lower in short day groups (intact and castrate) compared to the corresponding long day controls. These results support the view that aromatization is impaired in the brain of hamsters maintained on short daily photoperiods, and that changes in the quantity of estrogen formed in close proximity to ER can regulate a critical step leading to biological activation in the central nervous system. (HD 16714)

96.9 DIFFERENTIAL EFFECTS OF PROLACTIN ON THE UPTAKE AND RAPID RELEASE OF DOPAMINE (DA) FROM MEDIAN EMINENCE (ME) AND STRIATAL (S) SYNAPTOSOMES. Karen A. Gregerson* and Michael K. Selmanoff (SPON: D. Ruchkin). Department of Physiology, University of Maryland, School of Medicine, Baltimore, Maryland 21201.

The effects of hyperprolactinemia on the uptake and release of ^{3}H-DA was studied in crude synaptosomal preparations (P_2 fraction) from rat ME and S using a rapid filtration technique. Synaptosomes were prepared from intact male rats treated with ovine prolactin (oPRL) for 48h (4mg/kg, sc, every 8h) and from vehicle-treated controls. ME and S synaptosomes were prepared from comparable masses of tissue (10-15mg) and were run simultaneously. The synaptosomes were preloaded by incubation in 0.1μM ^{3}H-DA for 30 min at 30°C, then stimulated with 75mM K^+ or 75μM veratridine, and the effluents obtained by rapid filtration were analyzed for ^{3}H-DA content. ^{3}H-DA release under basal (5mM K^+) and depolarizing conditions was determined over 5 sec intervals.

In synaptosomes from vehicle-treated controls, uptake of ^{3}H-DA was about 2-fold greater into S than into ME synaptosomes. The majority of depolarization-evoked release of ^{3}H-DA from both ME and S synaptosomes was Ca^{++}-dependent, although a component existed which was not dependent on externally added Ca^{++}. The latter represented a greater fraction of the stimulated release from the ME (32%) than from the S (14%). In addition, the rate constant for the calculated initial phase of release from S was much greater than that for ME synaptosomes, suggesting that the apparent kinetics of DA release are markedly different in the ME as compared to the S.

^{3}H-DA incorporation into S synaptosomes was enhanced following exposure to oPRL, whereas the hormone treatment did not affect the percent of preaccumulated ^{3}H-DA released from S synaptosomes under either basal or depolarizing conditions. In contrast, hyperprolactinemia did not alter the amount of ^{3}H-DA taken up by ME synaptosomes but did selectively increase the evoked release of ^{3}H-DA from ME. Basal efflux was unchanged from controls. The difference in evoked release between oPRL-treated and control ME occurred when depolarization was induced with either high external K^+ or veratridine. Since the enhanced ^{3}H-DA efflux from ME synaptosomes from hyperprolactinemic rats was also evident during depolarization over a wide range of external Ca^{++} concentrations (0.01-3.0mM), the data suggest that this effect of oPRL is on a Ca^{++}-independent release mechanism in the ME.

In conclusion, the apparent kinetics of DA release differ between the ME and S. Moreover, oPRL in vivo has differential effects on the in vitro release of DA from these two dopaminergic terminal projection fields.

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96.10 DOSAGE AND TEMPORAL EFFECTS OF ESTROGEN ON AUDITORY EVOKED POTENTIALS RECORDED FROM THE FROG'S AUDITORY MIDBRAIN. K. F. Ramey* (SPON: A. S. Feng). Dept. of Physiol. Biophysics., Univ. of Illinois, Urbana, IL 61801.

Acoustic communication plays an important role in the reproductive behaviors of frogs and toads. Such behaviors are governed by both extrensic environmental as well as intrinsic physiological factors including sex hormones. Hormones have been shown to be taken up in and act directly upon the neurons in the anuran central nervous system. A recent study in our laboratory (Yovanof and Feng, 1983) showed that intraventricular injections of a small amount of 17-β-estradiol hemisuccinate, but not vehicle injections, significantly increased the auditory evoked potentials (AEP) recorded from the torus semicircularis of female northern leopard frogs (Rana p. pipiens). Increase in AEP was observed only after the second hormone injection made several hours after the first one. In this study, the dosage and temporal effects of estrogen were investigated.

Female northern leopard frogs were ovariectomized two weeks prior to the recording session in order to achieve a uniform estrogen background level among animals and thus obtain a more accurate estimate of the estrogen dose effect on AEP. Each frog was anesthetized and surgically prepared for AEP recordings from the torus semicircularis with a sharpened tungsten microelecrode. Acoustic stimuli consisted of isointensity (80 dB SPL) bursts of pure tones at four frequencies comprising the four dominant spectral components of the conspecific mating calls. Following an initial control recording period for establishing the baseline AEP, a varying amount of 17-β-estradiol hemisuccinate dissolved in 50% ethanol was injected into the third ventricle via a 1-μL microsyringe and the AEP observed for a period of 2-5 hours. Then a second hormone injection (same amount as in the first injection) was made and the AEP observed for up to 5 hours.

Pilot studies have shown that when the time between injections was 5 hr, a dual injection of 0.4 μL would cause a significant increase in the recorded AEP. In contrast, injection dosages of 0.2 μL showed no change in the AEP. When this time was decreased to 2 hr, no increase in the AEP was observed even at dosages as high as 0.5 μL. The results of additional experimentation will be presented and discussed to conclusively establish this trend.

This study indicates that the effects of estrogen on the auditory responses of female frogs is both dosage and time dependent. This concept is not only important in analysis of seasonal behaviors of these frogs but may also lend to future theories of hormonal mechanisms in neural tissue.

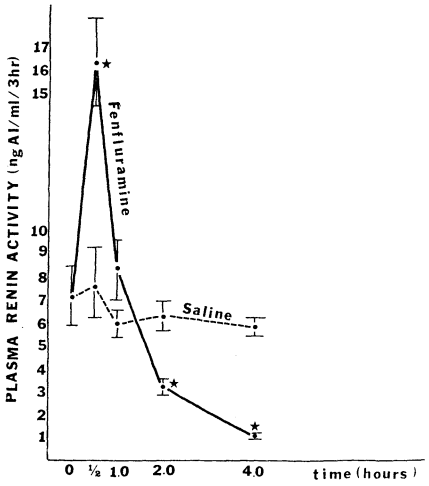
96.11 THE INFLUENCE OF OLFACTORY BULBECTOMY (ANOS), BLINDING (BLD) AND PINEALECTOMY (PX) ON 24-HOUR PLASMA PROLACTIN (PRL) LEVELS IN NORMAL AND NEONATALLY ANDROGENIZED (NA) FEMALE RATS. R.R. Gala, D.R. Pieper,* Jenn-Tser Pan,* W.P. Clarke* and D.J. Haisenleder.* (SPON: J.Benjamins). Dept. of Physiol., Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Female Sprague-Dawley rats were purchased pregnant and placed in a room with controlled lighting (14L-10D; lights on 0600 h) and temperature (24°C). The day of parturition was day 0 of life. On day 3 of life the litters were sexed, adjusted to ten pups/litter favoring females whenever possible and injected subcu with 1.25 mg testosterone propionate/0.1 ml of sesame oil. Normal female rat pups received no injection. Litters were weaned at 21 days of age. Between 22 and 26 days of age the following surgical procedures were performed on both NA and normal animals: Sham ANOS and PX, BLD + ANOS and BLD + ANOS + PX. At 10 wks of age all animals were vaginally smeared for a week to determine their estrous cycle pattern. At 15 wks of age the animals were ovariectomized, implanted with an atrial catheter and injected subcu with 0.5 mg of polyestradiol phosphate. Ovarian and oviductal weights were recorded. At 16 wks of age the animals were attached to a catheter extension at 0800 h and 0.3 ml of blood was obtained at 0900, 1100, 1400, 1700, 2000, 2300, 0200, 0500 and 0900 h replacing the fluid removed each time with sterile, warm (37°C) heparinized (50 units/ml) saline. Animals were sacrificed after sampling and uterine weights were recorded. Animals with incomplete surgery were removed from the experiment.

Sham NA animals had constant vaginal estrus while BLD + ANOS animals had constant vaginal diestrus. PX returned the BLD + ANOS animals to a constant estrous smear. Normal BLD + ANOS animals could be divided into two groups: one group had constant diestrus smear while the other group cycled normally. Ovarian and uterine wts were decreased by BLD + ANOS in both NA and in normal rats with diestrus smears. Normal BLD + ANOS animals that cycled had significantly larger organ weights. BLD + ANOS + PX animals had organ weights comparable to those of sham animals. Normal sham animals had an estrogen-induced afternoon PRL surge while NA sham animals had elevated morning basal values and an attenuated afternoon surge. Both normal and NA BLD + ANOS animals had a marked decrease in 24-hour plasma PRL levels and the surge exhibited a free-running rhythm. In BLD + ANOS + PX animals the plasma PRL levels were returned to values comparable to those of sham animals and PRL surges were again observed to have a free running rhythm. The results indicated that the NA animals respond similarly to BLD + ANOS as do normal animals and that PX eliminates the suppressing effect of BLD + ANOS on endocrine organ weights and plasma PRL levels.
(Supported in part by NIH Research Grant #HD 14671).

96.12 EFFECT OF THE SEROTONIN RELEASER FENFLURAMINE ON RENIN, PROLACTIN AND CORTICOSTERONE SECRETION. L.D. Van de Kar and Cynthia L. Bethea. Loyola Univ., Stritch Sch. of Med., Maywood, IL and Oregon Regional Primate Res. Ctr., Beaverton, OR.

The serotonin releasing drug fenfluramine caused a dose dependent stimulation of renin and prolactin secretion in male rats. The effect on renin, however was different in that the initial (30 minute) stimulatory effect was followed by an inhibiting effect after 2 hours. At 4 hours plasma renin activity was as low as the detection limit of the assay whereas plasma prolactin levels were still slightly higher than saline level. This inhibitory effect of fenfluramine on renin secretion is also dose dependent and is maximal at 10 mg/kg (i.p.). Pretreatment of the rats with the serotonin uptake inhibitor fluoxetine (10 mg/kg i.p.) caused an inhibition but not complete blockade of the effect of fenfluramine on renin secretion at 30 min. and 4 hours. These results suggest that the effects of fenfluramine on renin secretion are partly mediated by brain serotonin but that other mechanisms may also be involved.



(Supported by Loyola Potts # 842-32-000).

SYNAPSE ELIMINATION, COMPETITION, AND NEURON DEATH I

97.1 THE RELATIONSHIP OF CONVERGENCE AND POSTSYNAPTIC GEOMETRY IN MAMMALIAN SYMPATHETIC GANGLIA. Dale Purves and Jeff W. Lichtman, Washington University School of Medicine, Department of Physiology, St. Louis, MO. 63110.

Recent studies have shown a striking relationship between the geometrical complexity of individual parasympathetic ganglion cells and the number of axons that innervate each neuron in maturity. Thus in the rabbit ciliary ganglion, neurons that lack dendrites are innervated by a single preganglionic axon, whereas neurons that have dendrites are innervated by a number of different axons which increases in proportion to the complexity of the dendritic arbor (Purves and Hume, 1981). The purpose of the work reported here was to assess this relationship in a mammalian sympathetic ganglion, the superior cervical, in which the geometry of neurons is considerably more complex than in parasympathetic ganglia.

Superior cervical ganglia from adult guinea-pigs, hamsters and rats were dissected in continuity with the sympathetic chain and the upper thoracic ventral roots. Individual neurons in the the ganglia were impaled with microelectrodes in vitro and the average number of inputs received by each ganglion cell was determined by monitoring the increments in the postsynaptic response while individually stimulating different ventral roots. In separate experiments superior cervical neurons were impaled with microelectrodes filled with 5% horseradish peroxidase and labeled by iontophoresis. After a suitable diffusion time the morphology of the cells was demonstrated in whole mounts by application of the Hanker-Yates method.

Guinea-pig ganglion cells had, on average, 11-12 primary dendrites arising from the neuronal soma, whereas hamster ganglion cells had an average of only 6-7. In accord with this morpholgoical difference, guinea-pig neurons had an average of about 11 inputs measured electrophysiologically, whereas hamster ganglion cells had an average of only 6-7 different preganglionic inputs (about 100 neurons were examined in each species). Both the geometry and the number of inputs to rat superior cervical ganglion cells lay between the values in guinea-pig and hamster.

We conclude that, as in the parasympathetic nervous system, the convergence of preganglionic axons onto target neurons in sympathetic ganglia is closely tied to the geometry of the postsynaptic cells. The possible ways in which postsynaptic geometry might regulate the number of different axons that converge upon individual ganglion cells will be discussed.
Reference: Purves, D. and Hume, R.I. J. Neurosci. 1: 441-452.
Supported by NIH Grants NS 18629 and 11699.

97.2 REGIONAL INNERVATION OF RABBIT CILIARY GANGLION CELLS BY THE TERMINALS OF INDIVIDUAL PREGANGLIONIC AXONS. Cynthia J. Forehand and Dale Purves. Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO. 63110.

In the rabbit ciliary ganglion neurons with dendrites maintain inputs from several different axons during the period of synaptic rearrangement that occurs in early postnatal life. Neurons without dendrites, on the other hand, lose most of their initial inputs during this time and are innervated in maturity by the terminals of only one or two axons (Purves and Hume, 1981; Hume and Purves, 1981). We have explored the basis of this phenomenon by intracellularly staining both preganglionic axons and the neurons they innervate with horseradish peroxidase (HRP).

In general, the innervation of geometrically complex (multiply innervated) neurons by individual preganglionic axons is regional. That is, the synaptic contacts made by an axon onto such neurons are limited to a portion of the postsynaptic surface that usually includes the cell body and some, but not all, of the dendrites. Moreover, an individual preganglionic axon can follow a particular dendrite for 100 microns or more, elaborating boutons along its course while ignoring the surfaces of adjacent postsynaptic cells.

Electron microscopical examination of multiply innervated HRP-labeled ganglion cells shows that each of their several dendrites receives innervation. Therefore, the dendrites of a labeled ganglion cell which are not contacted by one labeled preganglionic axon are likely to be innervated by another input.

This regional innervation of target neurons is consistent with the view that dendrites allow multiple innervation to persist by providing relatively separate postsynaptic domains for individual preganglionic axons. Such regional innervation may mitigate competitive interactions between the several axons which initially innervate the same neuron.
References:
Hume and Purves (1981), Nature 293: 469-471.
Purves and Hume (1981), J. Neurosci. 1: 441-452.
This work was supported by NIH grants NS11699 and NS18628 and by the Muscular Dystrophy Association. C.J.F. was supported by training grant No. T32NS-07071-04.

97.3 DENDRITIC SPINES MAY REDUCE SYNAPTIC COMPETITION. Barry Horwitz. (SPON: John Rinzel). Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20205.

Evidence suggests that during development there is a competition among axons for synaptic sites, and as a result, only some of the innervating axons become stabilized. Electrical activity seems to play an important role in this competition. There are many who feel that synaptic competition also occurs during learning. Because dendritic spines are the primary locus of synapses for spiny neurons in the cerebral cortex, it is important to investigate the role spines play in synaptic competition.

I previously proposed (Soc. Neurosci. Abstr 8:709, 1982) an electrophoretic mechanism by which electrical activity at a synapse increases the probability for structural change near the synapse. The electrophoretic attenuation of a postsynaptic potential, as it spreads electrotonically from the site of synaptic activation, generates an intraneuronal electric field which permits the electrophoretic migration of charged molecules, both along the inner surface of the cell membrane and in the cytoplasm. These fields thus may locally organize the distribution of charged metabolites, allowing differential biochemical activity at different synaptic locations.

Here, this mechanism is applied to dendritic spines. Theoretical calculations are presented which show that synaptic activity at a spine head can establish large electrical fields along the spine, whereas synaptic activity at the base of the spine generates very small electrical fields in the spine. The thinner the spine, the stronger the fields. It is shown that the strong electric fields can be several times larger than those which have been used to produce measurable rearrangements of membrane-bound molecules (Poo, Ann. Rev. Biophys. Bioengn. 10: 245-276, 1982). If one assumes that these electric fields can lead to electrophoretic migration of charged metabolites necessary for either synaptic stabilization or enhancement, significant accumulation should occur within spines. Because the electric fields are small along the spine when electrical activity is generated elsewhere in the neuron, charged metabolites should not easily be drawn out of a spine. As a result, it is proposed that dendritic spines provide an anatomical means to reduce the competition between neighboring afferent inputs.

97.4 HISTOCHEMISTRY OF SINGLE MOTOR UNITS DURING SYNAPSE ELIMINATION IN RAT SOLEUS MUSCLE. W.J. Thompson, L. Sutton* and D.A. Riley. Dept. of Zoology, Univ. of Texas, Austin, TX 78712 and Dept. of Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Motor units in adult mammals each contain muscle fibers of a single histochemical type. How these uniform motor units arise during development is incompletely understood. We have investigated the fiber type composition of single motor units during the period of synapse elimination in neonatal rat soleus muscle.

Soleus undergoes a postnatal synapse elimination in which each soleus motor neuron reduces the number of fibers it innervates in the muscle. The time course of this process is such that up until postnatal day 10 every muscle fiber is polyneuronally innervated and it is only at day 16 that the process is nearly complete and most fibers are singly innervated (Brown et al., J. Physiol. 261: 387). As early as day 8, staining for myofibrillar ATPase after alkaline preincubation reveals that there are two distinct muscle fiber types present in soleus: ca. 55% of the fibers are darkly stained and 45% lightly stained (Riley, Exp. Neurol. 56: 400). The proportion of each fiber type does not change between 8 and 16 days. Thus, distinct fiber types are present in soleus prior to the establishment of the final pattern of innervation.

Using the technique of glycogen depletion we have identified muscle fibers belonging to a single motor unit. At the conclusion of most synapse elimination at 16 days, motor units are composed almost exclusively of one of the two fiber types. At 8 days, motor units contain, as expected, many more muscle fibers. However, even at 8 days these units are still greater than 70% homogeneous in fiber type. Thus, even at 8 days when motor neurons innervate at least two times their adult complement of fibers, this innervation is non-random, being largely confined to one of the two types of fibers present in the muscle. This non-random innervation might be present from the very earliest stages of muscle innervation or alternatively it could arise from an earlier random pattern by selective synapse loss.

97.5 THE PREVENTION OF SPINAL MOTONEURON DEATH IS ASSOCIATED WITH AN INCREASE IN ACETYLCHOLINE RECEPTORS IN SKELETAL MUSCLE. R. W. Oppenheim and S. Bursztajn. Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103 and Department of Neurology, Baylor University, Houston, TX 77030.

The suppression of neuromuscular transmission during the period of naturally-occurring motoneuron death in the chick embryo results in the survival of virtually all of the neurons that would have died. Previously, we suggested that the sequence of events leading to the prevention of motoneuron death may include an alteration in the number and/or distribution of acetylcholine receptors (AChR) in the target muscles (J. Comp. Neurol. 1979 v. 187). Chick embryos were injected once daily on embryonic days (E) 6-10 with 2.0 mg of curare. Embryos were sacrificed on either E11 or E16. Brachial (Br) and lumbo-sacral (L-S) spinal cords were processed for the determination of motoneuron numbers in the lateral motor columns (LMC); several different fore- and hindlimb muscles (slow and fast) were dissected from the same embryos. Muscles were washed with saline and fixed in 2% paraformaldehyde for 1 hr. After an overnight wash in phosphate buffered saline, muscles were incubated in 20 mM of ^{125}I-α-bungarotoxin (αBTX) (10-18 μCi/μg). For light microscopic autoradiography muscles were processed by two different procedures: 1) half were mechanically dissociated into individual muscle fibers and placed on glycerinated slides; 2) the other half were dehydrated, embedded in Epon and sectioned. Computer-aided analysis of the autoradiographs revealed a significant increase ($p < 0.05$) in the number of AChR clusters in all muscles from curare-treated embryos (E11 and E16). There was also a highly significant increase ($p < 0.001$) in the number of motoneurons in the LMC of Br and L-S cords from curare-treated embryos on E11 and E16. The number of AChR in muscles of control and curare-treated embryos per muscle wet weight and per mg of protein was also determined using ^{125}I-αBTX binding to isolated muscle membranes. All muscles from curare-treated embryos (E11 and E16) had significantly more ($p < 0.05$) AChR sites per mg of wet weight than those from control embryos; there were no differences in AChR number per mg of protein between muscles from curare and control embryos. These results indicate that the prevention of motoneuron death by neuromuscular blockade is associated with an increase in the number of AChR receptors in skeletal muscle. Experiments in progress will evaluate whether the increased number of AChR is also a necessary condition for the prevention of naturally-occurring motoneuron death.

97.6 THE FINE STRUCTURAL CHANGES IN FETAL PYRIFORM CORTEX FOLLOWING DEAFFERENTATION. H. Newman-Gage* and L.E. Westrum. Depts. of Biological Structure and Neurological Surgery, University of Washington, Seattle, WA 98195.

The ultrastructural patterns of degenerative change in fetal animals has been little studied in contrast to the well-described degenerative patterns in adult systems. Evidence from studies in pyriform cortex of early postnatal rats suggests a markedly different sequence of events in developing brain from that in the mature brain (Westrum, L.E., Anat. Embryol., 160:153-172, 1980). Clearly, these observations are of importance in understanding the increased capacity for plastic rearrangement in developing systems. The present study investigates deafferentation-induced degeneration during the period of initial synaptogenesis in fetal pyriform cortex. Earlier studies of normal development in this area show that synapse development begins on embryonic day 17 (E17 of 21.5) and is increasing rapidly by E19 (Newman, H. and Westrum, L.E., Neurosci., 8:866, 1982). Transuterine unilateral olfactory bulb lesions are made via needle aspiration in pups aged E17 or E19 with acute survival times of 6, 12, and 18 hours.

At 6 hour survival in both ages, the lateral olfactory tract (LOT) is characterized by marked swelling of growth cones and axons accompanied by disorganization and disappearance of organelles leaving a pale homogeneous cytoplasmic matrix. In addition two other forms of degenerating profiles are commonly seen: swollen profiles with flocculent aggregations of matrix and non-swollen profiles filled with a dense homogeneous granular cytoplasm. Normal appearing axons often contain organelles that appear to be degenerating. The superficial portion of layer I (Ia) contains expanded profiles similar to those described above. Many may be identified as growth cone processes. Occasionally, degenerating Type I synapses are seen in addition to apparently unaffected developing synapses of both Types I and II. Deep layer I (Ib) contains relatively fewer swollen profiles and many more developing synapses. From 12-18 hours all of these changes are progressively advanced. In addition, many profiles now have ruptured membranes and are being phagocytosed resulting in an exaggerated extracellular space. In conclusion the study shows the extreme rapidity of degeneration in fetal brain as compared to postnatal or adult tissue and describes the nature of degeneration in growing processes. (Supported in part by N.I.H. Grants No. NS 09678, NS 17111, and DE 04942. Lew is also an affiliate of the CDMRC.)

97.7 NEURONAL DEATH IN THE CHICK EMBRYO'S ISTHMO-OPTIC NUCLEUS (ION): SUSTAINING ROLE OF AFFERENTS FROM THE TECTUM. P.G.H. Clarke* (SPON: C. Marchand). Anatomy Institute, Lausanne University Medical School, 1001 Lausanne, Switzerland.

More than half the neurons in the ION are known to die between stage 38 (embryonic day 12 - E12) and stage 42 (E16). There is strong evidence that the survival of ION neurons during this period depends on their receiving some kind of maintenance from their axonal terminal field in the retina, but it has been less clear whether their afferents help to maintain them. The largest source of afferents to the ION is the ipsilateral optic tectum, and damaging the tectum is known to increase the ION neuronal death, but to interpret this result it was necessary to perform a series of control experiments, as reported below.

1) The lesions were restricted to the lateral and ventral parts of the tectum, since the isthmo-optic tract runs at its dorsomedial edge, but even with this precaution early (e.g. stage 25, E4) tectal lesions led to severe nonspecific effects, e.g. collapsed ventricles, failure of isthmo-optic fibres to reach the eye; but late (e.g. stage 38, E12) lesions avoided these difficulties. 2) The late lesions led to increased neuronal loss in the ipsilateral ION from stage 40 (E14) onwards. This might have been due to: 3) the shrinkage and subsequent misidentification of neurons, or to 4) migration of neurons out of the ION. I eliminated these possibilities: 3) all the ION neurons were labeled selectively with ^{3}H-thymidine applied at stage 27 (E5), whereas even in embryos fixed after the cell loss period the small (glial?) cells were unlabeled, so none of them can have been shrunken neurons. 4) Retrograde tracers were injected into an eye just before the period of cell loss, and the labeled neurons outside the ION were counted after short or long survival times; the numbers were similar in each case, so there cannot have been outward migration. 5A) The additional ION cell loss was probably not due to multistage retrograde influences, since the only retinal cells to degenerate in response to the tectal lesions were the retinal ganglion cells, and the amacrine sublayer (which contains the ION's target cells) was unaffected. 5B) Furthermore, ION neurons whose death was induced by a tectal lesion behaved differently from those which died following selective destruction of the amacrine cells by intraocularly injected kainic acid, since only in the latter case did the dying ION neurons phagocytoze extracellular HRP.

Hence, receiving tectal afferents is important for the survival of ION neurons during the latter half of the neuronal death period.

97.8 NATURALLY OCCURRING GANGLION CELL DEATH IN THE POSTNATAL CAT RETINA. H. E. Pearson, B. R. Payne and T. J. Cunningham. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

The visual system of the cat shows protracted development during postnatal life, as evidenced by continued synaptogenesis, maturation of physiological response properties and the existence of a lengthy critical period for the influence of visual experience. In other mammalian species, postnatal development has been shown to include naturally occurring neuron death and there is suggestive evidence that this phenomenon may contribute to the maturation of the retina in the cat (Stone et al. '82; Williams et al. '83). We have investigated this possibility directly by examining the retinae of kittens between the ages of 2 and 10 days postnatal for the presence of degenerating neurons.

Retinae were taken from kittens at ages 2,4,5,8 and 10 days postnatal. Following perfusion with aldehyde fixative, whole mounts of the retinae were made and drawn in outline. From each retina, a sample area was taken from the periphery of superior retina centered along the vertical meridian. The tissue samples were processed for plastic embedding.

Degenerating neurons were observed in all three cellular layers of the retina at all ages studied and early stages of neuron degeneration were characterized by marked condensation of nuclear chromatin. The morphology of degenerating cat retinal ganglion cells is therefore similar to that described for retinal ganglion cells of neonatal rats and embryonic chicks. Counts of degenerating profiles were made in the ganglion cell and inner nuclear layers from regularly spaced one-micron sections. Calculation of the surface areal density of degenerating neurons was made following measurement of the retinal area sampled. In the ganglion cell layer, we also expressed the numbers of degenerating neurons as a proportion of live cells present in that layer. The results from these analyses show that considerable numbers of neurons die naturally during early postnatal development in both the ganglion cell and inner nuclear layers of the cat retina. Thus far, we have observed maximal numbers of degenerating cells on postnatal days 5 and 8.

This documentation of naturally occurring neuron death by counts of actual degenerating cells shows that the final numbers of ganglion cells in the cat retina are determined after birth. Data obtained after target manipulation in cats and other species, including our previous results on ganglion cell death following visual cortex ablation, suggest that the survival of ganglion cells is dependent in part on the availability of target space. The regulation of neuron numbers in cat retina during normal postnatal development may depend, therefore, on interactions with appropriate targets. Supported by National Society to Prevent Blindness and NS16487 from NINCDS.

97.9 TRANSIENT CEREBRO-CEREBELLAR PROJECTIONS IN KITTENS. IS THEIR TRANSIENCE DUE TO NEURONAL DEATH? D.L. Tolbert* and W.M. Panneton. (SPON: L.C. Massopust). Murphy Neuroanatomy Research Laboratory, Depts. Anat. and Surg. (Neurosurg.), St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Direct projections from the cerebral cortex to the cerebellum have been observed in neonatal kittens. Cerebro-cerebellar axons arise from pyramidal neurons in layer V and project bilaterally to the internal granule cell layer of the cerebellar cortex as well as to the underlying deep nuclei. These projections are clearly transient in nature. Cerebro-cerebellar axons develop late in the 1st postnatal week, reach their maximum density in the 2nd postnatal week, and then quickly regress and disappear.

Since we have shown using fluorescent dye techniques that some cerebro-cerebellar axons are collaterals of cortical projections to the caudal medulla, experiments were carried out to determine if the transient nature of cerebro-cerebellar projections was due to selective elimination of collaterals or to neuronal death. Unilateral injections of 1% True Blue (TB) were made into the cerebellum of kittens 8-11 postnatal days old. At 12-85 postnatal days of age, injections of 1% Nuclear Yellow (NY) were made into the brain stem on the opposite side, after which the animals were sacrificed and then processed for fluorescent microscopy.

In all of these experiments only neurons labeled with NY, identifying a projection to the brain stem, were observed in the cerebral cortex. There were no cells labeled with TB present in the cortex. This lack of TB labeling of neurons in the cortex contrasted sharply to labeling of numerous neurons with TB in precerebellar nuclei of the brain stem which fluoresced brightly with this dye, even following long post-injection survival periods. Thus the absence of TB labeling in the cerebral cortex following injection into the cerebellum would indicate that cerebro-cerebellar projections arise from a population of neurons which subsequently degenerate with cortical maturation. Other possibilities which may account for the above findings are currently being explored. However, if the transient nature of cerebro-cerebellar projections is indeed due to neuronal death, then this pathway is different from those described in other studies using similar fluorescent dye techniques which showed that most of the loss of transient projections descending to medullary levels from the cortex was due to selective elimination of axon collaterals rather than to neuronal death (Stanfield, et al., Nature 298:317-373, 1982). (Partially supported by grant PHS 5 SO7 RR05388-20.)

97.10 CELL DEATH IN THE NEOCORTEX OF THE NORMAL MOUSE AND THE REELER MUTANT. A.L. Pearlman, J.O. Bautista, and J.P. Cohen. Depts. of Physiology and Neurology, Washington University School of Medicine, St. Louis, Missouri 63110.

Naturally occurring neuronal death is an important feature in the developmental history of many neural structures, but its role in the development of the mammalian neocortex has only recently been established. This study was undertaken to analyze the extent and time course of cell death in the neocortex of neonatal mice. In addition, we have studied cell death in the neocortex of the reeler mutant mouse, in which a developmental abnormality results in a disruption of the normal laminar pattern of neocortex and a striking anomaly in the relative positions of cortical neurons. Our prior electrophysiological and neuroanatomical studies and those of other laboratories have shown that the connections of reeler neocortex are established appropriately despite the abnormality in neuronal position. We wondered whether cell death might be responsible for eliminating neurons that were unsuccessful in establishing their proper connections in reeler with the result that only normally connected neurons remain. If that were the case, cell death ought to be present to a greater extent in the neocortex of reeler than it is in normal cortex.

We examined thionin stained serial sections (10μm) of the neocortex of newborn mice from the first postnatal day (P0) through P25, identifying dying cells by the presence of a very densely stained, homogeneous and sometimes fragmented nucleus. In normal neocortex cell death occurs primarily in the first 8-10 postnatal days, peaking on P5-6. It appears to spread in a broad wave from anterior to posterior and from medial to lateral, and to involve the deep cortical layers before the superficial layers. Cell death occurs in the same temporal and spatial pattern in the reeler neocortex, except that it involves neurons scattered at the depths of the cortex at the time when the most superficial neurons are dying in normal cortex. We also determined the ratio of dying to living cells from P0 through P25 in the sections that contained the posterior end of the corpus callosum and found no difference between reeler and normal. These findings indicate that cell death is an event that occurs at a particular time in the maturational history of selected cortical neurons, even though the cells may be abnormally positioned. The normal pattern of connections seen in the reeler neocortex can not be attributed to the elimination of an excess number of abnormally connected neurons by the process of cell death.

(Supported by NEI grant EY00621.)

97.11 STRAIN DIFFERENCES IN DOPAMINE NEURON NUMBER DEVELOP POSTNATALLY. H. Baker, S. Alden*, T.H. Joh and D.J. Reis. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

Differences in tyrosine hydroxylase (TH) activity in the substantia nigra-A10 area in adults of two inbred mouse strains are attributable to greater numbers of dopamine (DA) neurons in adult BALB/cJ than CBA/J mice. During development the strain differences in nigral TH activity are not present at birth but appear postnatally (Baker et al., Dev. Br. Res. 4: 157, 1982). Since neurogenesis of dopamine neurons is completed by the second week of embryogenesis, the postnatal appearance of the TH activity differences suggests that the differences in neuron number also appear after birth. To determine the age at which strain differences in neuron number could be detected within the substantia nigra, mice at 5, 10, 15 and 30 days postnatal age were perfused, the brains embedded in paraffin, and 10μ sections stained by the peroxidase anti-peroxidase technique using specific antibodies directed against tyrosine hydroxylase. Approximately 5-10 animals per strain per age were analyzed, but large numbers of mice were processed to insure that parameters such as sex, weight, and staining intensity were closely matched across strains.

In agreement with the biochemical findings at 5 days, there were no strain differences in the number of immunocytochemically demonstrable DA neurons. Similarly, by postnatal day 30, BALB/cJ mice had significantly greater numbers of immunocytochemically stained neurons than CBA/J mice, although the adult level of strain differences had not yet been reached. At postnatal day 10, however, although small but significant differences in TH activity exist, no differences in the number of DA neurons were detectable. At 15 days, there was a trend towards greater numbers of DA neurons in BALB/cJ than CBA/J mice; however, the data did not reach the significance level and contrasted with the biochemical findings where TH activity at 15 days is significantly greater in BALB/cJ mice. The disparity between the biochemical and neuron number data at this age may be attributable to the rapid changes in TH activity, and perhaps in neuron number, occurring at this time and resulting in a higher variability in the cell counts at this developmental stage. We conclude that the difference in DA neuron number in these two mouse strains develops postnatally. (Supported by NIMH Grant 33190.)

SENSORY SYSTEMS IN INVERTEBRATES II

98.1 AN INVESTIGATION OF THE ROLE OF MICROTUBULES IN SENSORY TRANSDUCTION BY AN INSECT MECHANORECEPTOR. J.E. Kuster* and A.S. French, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Microtubules are prominent cellular components of mechano-sensitive and chemo-sensitive sensilla associated with the insect cuticle, and they have been suggested to play several important roles in sensory transduction. Microtubule dissociating agents such as colchicine and vinblastine have been shown to interfere with transduction in these sensilla and this has been attributed to their anti-microtubule activity. We have now studied the effects of colchicine, vinblastine and lumicolchicine on the dynamic properties of sensory transduction in the cockroach femoral tactile spine and have simultaneously examined the effects of the drugs upon the ultrastructure of the sensory ending.

All of the agents reduced the sensitivity of the mechano-receptor and in the cases of colchicine and vinblastine this was accomplished without any effect on the dynamic properties of transduction. In the case of lumicolchicine there was a shift in the relative sensitivity toward rapid movements. All of the drugs also caused a reduction in the conduction velocity of the afferent action potentials in the axon arising from the sensillum.

The actions of the drugs on the ultrastructure of the sensory ending were quite disparate. In the case of colchicine there was a complete loss of microtubules in the tubular body and sensory dendrite before the cessation of mechanical sensitivity. Vinblastine was less effective in eliminating the microtubules although more effective in interfering with transduction. Lumicolchicine, which is produced by ultra-violet irradiation of colchicine, is known to be less effective than colchicine in dissociating microtubules. Its major morphological effect was a significant disruption of the dendritic membrane.

These results show that the microtubules are not essential for mechanotransduction in this receptor and that the effects of the chemical agents used are more likely to arise from their actions on the cell membrane than upon the microtubules.

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98.2 SENSORY INTERNEURONS IN THE LOCUST FLIGHT CONTROL SYSTEM: H. Reichert and C. H. F. Rowell*. Department of Zoology, University of Basel, Basel, Switzerland.

We study the integration of descending sensory information with the rhythm generating elements of the flight motor in the locust. We record with Lucifer filled electrodes from the neuropil of the thoracic ganglia during fictive flight. The effects of ocelli, windhair and compound eyes on impaled neurons are determined as are the interconnections among the neurons. These sense organs in nature report deviations from the flight path and elicit correctional steering maneuvers.

Second order interneurons convey processed ocellar information to the thoracic ganglia. Several of these phasic off units are also excited by the wind-hairs. These neurons make only weak and variable direct connections to flight motoneurons. However, they elicit large, short latency PSPs in at least 2 different populations of thoracic interneurons. One of these populations receives similar synaptic input from stimulation of any of the ocelli. It therefore seems unlikely, that they play a role in correctional steering during flight. During flight no phasic modulation of these interneurons occurs.

A second population of thoracic interneurons receives asymmetric ocellar input, being excited by stimulation of one lateral ocellus or of one lateral and the medial ocellus and often receiving inhibition from stimulation of the remaining lateral ocellus. The response properties of these neurons are appropriate for a role in roll/pitch correctional steering. Some of these interneurons make strong, short latency connections to flight motoneurons. Additionally, they are phasically modulated at flight frequency during flight. Depending on phase relationships, EPSPs caused in these neurons by ocellar stimulation are either augmented or shunted by this subthreshold modulation. Descending sensory information thus seems to be gated by the flight rhythm generator and can influence flight motoneurons only in specific, presumably appropriate, phases of the flight cycle.

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98.3 THERMOGRAPHIC IMAGING OF THE SENSORY DERMATOME. S. Uematsu* (SPON: D. ZEE). Johns Hopkins Hospital, School of Medicine, Baltimore, MD 21205.

Sensory examination of the skin is based on the patient's subjective expression of sensation; therefore, results of the examination are difficult to objectify. However, it is generally believed that cutaneous temperature alters in the vicinity of the peripheral nerve impairment. If this is true, then thermographic imaging of the sensory dermatome may be of practical clinical use.

In this study, all measurements were made using a telethermograph with a built-in computer for data compilation and analysis. Cutaneous temperature was measured in 37 segmental areas of the body on 32 controls (12 to 65 years old) and in selected areas on 38 patients with peripheral nerve impairment. Average temperatures were obtained in corresponding areas of interest on each side of the body and then compared. In normal controls, the average temperature differences between sides of the body were extremely small: on the trunk, the difference was 0.17 ± 0.042°C; on the extremities, 0.20 ± 0.073°C; and on fingers and toes, 0.45 ± 0.129°C. The temperature difference between the median and ulnar side was 0.37 ± 0.28°C in the forearm and the palm. The difference between the fifth lumbar and first sacral dermatome was 0.53 ± 0.42°C. In contrast, in nerve-damaged patients, skin temperature on the side with impaired nerve was 1.63 ± 1.002°C different from the corresponding normal site on the body. The difference is approximately ten times that of the normal control ($P<0.0001$).

Outlines of the sensory dermatome obtained by pin pricks, neurological examinations, and thermogram matched very well in all 38 cases. In cases of completely sectioned nerve (neuronotmesis), the anesthetic area was 2 to 4°C warmer than surrounding skin. In irritative lesion (e.g. lumbar disc herniation), the hypesthetic area was colder then surrounding area.

These results indicate that computerized color telethermography makes thermographic imaging of the sensory dermatome practical for use in clinical examination.

98.4 RESPONSES OF VIBRATION RECEPTORS IN THE MEDICINAL LEECH TO NEAR-FIELD STIMULATION. P.D. Brodfuehrer and W.O. Friesen. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Water-wave stimulation can elicit swimming in intact medicinal leeches. This same stimulus will also initiate swimming activity in dissected leeches via activation of segmental vibration receptors (SMRs), provided that serotonin is added to the saline (Brodfuehrer and Friesen, Neurosci. Abstr. 8:529, 1982). To study the responses of the SMRs to vibratory stimulation, we developed a small vibrating probe that could stimulate individual sensilla in a precisely controlled manner.

Suction electrode recordings from segmental dorsal posterior (DP) nerves of isolated body wall flaps give the following results. (1) A single vibratory probe stimulus near a sensillum evokes a long duration (8-12 ms), graded compound action potential (CAP). As the stimulus intensity (i.e. probe displacement) increases, the amplitude of this SMR response increases and the latency decreases. (2) The conduction velocity of the sensory units in the DP nerve is 0.34 m/s (S.D.=0.09, N=9). This value is approximately one-third of the conduction velocity of touch cell axons. (3) The SMR response recorded from the DP nerve is not eliminated by high Mg^{++} (40 mM) saline. However, the neuronal responses evoked in nerve cord connectives by near-field stimulation are reversibly eliminated by high Mg^{++} saline. Thus no chemical synapse occurs between the peripherally situated SMRs and the segmental ganglia, but a synapse is interposed between the SMRs and intersegmental neurons. (4) At stimulation frequencies of less than 1 Hz the SMR response is constant, while at frequencies greater than 25 Hz the response exhibits a reduced amplitude and an increased duration. At intermediate frequencies only the shape of the CAP fluctuates. Thus at high stimulus frequencies not all of the sensory units are activated by each stimulus, perhaps due to receptor adaptation. (5) Finally, the SMR response can be elicited with sinusoidal stimulation at frequencies as low as 1 Hz. This suggests that the SMRs could be activated by the swimming movements of the leech.

In conclusion, the SMRs are able to faithfully follow near-field stimulation at rates up to 10 Hz. Water-wave stimulation also provides vibratory stimulation at about 10 Hz (Friesen, J. exp. Biol., 92:255-275, 1981). Thus the SMRs are probably not adapting during water-wave stimulation that elicits swimming. Furthermore, the SMRs could provide sensory feedback to modulate the expression of the swimming movements. Supported by NSF grant BNS 81-0243.

98.5 SENSORY INPUT TO MOTOR NEURONS AND INTERNEURONS GENERATING THE HEARTBEAT RHYTHM OF THE LEECH. R. Davis and J.G. Nicholls, Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The rhythmical activity of the leech heart is produced by the activity of endogenously active interneurons (HN cells) within the CNS that periodically inhibit the motor neurons (HE cells) supplying the cardiac musculature (Thompson, W.J. and Stent, G.S., J. Comp. Physiol. 111: 309-333, 1976). The connections that make up this circuit have been studied in detail but no input from the periphery has as yet been described. Presumably such sensory information could allow the circulation to respond to needs of the animal under various circumstances.

In the present experiments the activity of motor neurons and interneurons has been measured with intracellular microelectrodes while peripheral nerves were stimulated electrically. In ganglia towards the head (that contain HN interneurons) stimulation of the peripheral nerve roots leads to ipsp's in the interneurons and large epsp's in the motor neurons. In HE motor cells of midbody segments, peripheral root stimulation leads to both inhibitory and excitatory synaptic potentials. The time course, amplitude and sign of the response depend on the particular peripheral root that is stimulated.

The specific modalities and receptive fields of the sensory inputs have not yet been identified. Nevertheless, it is clear that mechanical stimuli applied to regions of the periphery can also evoke synaptic potentials in the motor neurons that dramatically alter their pattern of firing.

98.6 DENDRITIC STRUCTURE PREDICTS THE RECEPTIVE FIELDS OF IDENTIFIED INTERNEURONS G.A. Jacobs and R.K. Murphey Dept. of Biology, SUNY Albany, Albany, New York, 12222

The response properties of a neuron are a function of the inputs it receives and how those inputs are distributed over its dendritic surface. We are studying how the structure of wind sensitive interneurons (INs) in crickets determines their receptive fields. Different INs respond preferentially to wind stimuli from different directions around the animal's body. Wind direction is encoded by four groups of afferents on each cercus which respond to four different wind directions. Each group of afferents arborizes in a separate neuropil region of the hemiganglion it innervates (Bacon and Murphey, this vol.). Thus an IN could obtain its receptive field by virtue of the position of its dendrites with respect to distinct populations of afferents.

In order to assess the role of structure in determining receptive fields we have studied an identified neuron with dendrites in three discrete regions of neuropil representing three different wind directions. This neuron is maximally sensitive to wind from one side of the animals body yet it has dendrites on both sides of the ganglion. To break this response down into its component parts we first determined the receptive field for the neuron on one side of the ganglion by blocking all the afferents which terminate on the other side. We have shown that the response of the neuron is oriented differently to inputs from the two different sides.

In order to assess these various inputs we created patches of hairs of known directional sensitivity. If we chose a group of hairs known to overlap a particular dendrite the cell was now tuned exclusively to this direction. In order to determine how the neuron sums inputs from various directions we are creating patches of hairs of equal numbers known to overlap two different dendrites and then examining the response to each patch independently and finally to both patches simultaneously. Thus the directional preference of this neuron can be thought of as the sum of three different wind directions each of which is detected by a separate dendritic region.

These experiments do not provide a definitive test concerning the location of the various inputs on a given cell. In order to precisely localize the inputs in a more dynamic way we are trying to ablate a specific dendrite and determine the effect of its absence on the receptive field of this neuron. In collaboration with Dr. J.P. Miller we will try to selectively photoinactivate a specific dendrite on a living neuron to answer this question. Supported by NSF Grant # BNS 81 19799

98.7 ALTERATIONS IN THE VISUAL SYSTEM OF A "REDUCED EYE" MUTANT GRASSHOPPER. J.D. Steeves, D.J. Emery* and K.A. Bell*. Dept. of Zoology, UBC, Vancouver, B.C. V6T 2A9.

The "reduced eye" mutant grasshopper of the species Melanoplus sanguinipes is characterized by small flat compound eyes, which lack facets (Chapco, 1980, Can. J. Genet. Cytol. 22:439-441). Our examination of this mutant indicates normal motor capabilities in terms of walking, jumping, flying and feeding. Escape responses normally elicited by visual and auditory inputs are absent, however jumping can be evoked with tactile stimulation.

Scanning electron micrographs reveal that the "reduced eye" mutant does not have lateral ocelli, and the median ocellus appears only as a small convex thickening of the cuticle in the center of the frons. Extracellular recordings from the ventral nerve cord, as well as electroretinograms, indicate that neither the compound eyes nor the dorsal ocelli function in this mutant. Attempted cobalt backfills of the compound eyes and median ocellus, in addition to gross dissections of the "reduced eye" mutant brain, show that the compound eyes and median ocellus fail to innervate the CNS. The optic lobes are substantially reduced in size and there is no evidence of any ocellar nerves.

Histological examination confirms these findings, and further reveals the incomplete differentiation of the retina in the compound eyes and the accompanying absence of the underlying optic lamina. The optic medulla and lobula are present, however the medulla lacks the characteristic neuronal projection patterns found in wild type animals. Similarly, there are only a few poorly differentiated cells underlying the cuticlar thickening of the "vestigial" median ocellus in the mutant. The development of the lateral ocelli is completely suppressed.

It is hoped that this "reduced eye" mutant grasshopper will be useful in the analysis of behaviors which are usually dependent on visual input. (Supported by NSERC)

98.8 NARROW YELLOW ELECTRORETINOGRAPHIC SPECTRAL SENSITIVITIES AND IN SITU MSP OF A VISUAL PIGMENT AND OF A RED SCREENING PIGMENT FROM THE RHABDOMERIC REGION IN TWILIGHT ACTIVE FIREFLIES. Abner B. Lall*, G.K.Strother*, H.H.Seliger*, J.E.Lloyd*and W.H.Biggley* (SPON: B. L. Partridge). McCollum-Pratt Institute and Dept. of Biology, The Johns Hopkins University, Baltimore.MD. 21218.

North American fireflies which restrict their activity periods to twilight possess narrow yellow visual spectral sensitivity, VSS, (1/2 bandwidth about 50 nm) as determined by electroretinography. The peaks of the VSS of Photinus scintillans, macdermoti, marginellus, pyralis and collustrans were at 580 nm, 570 nm, 565 nm, 565 nm amd 560 nm respectively and in each case VSS peak coincided with the peak of the species bioluminescence emission at 578 nm, 570 nm, 565 nm, 564 nm, and 560 nm respectively. We now report, the biochemical basis for the observed VSS. In situ MSP studies of the rhabdomeric slices from P. pyralis and scintillans compound eyes reveal the presence of a visual pigment absorbing in the green. The peaks of the difference spectra were at 550 nm. Besides the photopigment, a magenta red screening pigment was also located in the rhabdomeric slices. The absorption spectrum of this screening pigment had a narrow peak at 515 nm (1/2 bandwidth = 60nm) with an extremely sharp drop on the long wavelength side. These data confirm our hypotheses that the presence of unsually narrow yellow VSS obtained in P.pyralis & scintillans (Seliger, H. H., Photochem. Photobiol. 36, 681, 1982) can be accounted for by the presence of V550 and the red screening pigment. It has been suggested that the selection for narrow yellow VSS in the compound eyes and concomitant yellow bioluminescence would maximize signal to noise (foliage reflected sunlight) ratios in twilight acti ve fireflies (Lall,A.B.,Science. 210:560, 1980). Spectroscopic measurements made in the insects natural habitat confirm that foliage reflected ambient sunlight during twilight peaks in the green. It is hypothesized that the red screening pigment partially absorbs this light causing a reduction in the background interference during twilight, thereby maximizing signal to noise ratios and subsequently facilitate the detection of conspecific yellow bioluminescence. Support by NSF grant # BNS80-12215

98.9 POSSIBLE TRICHROMACY IN A CRUSTACEAN VISUAL SYSTEM. Robert Schehr* and E. Macagno. Department of Biological Sciences, Columbia University, New York, N.Y. 10027

We have examined photoreceptors in the single compound eye of Daphnia magna with intracellular electrodes; some of these cells were injected with horseradish peroxidase.

Results of recordings in many of the 22 ommatidia that comprise the bilaterally symmetric compound eye show that there are, probably, three major classes of photoreceptor spectral sensitivities with maxima at about 450, 510 and 590nm. There is however considerable spread in the distribution of maximum sensitivities. These sensitivities were calculated from measurements of the maximum depolarizing displacement of the membrane potential after a stepwise change in illumination. If other features of the response waveform were used the spectral sensitivities were different in some cases, suggesting the possibility of interactions between photoreceptors of different spectral sensitivity type.

We also classed cells at a given location in the eye according to spectral sensitivity. Each ommatidium has eight photoreceptors and they may be numbered according to their position around the rhabdom (Flaster et al, in Neuronal Development, N. Spitzer, ed., 1982). By examining serial one micron and occasional thin sections made perpendicular to the optical axis of an ommatidium we could identify cells filled with horseradish peroxidase reaction product with respect to position around the rhabdom and identify the ommatidium by virtue of the arrangement of lenses in the eye. It is our impression that all three classes of spectral sensitivities occur in all ommatidia, although the sample is small. Whenever cells in the same relative position in different ommatidia were filled they were of the same type.

The synaptic connections that these identified photoreceptors make with the cells of the laminar cartridges (Macagno et al. PNAS 70: 57, 1973) show that channels containing only information from a single spectral sensitivity class are maintained at this level.

98.10 DROSOPHILA MUTATION THAT DISRUPTS THE RHABDOMERE STRUCTURE AND DISPLAYS A DOSAGE EFFECT ON A RETINA SPECIFIC POLYPEPTIDE. Hiroyuki Matsumoto*, Quentin Pye*, Kunio Isono*, and W. L. Pak. Dept. of Biol. Sciences, Purdue Univ., W. Lafayette, IN 47907.

The ninaC locus, mapping at 24 map units on the second chromosome, was identified by mutations that decrease the amplitude of the prolonged depolarizing afterpotential (PDA) and reduce the rhodopsin content. Cytogenetic localization placed the gene between 26F and 28B. Ultrastructural studies of several allelic mutants of ninaC revealed that the rhabdomeres of both the peripheral and central photoreceptors are markedly decreased in size. To determine if there are any alterations in photoreceptor layer specific proteins in ninaC mutants, SDS polyacrylamide gel electrophoresis was carried out on the ninaC mutants and on wild type.

Polypeptides specific for the photoreceptor layer were identified by comparing electrophoretic patterns obtained from (1) compound eye samples, (2) samples of heads with the eyes removed, (3) samples of compound eyes with the corneas removed, and (4) cornea samples. Results showed that five polypeptide bands, labeled B1 to B5, ranging in molecular weights from 170K (B1) to 120K (B5) are specific for the photoreceptor layer. This layer contains primarily photoreceptor cell bodies and pigment cells and does not contain photoreceptor synapses. Specificity of these polypeptides for the photoreceptor layer suggests that they are neither the components of enzyme systems involved in general metabolism nor the elements of synaptic functions.

Results of the electrophoretic analysis showed that the B1 polypeptide is missing in all mutants homozygous for any of the nine ninaC alleles. The other four polypeptides, B2 - B5, were also present in these ninaC homozygotes in amounts different from that in wild type. The amounts present depended on the particular ninaC allele carried by the mutant. A deficiency uncovering the ninaC region was constructed to carry out the following analysis. Flies carrying 0 and 1 copy of the normal ninaC gene showed, respectively, about 0 and 50% of the amount of B1 in wild-type flies, suggesting that the ninaC locus codes for the structural gene for the polypeptide B1. The results to date are consistent with the working hypothesis that the ninaC gene encodes the 170K B1 polypeptide and that this polypeptide may be involved in the formation and/or maintenance of the rhabdomeric membrane structure.

98.11 CIRCADIAN CLOCK IN LIMULUS BRAIN GENERATES SYNCHRONOUS EFFERENT OPTIC NERVE ACTIVITY TO ALL EYES. Leonard Kass, Leslie E. Eisele, and Robert B. Barlow, Jr. Institute for Sensory Research, Syracuse University, Syracuse, New York 13210.

A circadian clock in Limulus brain in situ generates efferent lateral optic nerve (LON) activity at night, leading to numerous changes in retinal structure and function (Science 197:86-89, 1977; Science 210:1037-1039, 1980). We developed an excised brain preparation to further characterize the efferent and afferent connections to this circadian clock. The Limulus brain and optic nerves were removed by dissection and were placed in a temperature-controlled chamber filled with organ culture solution. Glass suction electrodes were positioned along the various desheathed optic nerve stumps, allowing us to record from or to electrically stimulate the nerves.

Efferent optic nerve activity can be recorded for up to three days in the excised brain of Limulus. Like efferent activity recorded in situ, the discrete bursts of efferent activity recorded from the excised brain were first detected in the early evening, continued throughout the night, and stopped at dawn. The general rate of bursting activity was greater at night than during the day. The bursts of efferent activity recorded from one LON were synchronous with those recorded from the opposite LON, the median optic nerve (MON), and the ventral eye nerve. Electrically stimulating the MON induced efferent activity in both LONs. Illuminating the excised brain can inhibit the efferent activity. We have not detected efferent activity in other nerve trunks (non-optic) leading to the protocerebrum; nor have we succeeded in modulating the efferent activity in LONs by electrically stimulating other nerves leading to the protocerebrum. Thus, with regard to sensory systems, the circadian clock appears to primarily mediate functional changes in the visual system that occur from day to night.

Bisecting the isolated protocerebrum desynchronizes the bursts of efferent activity in opposite LONs, indicating that efferent cell bodies are located in both sides of the protocerebrum. Further lesions of the brain suggest that the location of the cell bodies may be limited to the lamina or medulla. Various studies are currently underway to identify the precise location of the efferent cell bodies and the associated circadian clock.

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98.12 RECEPTOR POTENTIALS OF IDENTIFIED MECHANORECEPTOR NEURONS IN THE COCKROACH LEG BEFORE AND AFTER THE FINAL MOLT. K.M.Chapman, J.B.Zuckerman* and D.-W.Shin*. Neuroscience Sect., Brown Univ., Providence, RI 02912.

In the final days before the immature cockroach molts, the bipolar neuron of each proprioceptive campaniform sensillum on the legs remains attached to the old exoskeleton, but its dendritic sensory process elongates roughly tenfold as molting fluid erodes the old cuticle and separates the epithelial cell layer from it (Moran et al, 1976). Behavior suggests that sensory function persists until the moment the outer sensory process is shed with the exoskeleton, and resumes immediately afterward. To study this process electrophysiologically, we have recorded receptor potentials noninvasively from the same sensillum in individual cockroaches (Blaberus discoidalis) during the weeks before, and shortly after their final molt. As in previous work on amputated legs, individual campaniform sensilla of Group 6 at the knee were stimulated with a fine tungsten compliance probe (Chapman, Mosinger & Duckrow, 1979) and transcuticular receptor potentials were recorded from the sensillar cap with a gel-filled pipet (Mann & Chapman, 1975; Swearingen & Chapman, in prep.). Trans-cap resistances were high (> 1 Gohm) on initial contact, but after gentle stimulation with 10-100 uN punctate force, fell to 50-500 Mohm, as in amputated legs, and remained so during subsequent weeks of observation. Until several days before the molt, receptor potentials were virtually identical to those found in amputated legs, with typically: (1) 0 to -20 mV "standing potential" in the unstimulated state, unlike the +20 to +70 mV found in many insect sensilla (Thurm & Wessel, 1979); (2) mechanically modulated negative-going 1-10 mV receptor potentials, with transfer functions on the 0.1-130 Hz range which remained reproducible for weeks; (3) evoked spikes superimposed on the receptor potential. About a week before molting, positive-going receptor potential modulations became common, with "irregular" changes in the transfer functions. In the final 2-3 days, receptor potentials became too small to measure, but modulated spike trains remained. Within a week the typical non-molting pattern reappeared. These findings suggest that the "irregular" transfer functions reflect elongation of the dendritic cable until molting fluid finally attenuates the generator current immediately before the molt. We are grateful for a gift from the Grass Foundation.

98.13 IDENTIFIED INTERNEURONS STEER FLIGHT IN THE DRAGONFLY. R. Olberg, Dept. of Biological Sciences, Union College, Schenectady, NY 12308

In the ventral nerve cord of the dragonfly, 7 giant interneurons have been identified, each of which produces asymmetrical wing movements when stimulated intracellularly. The interneurons fall into two distinct classes, (1) rotation-sensitive and (2) object-movement sensitive. Lucifer Yellow and/or cobalt sulfide staining revealed that the cell bodies and dendritic arborizations of rotation sensitive interneurons are located in the prothoracic ganglion. Here they receive inputs from the compound eyes, antennae, wind-sensitive hair fields on the head and prothorax, cervical proprioceptors, and in some cases the ocelli and/or abdomen. Each input is directionally selective, and all code for rotation in a given direction within the same plane in space. The wing movements produced by individual rotation-sensitive neurons are small, but appear to be components of reflex movements elicited by the rotation of the visual surround, rotation of the head, or directed puffs of air. Object-movement sensitive neurons are purely visual and descend from the brain. They are directionally selective for small-object (8° or less, visual angle) movement within their receptive fields. Intracellular stimulation revealed that they produce much larger wing movements. The two groups of interneurons appear to exert potent influence on flight direction, at least during gliding flight.

99.1 THE MAJOR PRODUCTS OF THE DYNORPHIN GENE ARE κ OPIOID LIGANDS. I.F. James*, W. Fischli and A. Goldstein. Addiction Research Foundation, 701 Welch Rd., Palo Alto, CA 94304.

The precursor for dynorphin contains at least five active opioid peptides, dynorphin A, dynorphin A-(1-8), dynorphin B, α-neo-endorphin and β-neo-endorphin, all of which have been found in mammalian tissue. There is strong evidence that dynorphin A is highly selective for κ opioid receptors; one of three well known types of opioid receptor. We have now assessed opioid receptor selectivity of the other main products from the dynorphin precursor using the guinea pig ileum myenteric plexus-longitudinal muscle preparation (GPI). In GPI there are two types of opioid receptor, μ and κ, which are distinguished by their sensitivity to the antagonist naloxone. Agonists acting at κ receptors are 10 times less sensitive to naloxone than those acting at μ receptors.

Dynorphin A, dynorphin B and α-neo-endorphin were all equally insensitive to naloxone (K_e values of 29±3, 33±9 and 22±3, respectively), compared to the prototypical μ agonist normorphine (K_e=3.1±0.3). Furthermore, all three of these peptides protected κ but not μ receptors from inactivation by the opioid site directed alkylating agent β-chlornaltrexamine, suggesting that they act exclusively through κ receptors in this tissue. Dynorphin A-(1-8) and β-neo-endorphin (K_e values 15±1.5 and 14±1, respectively) were more sensitive to naloxone than dynorphin A but less sensitive than normorphine. Values of K_e for these peptides were still intermediate between values for dynorphin A and normorphine when peptidase inhibitors were present in the bathing medium, indicating that the intermediate naloxone sensitivity was not caused by degradation to products with different receptor selectivity from the parent compounds. We propose therefore that dynorphin A-(1-8) and β-neo-endorphin act predominantly at κ receptors but partially at μ receptors in GPI.

Members of two different families of endogenous opioid peptides have now been associated with different opioid receptor types -- dynorphins with κ receptors and enkephalins with δ receptors. Perhaps each opioid gene, coding for a family of opioid peptides constitutes a cognate pair with a single receptor type. Hence in vivo dynorphins may always act at κ receptors and enkephalins always at δ receptors. It is not known what endogenous ligand may be selective for μ receptors.

99.2 MULTIPLE OPIOID RECEPTORS IN THE GUINEA PIG ENTERIC NERVOUS SYSTEM: UNMASKING OF RECEPTOR SUBTYPES, A.R. Gintzler* and D. Hyde* (Spon: F. Kao). Dept. of Biochemistry, Downstate Med. Ctr., Brooklyn, New York 11203.

The presence of δ opioid receptors have been demonstrated in the myenteric plexus of the guinea pig by radioligand binding experiments but their functional significance is not clear. The naloxone K_e for the δ preferring agonist D-ala^2-D-leu^5-enkephalin (DADLE) indicates that the u and not the δ receptor mediates the inhibitory action of these peptides on responses to 0.1 Hz electrical stimulation. However, the demonstration in the guinea pig ileum of differential tolerance to normorphine and DADLE suggests that myenteric δ receptors mediate at least a portion of this effect in tolerant/dependent preparations (Gintzler and Scalisi, Brain Research 238:254-259, 1982). The present experiments were performed in order to clarify this apparent discrepancy and to demonstrate the presence of functional δ receptors in naive preparations.

The population(s) of receptor mediating responses of the naive gut (0.1 Hz contractions) to u and δ agonists were compared on the basis of their reactivity with the nonequilibrium opioid antagonists, B-chlornaltrexamine (B-CNA) and B-funaltrexamine (B-FNA), (q" value). The "q" value for normorphine and DADLE calculated following pretreatment with B-CNA was 0.12 ± 0.02 and 0.43 ± 0.06 respectively. After incubation with B-FNA, (selective for u receptors), the "q" value for normorphine was 0.24+0.046 while that for the highly selective δ agonist Tyr-D-Ser-Gly-Phe-Leu-Thr (DSLET) was 0.1 ± 0.037. These results indicate that the above agonists do not act via identical populations of receptor in the naive ileum despite the similarity of their naloxone K_e value (1-2nM). In addition, the naloxone K_e for DSLET was determined before and after the u subtype was eliminated by pretreatment with B-FNA. In untreated ilea, the naloxone K_e for DSLET indicated an action on u receptors. However, following destruction of the u subtype, the naloxone K_e for this agonist increased approximately 10 fold approximating the value observed in the mouse vas deferens.

These results indicate that the delta preferring agonists acts via two populations of myenteric opioid receptor to inhibit the 0.1 Hz contraction height. Following the elimination of the u opioid receptor the DSLET-induced inhibition that remains results from the occupation of another opioid receptor subtype. The marked lack of cross-reactivity between DSLET and K receptors would suggest that this subclass is of the δ type. These data also indicate that naloxone K_e values by themselves may not be sufficient criteria for concluding that a particular receptor subtype is not present in a given tissue or not associated with a particular function. Supported by NIDA Grant DA02893.

99.3 DIFFERENTIAL PROPERTIES OF MU RECEPTORS IN THE GUINEA-PIG ILEUM (GPI) AND MOUSE VAS DEFERENS (MVD) PREPARATIONS. Susan J. Ward*. (SPON: T. Truscott). Sterling-Winthrop Research Institute, Rensselaer, NY 12144.

Incubation of the GPI with the highly selective non-equilibrium antagonist B-funaltrexamine (B-FNA), inactivates those mu receptors at which normorphine interacts, such that, following pretreatment with B-FNA (500 nM), the pA_2 value for the interaction of normorphine (NM) and naloxone is similar to that for the interaction of ethylketazocine and naloxone, but different from that for the interaction of NM and naloxone in control preparations incubated with nalorphine (500 nM). Similarly, those mu receptors at which morphine and methadone interact are also blocked by B-FNA, and the pA_2 values for the interactions of these agonists with naloxone are lowered by pretreatment of tissues with B-FNA. In contrast, the pA_2 values for the interactions of naloxone with D-Ala^{2}D-Leu^5-enkephalin, D-Ala^{2}met-enkephalin, D-Ala^{2}met-enkephalinamide, D-Ser^2, Thr^6,Leu-enkephalin and Tyr-D-Ala-Gly-MePhe-NH-CH_2-CH_2-N(O)$(CH_3)_2$ (Rx 78, 3030) were not altered by pretreatment of tissues with B-FNA, despite the fact that B-FNA did antagonise the agonist actions of these compounds.

In contrast to the effects of B-FNA on the GPI, on the MVD, pretreatment of tissues with B-FNA resulted in the lowering of pA_2 values for the interactions of naloxone with both NM-like and Rx 78,3030-like agonists.

Similar experiments using the non-equilibrium mu antagonist naloxonazine instead of B-FNA (0.2 or 1 uM) demonstrated that the pA_2 values for the interactions of naloxone with NM-like and Rx 78,3030-like were lowered by pretreatment with naloxonazine in both the GPI and the MVD.

In experiments using the non-equilibrium antagonist B-chlornaltrexamine (B-CNA), B-CNA was a more potent non-equilibrium antagonist of NM than of Rx 78,3030 on the GPI, and 'q', the proportion of receptors not occupied by B-CNA (100 nM) was greater when Rx 78,3030 was the agonist in the GPI.

These experiments suggest that NM-like and Rx 78,30-30-like agonists interact with mu receptors in the GPI in a different manner, or that these two groups of compounds interact with different populations of receptors that are equally sensitive to naloxone in the GPI.

99.4 OPIATE RECEPTOR UPREGULATION IN EXPLANTS OF SPINAL CORD-DORSAL ROOT GANGLIA. A. Tempel, S. Crain, E.J. Simon and R.S. Zukin Department of Biochemistry and Neuroscience, Albert Einstein Coll. Med., Bronx, N.Y. 10461, (E.J.S.) Dept. of Psychiatry, New York University Medical Center, New York, NY 10016

High-affinity stereospecific opiate receptors have been demonstrated in explant cultures of fetal mouse spinal cord with attached dorsal root ganglia (DRG). We have used this system to examine the mechanisms underlying the regulation of opiate receptors in response to chronic antagonists. Cultures were grown in standard growth medium for two weeks, and then in this medium containing naloxone (10 μM) for an additional 7 days. For opiate binding assays, 7-9 cultures were pooled, and homogenates prepared. Aliquots of homogenate were incubated with ^{3}H-dihydromorphine (DHM, 5 nM) in the absence and presence of levorphanol (10 μM). Analyses of cultures exposed to naloxone for 7 days revealed a 50% increase in ^{3}H-DHM binding relative to that in control cultures exposed to medium only or to short-term naloxone (1 hour prior to assay). In order to answer the question as to whether antagonist-induced receptor upregulation requires the synthesis of new receptors, cultures were co-exposed to naloxone (10 μM) and cycloheximide (1 μM), a potent and relatively selective inhibitor of protein synthesis, for 5 days at 37°. These conditions have been shown to block protein synthesis by greater than 90%, as measured by incorporation of ^{14}C-leucine into total protein. Upregulation was observed in cultures so exposed. This result suggests that upregulation may be due to an unmasking of previously-existing receptors. The time-course of upregulation was also determined. No significant change in receptor density was observed following 1 day of naloxone treatment; a modest increase was observed after 2 days; and maximal upregulation was observed after 5 days of chronic exposure. The maximal increase of 50% was quite comparable to the 52% increase observed in assays of spinal cord after chronic naltrexone in situ (Tempel and Zukin, in preparation). These data demonstrate the feasibility of utilizing cord-drg explants as an in vitro model system for further analyses of cellular mechanisms underlying opiate supersensitivity and tolerance.
(Supported by research grants to S.M.C.: DA-02031 from NIDA and DA-01843 and DA-00069 to R.S.Z.).

99.5 KAPPA RECEPTOR ANTAGONISTS REVERSE "NON OPIOID" STRESS INDUCED ANALGESIA. A.E.Panerai, A.Martini+, P.Sacerdote+, L.Vicentini, P. Mantegazza+. Dept. Pharmacology, School of Medicine, University of Milano, Milano, Italy

The opiate receptor antagonist Naloxone reverses stress-induced analgesia when this is induced by a long, intermittent stress (1 sec every 5 sec for 20 min), but is ineffective on analgesia induced by a short, continuous stress (3 min). This observation suggested the definition of this stress-induced analgesia as "non opioid dependent". We show that two specific antagonists of the kappa opiate receptor: MR-2266 and MR-1452 MS completely reverse the "non-opioid" stress induced analgesia. We confirm that the two antagonists of the receptor: Naloxone and Naltrexone are ineffective in reversing the 3 min stress-induced analgesia, and show that also the mixed and delta receptor antagonist Diprenorphine and the delta receptor antagonist ICI 154129 are ineffective. Moreover, only the receptor antagonists Naloxone and Naltrexone can completely reverse the long, intermittent stress "opiod dependent" analgesia, while Diprenorphine is only partially effective. Both kappa receptor antagonists and the delta receptor antagonist are ineffective in reversing the "opioid-dependent" stress-induced analgesia.

Our results indicate that "opioid dependent" analgesia is inhibited by an endogenous opiate active on the receptor, e.g. B-endorphin, while the "non opioid" stress induced analgesia is mediated by an endogenous opiate active on the kappa receptor, e.g. Dynorphin. Endogenous opiates active on the delta receptor, e.g. Leu-enkephalin, do not seem to be involved in stress induced analgesia.

99.6 BINDING OF ^{3}H-DIHYDROMORPHINE IN NEWBORN RAT BRAIN MEMBRANES: CORRELATION WITH ENDOGENOUS OPIOID PEPTIDE LEVELS. William J. Shoemaker, R. Suzanne Zukin, Lynne Randolph*, and F.E. Bloom. Albert Einstein College of Medicine, Bronx, New York 10461 and The Salk Institute, La Jolla, CA 92037.

Both high and low affinity opiate binding sites have been described in newborn rat brain membranes using ^{3}H-dihydrgmorphine (DHM), as well as several other opioid ligands. We use ^{3}H-DHM to quantitate high and low affinity opiate binding sites in newborn rat pups that have been treated in a way that alters their brain ß-endorphin levels. Liquid diets that contain alcohol are fed to timed-pregnant female Sprague-Dawley rats in a model of the Fetal Alcohol Syndrome (FAS). The offspring of these alcohol treated mothers and their controls have previously been reported by us to have greatly increased levels of ß-endorphin in two regions of their brains at birth (Shoemaker et al. Soc. Neurosci. Abstr. 7:349, 1981). The increase in ß-endorphin levels is proportional to the blood alcohol levels of mother during gestation. The brain homogenates from these treated offspring are the subject of ^{3}H-dihydromorphine binding in homogenates at birth. Five brains from each treatment type were pooled for Scatchard analysis over 12 concentrations of ^{3}H-dihydromorphine (range 0.1 nM to 10 nM). The four treatment groups are as follows: (C) = mothers receive high quality lab chow; (BSA) = a liquid diet with low protein and 5% alcohol; (BSP) = the same diet as BSA with alcohol omitted, pair-fed to BSA; (RA) = a higher protein liquid diet containing 5% ethanol. In general, neonate brains bind only a small fraction of the adult level of DHM binding; however, the affinities for the two sites do not change. The effect of alcohol treatment to the fetuses (BSA) was to decrease the number of both high and low binding sites as measured by Scatchard analysis. This effect was not due to the undernutrition since the BSP (pair-fed control) did not show such alterations. The results from the RA group were difficult to interpret. However, there appears to be an inverse correlation between the level of brain ß-endorphin and the number of high and low affinity DHM binding sites. (Supported by NIAAA 03504.)

99.7 EVIDENCE FOR κ-RECEPTOR MEDIATED ANALGESIA IN THE DEVELOPING RAT Gordon A. Barr*, William Paredes*, Karen L. Erickson* & R. Suzanne Zukin (SPON: E.L. Gardner). Albert Einstein College of Medicine, Bronx, NY 10461 and Biopsychology Program, Hunter College, CUNY, New York, NY 10021

Mu, delta, and kappa opiate receptors are distinguished on the basis of: 1) differing physiological and behavioral profiles of different opiates; 2) differing sensitivities of opiate agonists to naloxone antagonism; and 3) differences in binding characteristics and selective protection (for a review see Zukin & Zukin, 1981). The question arises as to whether mu and kappa opiates elicit analgesia by an interaction with the same or with different opiate receptors. Evidence for separate receptors has been provided by findings that ketocyclazocine (KC) and morphine (M) have different anatomical sites of action and do not produce cross-tolerance. Data in support of a common receptor are the similar pA_2's for naloxone antagonism, and the ability of naloxazone to block KC- and M- induced analgesia. The present study examined the development of μ and κ binding sites and correlated these with the onset of M- and KC- analgesia. For receptor assays, high affinity ^{3}H-dihydromorphine binding was measured using homogenates of whole brain of rat pups of various ages. For κ receptor assays, ^{3}H-ethylketocyclazocine (^{3}H-EKC) binding was measured in the presence of μ and δ receptor blocking agents. For analgesia studies, tail flick nociception thresholds were determined by immersing the pups' tails in water (50°C) and recording tail flick latencies. M and KC were injected 30 minutes prior to testing. At birth κ receptor density, determined by Scatchard analysis, was ca. 65% of adult levels. κ site density remained nearly constant until 10 days of age, at which time it increased to adult levels. In contrast, high affinity ^{3}H-DHM sites were present at a very low density at birth. These sites increased gradually until about 13 days of age, at which time they increased markedly to nearly adult levels. In analgesia testing, KC was modestly active at very high doses from 3 to 9 days of age, but increased markedly in potency at 10 days of age. M was without any detectable effect until 12 days of age, at which time it produced analgesia at adult potency. Thus, the onset of KC-induced analgesia correlates with the observed increase in κ binding sites, and that of M- induced analgesia with the rise in μ receptors. The appearance of functionally-active κ receptors precedes the onset of μ receptors by approximately 3 days. In summary, the present study assigns a role for the κ binding sites in analgesia and differentiates its ontogenetic pattern from that of the μ receptor. We thank NIH Grants DA01843 and DA00069 (to R. Suzanne Zukin).

99.8 Phencyclidine Displaceable Binding of [^{3}H] Dextrorphan in Rat Brain Membranes. T.F. Murray and M.E. Leid*. Washington State University, College of Pharmacy, Pullman, WA 99164-6510.

Dextrorphan and certain psychotomimetic opioids have been reported to share discriminative stimulus properties with phencyclidine (PCP) in numerous behavioral studies. Moreover, we have previously demonstrated that dextrorphan is a competitive inhibitor of the specific binding of [^{3}H] PCP in rat brain membranes (Murray et al., Soc. Neurosci. Abstr. 8, 388, 1982). The purpose of the present investigation was to characterize the specific binding of [^{3}H] dextrorphan to crude synaptic membranes from the rat cortex. [^{3}H]-Dextrorphan (19 Ci/mmole, NEN) binding was measured by rapid filtration using glass fiber filters which had been presoaked in a 0.1% w/v poly-L-lysine solution . This treatment of filters completely eliminated PCP displaceable binding of [^{3}H] dextrorphan to filters in the absence of brain homogenate. Specific binding was defined as the total binding minus that occurring in the presence of a 10 μM nonradioactive PCP. The specific binding of [^{3}H] dextrorphan to synaptic membrane preparations was routinely determined after 2 hr. incubations at 0-4°C. Under these conditions the specific binding of [^{3}H]dextrorphan displayed tissue linearity between 200-800 μg protein per assay tube. The ratio of specific to nonspecific binding was enhanced in a medium of low ionic strength. The PCP displaceable component of binding ranged from 50 to 70% of the total in a 10mM HEPES buffer. The IC_{50} for PCP determined from logit-log analysis of competition data was 0.34 μM. The rank order potencies as displacing agents for [^{3}H] dextrorphan binding to cortical membranes was phencyclidine > (±) cyclazocine > (±) N-allyl-normetazocine which is compatible with behavioral data for these compounds. These relative potencies are also similar to those reported for the binding of these compounds to the sigma opiate/PCP recognition site (Zukin et al., Brain Res. 258, 277, 1983). Thus, our results suggest that [^{3}H]-dextrorphan may be a useful probe to investigate the recognition site which mediates the unique psychotominetic effects of drugs such as PCP, dextrorotatary opioids, and N-allylnormetazocine.

99.9 SIGMA OPIATE/PCP BINDING SITES IN DEVELOPING RAT BRAIN. Ratna Sircar* and Stephen R. Zukin, Dept. of Psychiatry, Albert Einstein College of Medicine, 1300 Morris Park Ave., Bronx, NY 10461

Stereospecific binding sites selective for phencyclidine (PCP), derivatives dioxalans and sigma opiates, with characteristics suggesting that these sites mediate the unique psychotomimetic effects of these agents, have been demonstrated in rat brain (Vincent et al., Proc. Natl. Acad. Sci. USA 76:4678, 1979; Zukin and Zukin, Proc. Natl. Acad. Sci. USA 76:5372, 1979; Quirion et al., Proc. Natl. Acad. Sci. USA 78:5881, 1981; Vignon et al., Eur. J. Pharmacol. 81:531, 1982; Hampton et al., Life Sci. 30: 2147, 1982; Zukin, Life Sci. 31:1307, 1982). In order to further the understanding of the functional roles of these receptors in the CNS, we have examined the time course of their development in the brain of the developing fetal and neonatal rat.

Brain homogenates from Blue Spruce hooded rats were incubated in 5 mM Tris-HCl, pH 7.4, 4°C, 45 min. with [^{3}H]PCP alone or with 10 µM PCP or varying concentrations of PCP or other drugs. Free ligand was separated from bound by rapid filtration over vacuum through GF/B filters (Whatman) pretreated with 0.01% poly-L-lysine. Apparent K_D and B_{max} values were obtained by Scatchard analysis. IC_{50} values for displacement of 10 nM [^{3}H]PCP by other drugs were calculated by linear regression. In each experiment, an adult brain was run in parallel as control.

Displaceable [^{3}H]PCP binding was detected at low levels in fetal brain homogenates on gestational day 13, increased gradually until day 17, then increased rapidly to adult levels by gestational day 21. The increase represents a change in B_{max}; no change in K_D occurred. As has been reported for adult rat brain homogenates, Scatchard plots of [^{3}H]PCP binding in fetal brain were linear, indicating a single case of binding sites throughout the developmental period examined.

Stereospecific [^{3}H]PCP binding, defined as [^{3}H]PCP binding displaced about 100 fold more potently by dexoxadrol than by levoxadrol, appeared suddenly at gestational day 20. Such stereospecific [^{3}H]PCP binding has been shown to be characteristic of pharmacologically-relevant CNS PCP binding sites, but not to be found in peripheral tissues, nonbiological materials or denatured CNS tissue (Hampton et al., Life Sci. 30:2147, 1982; Zukin, Life Sci., 31:1307, 1982). These results suggest differential development of membrane components responsible for non-stereospecific and stereospecific [^{3}H]PCP binding, and predict that the rat CNS becomes capable of response to the unique pharmacological effects of PCP-like or sigma opiate drugs just prior to parturition.

99.10 EVIDENCE THAT [^{3}H]DADL LABELS TWO CLASSES OF DELTA RECEPTORS IN SLIDE-MOUNTED SECTIONS OF RAT CAUDATE. R.B. Rothman*, W.D. Bowen*, and C.B. Pert. (SPON: C.B. Pert) Section on Brain Biochemistry, NSB, NIMH, Bethesda MD 20205.

Previous work has shown that [^{3}H]D-Ala^2-D-Leu^5-enkephalin (DADL) labels two anatomically distinct binding sites in rat caudate which differ in their sensitivity to ionic conditions (Bowen et al., PNAS, 78:4818, 1981). Further, Rothman and Westfall (Mol. Pharmacol., 21:538, 1982) demonstrated noncompetitive interactions between mu and delta receptors. A concept consistent with both sets of data is that [^{3}H]DADL labels two types of delta receptors, the class of delta receptor noncompetitively inhibited by morphine being the Type I opiate receptor.

To explore this idea, the displacement of [^{3}H]DADL binding to slide-mounted sections of rat caudate (90 min incubation at 25°C in 50 mM TRIS pH 7.4, 100 mM NaCl, 3 mM MnAc, and 2 uM GTP) by morphine, oxymorphone (OX), and leucine enkephalin (LE) were analyzed simultaneously with a [^{3}H]DADL binding isotherm according to a two-site competitive model and a two-site allosteric model. The allosteric model fit the data better than did the two-site competitive model. Further, only the allosteric model correctly predicted the noncompetitive interaction of OX with [^{3}H]DADL binding.

We conclude that OX is a noncompetitive inhibitor of [^{3}H]DADL binding. Our working hypothesis is that the population of delta receptors noncompetitively inhibited by OX is the Type I receptor. The population of delta receptors competitively inhibited by OX is the Type II receptor. These data are consistent with the idea that the Type I receptor is a conformationally malleable receptor complex, with distinct mu and delta binding sites, which can assume mu and delta conformational states.

99.11 QUANTIFICATION OF RECEPTOR DENSITIES BY AUTORADIOGRAPHY: TISSUE DEFATTING MINIMIZES DIFFERENTIAL ABSORBANCE OF TRITIUM BY GRAY AND WHITE MATTER. Miles Herkenham and Louis Sokoloff. Lab. Neurophysiol. and Lab. Cerebral Metabolism, NIMH, Bethesda, MD 20205.

A newly-developed means for quantifying neurotransmitter and drug receptor densities in discrete brain regions is densitometry of film autoradiographs previously exposed to tissue sections containing tritiated ligand bound to receptors. The technology for autoradiographic receptor localization and quantification has followed the developments in the field of local brain glucose metabolism mapped by [^{14}C]2-deoxy-D-glucose (2DG) uptake. In that field, recent attempts to increase the autoradiographic resolution by the use of [^{3}H]2DG resulted in the observation that gray matter and white matter differentially absorb low-energy tritium emissions (Alexander et al., Br. Res., '81, 223: 59-67). In fact, as much as 30-50% of emissions from white matter are absorbed relative to gray matter. Consequently, circulating methyl glucose, when labeled with ^{14}C, produces a homogeneous autoradiograph because of its uniform distribution; but when it is labeled with ^{3}H, it produces autoradiographs in which gray and white matter are clearly distinguished (Orzi et al., J. Cereb. Blood Flow Metab., in press).

This artifact can be minimized by defatting the sections in xylene prior to autoradiographic exposure (Herkenham and Pert, J. Neurosci., '82, 2: 1129-1149). To demonstrate this, adjacent slide-mounted sections, cut frozen from unfixed brains, were incubated in either 2.5nM [^{3}H]- or [^{125}I]DAGO-enkephalin (a selective mu opiate ligand), washed, dried and film exposed. Some sections were first fixed in formaldehyde vapors and defatted. The results show that fixation and defatting did not alter the relative densities of [^{125}I]DAGO binding in myelin-rich and myelin-poor regions. In contrast, the [^{3}H]DAGO labeling matched the pattern of [^{125}I]DAGO labeling only after the sections were defatted. Sections that were not defatted produced autoradiographs with exaggerated density differences at gray/white matter borders, resulting in a "contrast enhancement." In addition, receptor-dense fiber tracts appeared less optically dense, as did most of the reticular formation.

Even though 50% of the bound [^{125}I]DAGO is lost in the defatting process, the relative densities and distributions across structures are unaltered, indicating a non-selective loss of ligand. With ^{3}H as the isotope, the image after defatting is "corrected" for the differental quenching, even in autoradiographs of other opiate ligands which are lost in varying amounts (15-80%) during the defatting process. These results suggest the general applicability of tissue defatting to all [^{3}H]ligand-receptor binding studies, including those that report quantitative results as well as those that require the higher resolution and improved counterstaining achieved by tissue defatting and liquid emulsion coating.

99.12 HIGH RESOLUTION RADIOAUTOGRAPHIC VISUALIZATION OF OPIATE BINDING SITES IN RAT CENTRAL NERVOUS SYSTEM. E. Hamel and A. Beaudet, Lab. of Neuroanatomy, Montreal Neurological Inst., McGill University, Montreal, Quebec H3A 2B4.

The distribution of specifically labeled opiate binding sites was studied by light and electron microscopic radioautography in sections of rat striatum labeled in vitro with the met-enkephalin analog FK 33-824 (Sandoz). Brains were lightly fixed by intra-aortic perfusion of an ice-cold phosphate buffered solution containing weak concentrations of aldehydes, rapidly dissected out of the skull and sectioned on a vibratome. Fifty or 100 µm-thick slices were collected across the striatum and incubated at room temperature in Tris-sucrose buffer (pH 7.4) with 1nM ^{125}I-FK. After rinsing, the tissue slices were immersion-fixed in 4 to 6% glutaraldehyde, post-fixed in 2% osmium tetroxide, dehydrated in ethanols and flat embedded in Epon. Semi-thin (1 µm) and thin sections were then cut from the surface of each slice and processed for radioautography. Radioactivity measurements indicated that 50-70% of specifically bound FK molecules had been retained in the tissue during histological processing. Film radioautographs of whole incubated slices showed similar topographical distributions of specific FK binding sites before and after fixation and dehydration, consistent with the observed stabilization of receptor-ligand complexes by glutaraldehyde (Hamel and Beaudet, in press). In light microscope radioautographs, 85% of silver grains appeared scattered over the neuropil as compared to 15% over neuronal cell bodies. A significant proportion of the former (15%) was clearly associated with large dendritic profiles. However, in the majority of cases, underlying structures could not be identified by light microscopy. Of the grains localized over nerve cell bodies, 42% were associated with the cytoplasmic membrane. Tissue slices incubated in the presence of 1 µM non-radioactive FK or naloxone exhibited 75% fewer grains than those incubated with ^{125}I-FK alone. Most of these were detected over nuclei, nuclear membranes and unidentified structures of the neuropil. Electron microscope radioautography revealed that a large number of silver grains in the neuropil were located in the vicinity of axo-dendritic synaptic junctions involving mainly small axonic and dendritic processes. It also confirmed the association of silver grains with plasma membranes of neuronal perikarya and proximal dendrites. Some of these grains, however, did not appear to be associated with synaptic specializations. These preliminary results suggest that opiatergic terminals are associated predominantly with distal dendrites and, to a lesser extent, the soma and proximal dendrites of certain striatal neurons. Supported by fellowship (EH) and grant from the Medical Research Council of Canada.

100.1 EFFECTS OF L-GLUTAMATE AND L-GLUTAMIC ACID DIETHYL ESTER (GDEE) ON THE RESPONSE OF CORTICAL UNITS TO HYPOTHALAMIC STIMULATION. P. H. Cooper and C. D. Woody. Department of Anatomy and Psychiatry, UCLA Medical Center, Los Angeles, CA 90024.

Extracellular recordings of unit activity were made from twenty eight cells of the motor cortex of awake cats during extracellular iontophoresis of 1 M monosodium glutamate (buffered to pH 8.0) with push-pull currents ranging between 2 and 100 nA. Twenty four of the cells (86%) showed an increased firing rate in response to application of glutamate. Eight cells that demonstrated short latency (less than 20 msec) activation in response to hypothalamic electrical stimulation were tested with iontophoretic application of glutamate. Seven of these cells showed an increased firing rate in response to glutamate. Six of these cells were then tested with hypothalamic stimulation after extracellular iontophoretic application of 0.5 M GDEE (a glutamate blocker). The short latency response to hypothalamic stimulation was suppressed in four and reduced in one of these cells.

Other studies (Kim, E.H.-J., Woody, C.D. and Berthier, N.E. J. Neurophysiol., 49:767-779, 1983; Woody, C.D., Kim, E.H.-J. and Berthier, N.E., J. Neurophysiol., 49:780-791, 1983) have shown that hypothalamic stimulation, when added to presentations of a conventional CS and US, accelerates the rate of acquisition of a classically conditioned eyeblink reflex. Short latency activation of neurons of the motor cortex by hypothalamic stimulation is predictive of loci of stimulation within the hypothalamus that will produce this effect. It was also shown that hypothalamic stimulation had to be presented in an associative manner in order for this acceleration of conditioning to occur. The present findings indicate that cells of the motor cortex that respond to hypothalamic stimulation can also be activated by glutamate and, further, that the short latency response of neurons of the motor cortex to hypothalamic stimulation can be suppressed by extracellular application of GDEE, a glutamate blocker. (Supported by BNS 78-24146 and AFOSR F49620-83-C-0077.)

100.2 PATTERNS OF RESPONSE TO A BEHAVIORAL US AMONG NEURONS OF THE SENSORIMOTOR CORTEX OF AWAKE AND ANESTHETIZED CATS. D. Birt and C. D. Woody. Depts. Anatomy & Psychiatry, UCLA Med. Ctr., Los Angeles, CA 90024.

The temporal patterns of response to glabella tap were studied with intracellular recordings from 33 cortical units in awake and barbiturate anesthetized cats. Glabella tap is a satisfactory unconditioned stimulus (US) for the development of associative eyeblink conditioning. Unconditioned eyeblink responses to tap were recorded electromyographically from the levator oris and orbicularis oculi muscles.

The responses of most cortical units to tap-US fit one of two patterns. Half of the units responded with a sequence of increased activity (E), followed by a period of decreased activity (I), and then by a later period of increased activity (E). This pattern was termed E-I-E. Another fourth of the units showed early activation followed in many cases by inhibition, but without the later period of increased activity. This pattern was termed E-I.

Previous studies have shown that glabella tap elicits a two-component unconditioned eyeblink response, the first occurring 7-15 ms and the second 25-50 ms after tap delivery (Kugelberg, E., Brain, 75:385-396, 1952; Woody, C.D. and Brozek, G., J. Neurophysiol., 32:704-716, 1969). The time course of the E-I-E and the E-I unit responses resembled the time course of the two components of the unconditioned eyeblink response. However, the unit responses were not due to sensory feedback from the blink movement since similar response patterns were observed in awake animals (in which EMG identified blink responses occurred) as in anesthetized animals (during sequences of trials when no blink responses occurred). The blink movement in response to glabella tap is itself unnecessary for blink conditioning to occur (Crow, T.J. and Woody, C.D., Brain Res., 64:414-418, 1973).

A variety of stimuli that can serve as effective USs for classical conditioning produce response patterns in neurons of the sensorimotor cortex which resemble that of the E-I (or E-I-E) reported here. These include stimulation of the pyramidal tract (O'Brien, J.H. et al., J. Comp. Physiol. Psychol., 91:918-929, 1977; Bindman, L.J. et al., Soc. Neurosci. Abstr., 8:540, 1982), stimulation of the reticular formation (Segundo, J.P. et al., EEG Clin. Neurophysiol., 11:471-484, 1979; Kumar et al., Brain Res., 163:156-160, 1979), and stimulation of the hypothalamus (Woody, C.D. et al., J. Neurophysiol, 49:780-791, 1983). Weak clicks used as auditory conditioned stimuli (CSs) elicit the early E component alone. The possibility arises that the E-I pattern of response to the US may serve to distinguish USs from CSs and thereby play a critical role in the development of conditioned responses. (Supported by BNS 78-24146.)

100.3 SUBSTANTIA NIGRA LESIONS AND PAVLOVIAN CONDITIONING OF EYEBLINK AND HEART RATE RESPONSES IN THE RABBIT. K. T. Kao* and D. A. Powell (SPON: N.S. Shah). Neuroscience Laboratory, VA Hospital and University of South Carolina, Columbia, S.C.

Bilateral radio frequency lesions were made in the substantia nigra (SN) of New Zealand albino rabbits. A group of sham operate rabbits served as a control group. Following a 2-4 week recovery period, all animals were trained on delayed classical conditioning in which eyeblink (EB) and heart rate (HR) responses were concurrently assessed. Each session consisted of 100 trials. A 500 msec, 1216 Hz, 75 db (SPL) tone conditioned stimulus (CS) was followed immediately by a 250 msec, 3 mA paraorbital shock train as the unconditioned stimulus (UCS). The intertrial interval was 60 sec. The animals were trained to a criterion of 10 consecutive EB conditioned responses.

The lesioned group showed a deficit in EB acquisition compared to sham-lesioned animals. However, conditioned bradycardia appeared to be unaffected by the lesions. Furthermore, SN lesions did not affect unconditioned EB or HR thresholds to paraorbital electric shock, suggesting that lesion-induced motor and/or sensory deficits could not account for the conditioning deficits in the lesioned animals.

A previous report from our laboratory suggested the involvement of the caudate nucleus in Pavlovian somatomotor but not HR conditioning (Powell et al., Physiol. Behav., 1978, 20, 143-150). The present data suggest that the nigrostriatal system may also be important in mediating acquisition of somatomotor conditioning. Other extrapyramidal motor structures have also been implicated in simple Pavlovian somatomotor conditioning (Lavond et al., Physiol. Psychol., 1981, 9, 335-339; Moore, Desmond & Berthier, In: Conditioning: Representation of Involved Neural Functions, C. D. Woody (Ed.), 1983). However, the occurrence of conditioned bradycardia in the lesioned animals of the present study is inconsistent with the suggestion that the nigrostriatal system is a part of a "general learning system" (Thompson, Robert, Physiol. Psychol., 1982, 10, 186-198).

100.4 ROLE OF THE ACCESSORY ABDUCENS NUCLEUS IN THE NICTITATING MEMBRANE RESPONSE OF THE RABBIT. J.A. Harvey, G.J. Marek*, A.M. Johannsen*, S.E. McMaster*, T. Land* and I. Gormezano* (SPON: M.S. Stewart). Dpt. Psychology and Pharmacology, Univ. Iowa, Iowa City, IA 52242.

The corneal reflex of the rabbit, involving retraction of the eyeball by the retractor bulbi muscle and the correlated extension of the nictitating membrane, is being increasingly used as a model for examining the neuronal substrates of associative learning. The anterograde and retrograde transport of HRP was used to map the pathways of this reflex from cornea to the accessory (ACC) and abducens (ABD) nuclei which contain retractor bulbi motoneurons. Injection of HRP into ACC resulted in retrograde labeling of cells in ventral pars oralis of the spinal trigeminal nucleus, while HRP injected into ABD did not. Application of HRP to the cornea resulted in anterograde labeling of fibers in the ventral part of the trigeminal spinal tract and labeled fibers could be seen to enter the ventral pars oralis. These results suggested a disynaptic pathway for the nictitating membrane response (NMR) elicited by corneal stimulation and a primary role of motoneurons in ACC for mediating this defensive reflex. We, therefore, employed electrolytic and knife-cut lesions to examine the role of the VIth cranial nerve, and of the motoneurons in ACC and ABD that supply the VIth nerve, in the conditioned and unconditioned NMR. The amplitude of the unconditioned NMR, elicited by three intensities of air puff delivered to the cornea, was measured before and after these surgeries. Total destruction of the VIth nerve, with the resultant loss of all motoneurons in ACC and ABD, or interruption of all ACC inputs to the VIth nerve, while leaving the ABD and its projections into the VIth nerve intact, produced a large reduction in the magnitude of the NMR, though a small residual response to the air puff (less than 1 mm) could still be detected. This residual NMR was not affected by removal of all extraocular muscles while leaving the retractor bulbi intact. In contrast, severing the VIIth (facial) nerve, which alone had no effect on the NMR, eliminated this small residual responding. Therefore, a small component of the NMR elicited by air puff results from contraction of the obicularis oculi muscle. Interruption of all ABD inputs to the VIth nerve, while leaving ACC inputs intact, had no effect on the NMR elicited by air puff. Isolation of ACC inputs to the VIth nerve in animals that had acquired conditioned NMRs to a tone conditioned stimulus, that had been paired with corneal air puff as the unconditioned stimulus, produced a large and equivalent reduction in both the conditioned and unconditioned NMR. These results indicate that both the unconditioned NMR elicited by air puff delivered to the cornea and the conditioned NMR elicited by an auditory conditioned stimulus are mediated by motoneurons in the ACC, but not by motoneurons in the ABD.

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100.5 RED NUCLEUS AND SUPRATRIGEMINAL RETICULAR FORMATION: BRAIN STEM COMPONENTS OF THE CONDITIONED NICTITATING MEMBRANE RESPONSE. J. E. Desmond*, M. E. Rosenfield* and J. W. Moore. Department of Psychology, Univ. of Mass., Amherst, MA 01003.

Crystalline HRP (Boehringer Mannheim Grade 1) was implanted into the region of the accessory abducens nucleus (AAN) of 3 rabbits. This brain region contains motoneurons involved in defensive eye reflexes and extension of the nictitating membrane (NM). Following 24-48 hr survival time, the animals were perfused under deep nembutal anesthesia. Two 1 of 0.9% saline was followed by 2 1 of 10% formalin, and then 2 1 of 12% sucrose in 0.1M phosphate buffer. Brain stem and cerebellar tissue, cut at 40-60 um thickness, were reacted with the TMB procedure of Mesulam (J. Histochem. Cytochem., 26: 106, 1978) with the stabilization step omitted. Labeled cells and a network of fibers and apparent terminations were observed throughout the brainstem. Most relevant from the standpoint of current research into the anatomical substrates of the classically conditioned NM response is the observation of extensive bilateral labelling of small diameter cells of the supratrigeminal region (SR, i.e., the reticular formation located dorsal to N.V): SR has been implicated in the conditioned NM response (Desmond et al., Physiol. Behav., 28: 1029, 1982; Moore et al., Soc. Neurosci. Abstr., 7: 358, 1981). Labelling of cerebellar nuclei was mostly confined to contralateral fastigial nucleus. Many labeled cells were observed in contralateral magnocellular red nucleus (RN).

Sixteen rabbits received classical conditioning of the right NM response using a tone CS and electrostimulation of the right eye as the US. Single RF lesions of the medial portion of the left previously acquired conditioned response. This finding is not only consistent with the HRP data described above, but is also consistent with the idea that an essential anatomical substrate of the conditioned response includes a circuit from the cerebellum (McCormick et al., Proc. Nat. Acad. Sci. USA, 79: 2731, 1982) to the contralateral RN which projects contralaterally in turn to pontine motoneurons mediating the NM response.

100.6 CONDITIONED STIMULUS DURATION INFLUENCES LONG LATENCY HIPPOCAMPAL ACTIVITY DURING SHORT-DELAY CLASSICAL CONDITIONING OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE. A. G. Romano* and M. M. Patterson. Dept. of Psychology, Ohio Univ., Athens, OH 45701.

Previous research has demonstrated that multiple unit activity (MUA) in the pyramidal cell layer of the hippocampus exhibits a learning-dependent increase in frequency during short-delay classical conditioning of the rabbit's nictitating membrane (NM) response.

One training variable known to have an effect on rate of short-delay NM conditioning is conditioned stimulus (CS) duration after unconditioned stimulus (US) offset. This study examined the effect of post-US CS duration on rate of NM conditioning and conditioned hippocampal MUA. Rabbits were chronically implanted with electrodes in the pyramidal cell layer of the hippocampus one week prior to classical conditioning of the NM response. For one group of animals, a standard short-delay conditioning procedure was employed; a 350 msec tone CS overlapped and terminated with the offset of a 100 msec corneal airpuff US yielding a CS-US interval of 250 msec. For a second group of animals, a similar training procedure was employed except that CS duration was extended to 2.1 sec. During each trial, hippocampal MUA was bandpass filtered at 500-5000 Hz and recorded on magnetic tape. Off-line analysis of hippocampal MUA consisted of the creation of poststimulus histograms and the computation of standard scores of MUA for the 250 msec CS-US interval and 8 consecutive 500 msec periods following the onset of the US.

Animals trained with the short CS conditioned faster and exhibited more conditioned responses than animals trained with the long CS. Similarly, standard scores of hippocampal MUA during the CS-US interval and first post-US period increased faster for the short CS animals. Moreover, the poststimulus histograms for the short CS animals showed a pattern of activity resembling the topography of the NM response. The poststimulus histograms for long CS animals also bore a resemblance to the topography of the NM response but only up until the second or third post-US period. At this point during the trial, although there was no evidence of NM movement, hippocampal MUA showed a pronounced and long-lasting increase which did not decline to baseline levels until 1-2 sec after the offset of the CS. In most instances, the standard scores of the long CS group were several orders of magnitude greater during the later post-US periods than during the first post-US period when the NM response was being executed. Given that the hippocampus has often been implicated in short-term memory processes, it is possible that a short-term memory for a CS-US pairing is being expressed in the hippocampus as a result of cueing by the continued presence of the CS.

100.7 EFFECTS OF LESIONING CEREBELLAR NUCLEI ON CONDITIONED LEG-FLEXION RESPONSES. N. H. Donegan*, R. W. Lowery*, and R. F. Thompson (SPON. S. Levine). Dept. of Psychology, Stanford Univ., Stanford, CA. 94305.

McCormick et al. (1981, 1982) have shown that the cerebellum is an essential element of the neuronal circuitry responsible for the generation of conditioned NM/eyeblink responses in the rabbit --lesions of the dentate and interpositus cerebellar nuclei ipsilateral to the side of training selectively abolish the NM conditioned response (CR), leaving the unconditioned response (UR) unaffected. Here we report that lesioning the cerebellar nuclei produces a similar effect on conditioned leg-flexion responses.

Initial training involved presentation of a 350 msec tone conditioned stimulus (CS) that overlapped and coterminated with a 100 msec, 2 mA shock unconditioned stimulus (US) to the rabbits' left hindpaw. Subjects (N=7) received 45 trials per session (30 paired, 10 tone-alone, and 5 US-alone), one session per day for six days. Over the course of training, both hindlimbs developed substantial, equivalent conditioned flexion responses, measured as increased EMG activity to the tone CS. (Additional subjects (N=4) given unpaired presentations of the CS and US showed little or no change in EMG activity to the tone over sessions.) Subjects then received lesions of the left cerebellar nuclei and/or overlying cortex, were allowed seven days of recovery, and then received six days of retraining on the left side. Lesions of the dentate and interpositus nuclei (N=4) resulted in an abolition or substantial impairment of the leg-flexion CRs in both hindlimbs. Cortical lesions (N=3) resulted in an initial reduction of CR amplitude followed by a recovery approaching prelesion levels. When training was switched to the right hindlimb, both hindlimbs developed conditioned responses similar to prelesion training. This finding, that normal CRs can be generated by the left hindlimb when training is given on the side contralateral to the lesion (right), and the fact that leg-flexion URs to the pawshock US (measured by a stabilimiter device) were unaffected by the lesions, indicate that the lesions did not produce an overall performance deficit, but rather, a selective deficit in the expression of the learned behavior. When training was shifted back to the left side, conditioned responding in both hindlimbs returned to near zero levels in the nuclear lesioned animals.

These results (a) indicate that the lesion effects of McCormick et al. (1981; 1982) are not peculiar to the NM/eyeblink response systems and (b) suggest that the cerebellum is an essential part of the circuitry for classical conditioning of discrete striated muscle responses, at least with aversive USs.

100.8 THE CLAIRE-BISHOP AREA IN THE CAT AS A REPOSITORY OF VISUAL EXPERIENCE. D. N. Spinelli. Dept. of Computer and Information Science, University of Massachusetts, Amherst, MA 01003.

To further our understanding of how visual experience is recorded in the brain we trained cats to perform a visual experience which has unique identifying properties. Normally reared cats are shown a "danger" signal (i.e. two vertical bars) to one eye only and required to flex one of their forelegs (within one second) to avoid a mild negative reinforcer. Correct performance causes the danger stimulus to disappear and the appearance of a "safe" signal (i.e. two horizontal bars) which are shown to the other eye. This experience is unique in that animals with frontally located eyes always see the same things with both eyes. We have shown previously that a percentage of cells in area 17 become tuned to vertical bars for one eye and horizontal bars for the other: a class of cells which is not present in cats not so trained. In this study we find that the receptive fields of single cells in the Claire-Bishop area of cats which had been trained up to two years ago are extremely large, binocular, and primarily responsive (60% of the total) to horizontal or vertical bars. Most interestingly we find that a percentage of cells have receptive fields of 30° of visual arc or more and that, when binocularly activated, these cells respond best to vertical bars for one eye and horizontal bars for the other, as seen during training. Furthermore, the shape of these receptive fields consists of two excitatory areas whose shape and separation are strikingly similar to the one used in the stimuli. These unique properties clearly reflect the unique aspects of the experience. The genesis of receptive field properties under guidance of visual experience will be discussed.

This work was supported in part by Air Force Contract F33615-80-C-1088.

100.9 DISRUPTION OF SHORT AND LONG-TERM MEMORY FOLLOWING REVERSIBLE INACTIVATION OF THE HIPPOCAMPUS. Douglas C. Smith, Robert A. Jensen and T. M. Reeves, Developmental Biopsychology Program and School of Medicine, Southern Illinois University-Carbondale, IL 62901.

Using a passive avoidance paradigm, Kesner and Conner (1972) demonstrated that electrical stimulation of the hippocampus (HC) disrupted long-term (LTM), but not short-term memory (STM). In contrast, stimulation of the midbrain reticular formation disrupted STM, but not LTM. While these and similar results have been taken to indicate that two separate neural pathways mediate STM and LTM, the lack of a localized effect of electrical stimulation makes these conclusions tenuous. We now report that discrete injections of lidocaine, a local anesthetic which completely and reversibly inactivates all neural activity in the area affected, into the HC produce both STM and LTM deficits.

In a one-trial inhibitory avoidance task, 20 Long Evans rats received a 1.5 ma, 1.0 sec shock immediately upon entering the dark compartment from the light side of a two compartment alley. For the experimental group, 1.0 µl of lidocaine was bilaterally injected into each HC through implanted cannulae within 5 sec following termination of the shock and were then tested for retention 60 sec and 24 hrs later. Control rats (C) received the same training and testing procedure but no lidocaine. Data were analyzed using a mixed design analysis of variance with repeated measures on one factor. Mean post-learning-trial latency to enter the dark at 60 sec was 290 sec (ceiling 300 sec) for C rats, and 171 sec for HC injected rats ($F_{1,18}$=7.19, p<.025). At 24 hrs, mean latency was 292 sec in C rats and 120 sec for HC-injected rats ($F_{1,18}$= 17.94, p<.001). For HC-injected rats, 60 sec and 24 hr latencies to enter the dark did not differ ($F_{1,9}$= 1.4, p>.20). Preliminary electrophysiological investigations indicate that 1 µl of lidocaine affects a brain area approximately 2 mm in diameter.

We conclude that when highly discrete regions of the hippocampus are inactivated for a short period of time following a learning trial, deficits in both STM and LTM are produced. However, the memory deficit was not complete, i.e., there was some retention evident in those animals that received a lidocaine infusion into the hippocampus. This residual retention may reflect the fact that only a small region of the hippocampus was rendered dysfunctional by the lidocaine. Taken together, these results argue against the idea that different neural substrates mediate STM and LTM. Supported by grant 2-11401 from the Office of Research and Development of Southern Illinois University-Carbondale to D.C.S.

100.10 PLACE NAVIGATION IN RATS AS A FUNCTION OF AGE. F. Schenk, F. Inglin and R.G.M. Morris. Institute of Physiology, University of Lausanne, 1011 Switzerland and Physiological Laboratory, University of St. Andrews, Scotland.

Hippocampal damage have been shown to cause a significant deficit in place but not in cue guided navigation (Morris et al., Nature, 297: 681-3, 1982; Morris & Schenk, in prep.). This study examined whether the maturation of the hippocampus in young Rodents would be accompanied by significant changes in place navigation.

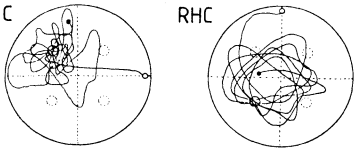
Young hooded rats were trained to escape from opaque water onto a small platform (Ø 8.0 cm) at a fixed position in a circular swimming pool (Ø 100 cm). Only spatial room cues were available for locating the platform position in the condition "place only"; a small piece of wood (5 cm height) was placed onto the platform as an additionnal local cue in the condition "place + cue". Five litters (8-10 pups) were weaned at 20 days. Training consisted in four daily sessions of resp. 4, 4, 8 and 8 trials, beginning when the individuals were either 21 (n=24) or 35 days old (n=23).

The young rats (n=17) were slower to escape than their 35-day-old littermates (n=16) ($p < 0.01$), and showed longer path length ($p < 0.05$) throughout training in the condition "place only". On a subsequent "transfer test" during which the hidden platform was removed from the pool, young rats were less accurate than older subjects in passing through its exact former location ($p < 0.05$). The behaviour of the 21-day-old rats (n=7) was more similar to that of the 35-day-old subjects (n=7) at the end of "cue + place" training. However, removal of the wooden cue for the subsequent 15 trials "place only" condition) strongly impaired the performance (path length and latency, $p < 0.001$) of the young but not of the older rats.

Complementary data from 28 and 42-day-old subjects help discussing critical days for the maturation of place navigation in young rats. Detailed analysis of the pathways suggests that onset of searching behaviour impairs performance between 26 and 32 days of age.

100.11 DISSOCIATION BETWEEN 'PROCEDURAL' AND 'DECLARATIVE' ASPECTS OF SPATIAL MEMORY AFTER RECOVERY FROM RETROHIPPOCAMPAL LESIONS BY RATS. R.G.M.Morris*and F.Schenk* (Spon:W.Heitler). Psychol.Lab.Univ.St.Andrews,SCOTLAND & Institute de Physiol., Univ.de Lausanne,SWITZERLAND.

This study examined whether retrohippocampal lesions would cause a comparable deficit in place-navigation as that after hippocampal damage (Morris et al.,Nature,1982,297,681-683) and whether extensive pre- and/or postoperative training could ameliorate any deficit observed. Rats with bilateral RHC lesions (including entorhinal cortex, pre/para subiculum and the tip of the angular bundle), sham surgery (SH) or no treatment (C) (Ns=11, 12 & 8) were allowed to escape from opaque water onto a small platform hidden (1cm below water) at a fixed position in a large (132cm diam) pool. Spatial room cues were readily visible but no local cues marked the position of the platform. RHC lesion rats were slower to escape than SH and C rats throughout training ($p<0.0001$). This deficit disappeared when these, and other rats (RHC, SH & C; Ns=8, 4 & 4) which had received pre-op training also, were then trained with a visible platform (1cm above water). Finally, the rats were returned to the hidden platform task (the RHC lesion deficit reappeared) and then given a 60 sec transfer test during which the hidden platform was removed from the pool. The paths taken by the rats (Fig 1) showed



that RHC lesion rats failed to search near the now absent platform, but often passed through its exact former location during semi-stereotyped traverses of the entire pool. The groups differed with respect to time spent in the training quadrant ($p<0.0001$) but not crossings of the correct location ($p>0.10$). Thus rats with RHC lesions can learn the procedural rule of going to a particular place, but do not recognise it or initiate localised search upon failing to find it. This lack of declarative spatial knowledge (Squire and Cohen, 1983) was also reflected in the failure of "recovered RHC" rats to discriminate two visible platforms by their spatial location in a later experiment. Detailed histological examination (CV and Fink-Heimer) has shown the lesions to be complete.

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100.12 LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS IMPAIR LONG-TERM MEMORY IN RATS. H.C. Fibiger, C.L. Murray* and A.G. Phillips, Div. Neurological Sciences, Dept. Psychiatry; Dept. Psychology, University of British Columbia, Vancouver, B.C.,Canada, V6T 1W5.

It has been demonstrated that there is a loss of cholinergic neurons in the basal forebrain of patients dying with Alzheimer's disease and this appears to be the basis of the consistent decrease in cortical choline acetyltransferase (ChAT) activity that is observed in this syndrome. At the present time, the functional consequences of the loss of this cholinergic innervation of the cortex are not known. Inasmuch as there is a profound impairment of certain types of memory in Alzheimer's patients, the present experiments were designed to investigate spatial memory in rats that received bilateral ibotenic acid lesions of the nucleus basalis magnocellularis (nBM), the origin of a major cholinergic projection to the cortex in this species.

Under halothane anesthesia, male Long-Evans rats (300-350 gm) received bilateral injections of ibotenic acid (5 µg in 1 µL) or vehicle at co-ordinates AP+7.2, L±2.4, and DV+2.7 mm from stereotaxic zero with the animal's head held in the horizontal plane. Six weeks postoperatively the animals were food deprived to 85% of free-feeding weights and received training in a 16 arm radial maze in which 9 of the arms contained food. The animals were trained to run this maze for food reinforcement over a period of 36 days, during which measures of working or short term memory (defined as the number of entries into previously unentered baited arms) and reference or long term memory (defined as the total number of entries into the baited arms) were obtained (Olton, D.S. and Samuelson, R.J., J.exp.Psych., 2:97,1976; Olton, D.S. and Papas, B.C., Neuropsychologia, 17:669,1979).

Unlike the control group, animals with lesions of the nBM failed to show evidence of spatial reference memory over the 5 week training period. On the other hand, working memory was not disrupted by these lesions. At the end of the 5 week training period, the nBM lesioned group received intraperitoneal injections of physostigmine sulfate (0.5 mg/kg) 30 min before each daily session. The reference memory performance of the physostigmine treated animals improved rapidly and significantly to approach that of the control group. Cortical ChAT activity was found to be reduced by approximately 50 percent whereas hippocampal ChAT activity was not affected by the lesions. The data indicate that the cholinergic innervation of the cortex is an important neuronal substrate for long term spatial memory and suggest that the loss of cortical ChAT activity in Alzheimer's patients may be responsible for some of the memory deficits associated with this disease.

100.13 THE ROLE OF THE POSTERIOR PARIETAL ASSOCIATION CORTEX IN THE PROCESSING OF SPATIAL EVENT INFORMATION. B. V. DiMattia* and R. P. Kesner. Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

Damage to the posterior parietal association cortex (PPC) in humans results in deficits for information concerning spatial aspects of their environment. These patients typically exhibit an inability to discriminate near from far objects, an inability to read or draw maps or diagrams of familiar spatial locations, an impaired recent spatial memory and a general loss of "topographic sense". In addition, there is a deficit in attention to spatial stimuli, which appears to be a function of lesion size and level of difficulty of the task.

In order to test whether there might be a correspondence between rats and humans regarding the role of PPC in the processing of spatial event information, the effect of PPC lesions in the rat on memory for a list of five spatial events (arms on an Olton eight-arm radial maze) was examined. Animals were trained to employ either a win-stay or a win-shift rule on the maze and serial position functions were generated for each group of animals. These tasks were chosen because it is assumed that they have differential attention requirements, because (a) the win-shift rule is considerably easier for the rat to learn than the win-stay rule and (b) rats that have learned the win-stay rule show a serial position curve which includes significant primacy and recency effects, whereas only recency effects are seen with animals that have learned the win-shift rule.

Half of the win-stay and half of the win-shift animals were given bilateral aspiration lesions of PPC while the remaining animals from each group received sham operations. In the win-stay task, rats' ability to perform was deleteriously affected by bilateral removal of PPC, i.e., there was a disruption of memory performance for every position on the list, including both primacy and recency positions. However, comparable lesions of PPC did not produce any post-operative impairment of memory in the win-shift task. Sham operated control animals did not show a deficit in either task.

Thus, the PPC lesions only affected memory for spatial events in the task that is assumed to have greater attentional requirements for spatial information. An alternative possibility is that the win-stay task requires the utilization of more spatial information than the win-shift task. The results of the present study provide support for an equivalency of mnemonic function of the PPC in both rats and humans.

100.14 DISSOCIATION OF ITEM AND ORDER MEMORY IN RATS FOLLOWING MEDIAL PREFRONTAL CORTEX LESIONS. R. P. Kesner and T. H. Holbrook* (SPON: M. E. ELLIS). Dept. of Psychology, University of Utah, Salt Lake City, UT 84112.

Humans with prefrontal lobe damage have difficulty in remembering the temporal ordering of events. When these patients are tested for item and order memory for a list of events, they show poor memory for order information, but have normal memory for item information. In order to test whether there is a correspondence in function of prefrontal cortex in rats and humans, rats were tested for item and order memory for a list of items (places on a maze).

Rats were trained on an eight arm radial maze for Froot Loop reinforcement. After extensive training each animal was allowed on each trial (one per day) to visit four arms in an order that was randomly selected for that trial (study phase). The sequencing of the four arms was accomplished by sequentially opening of Plexiglas doors (one at a time) located at the entrance of each arm. Immediately after the animal had received reinforcement from the last of the four arms, the test phase began. The test for order memory consisted of opening of either the first and second, second and third, or third and fourth door that occurred in the sequence. The rule to be learned leading to an additional reinforcement was to choose the arm that occurred earlier in the sequence. The test for item memory consisted of opening of a door that was previously visited for that trial and a door that was not. The rule to be learned resulting in an additional reinforcement was to choose the arm previously visited during the study phase of the trial (win-stay rule). The order of presentation of the two tests was varied randomly.

Following extensive training, animals performed better than chance for each item position for both tests. The animals then received medial prefrontal cortex aspiration lesions. Results indicated a complete loss for order memory. This order deficit appeared even when (a) the list length was only two items, (b) the items presented during the study phase of a 5-item list were constant from trial to trial and, (c) the animal was allowed to self-order during the study phase.

In contrast, there was excellent memory for the first item of the list with a deficit for the remaining items on the item memory test. In addition, memory was excellent for all items of a 5-item list when the study phase was constant from trial to trial.

Thus, rats can remember the occurrence, but not the temporal ordering of spatial events. This suggests that there is a correspondence between rats and humans with respect to the mnemonic functions of prefrontal cortex.

BIOGENIC AMINES I

101.1 A NEW RECEPTOR FOR 5-HT: CHARACTERIZATION AND LOCATION IN THE NERVOUS SYSTEM OF THE GUT. Theresa Branchek, Mandes Kates*, and Michael D. Gershon, Dept. Anatomy and Cell Biology, Columbia Univ., P&S, New York, NY 10032.

Although the existence of enteric serotonergic neurons is now well established, relatively little is known about enteric serotonin (5-HT) receptors. We have therefore sought to characterize these receptors utilizing a filtration assay and radioautography with ^{3}H-5-HT as a radioligand. Saturable, reversible, specific, high affinity (K_D = 5nM) binding of ^{3}H-5-HT was found to membranes isolated from the submucosa-mucosa of the gut and from the longitudinal smooth muscle with attached myenteric plexus. Specific ^{3}H-5-HT binding sites were localized by radioautography to discrete patches in the gut wall corresponding to the location of the myenteric plexus and to non-epithelial components of the submucosa-mucosa. The binding of ^{3}H-5-HT was not inhibited by antagonists active at the following receptors: a- and B-adrenoceptors (phentolamine, propranolol), H_1 histamine (diphenhydramine, mianserin, cyproheptadine), H_2 histamine (cimetadine), or dopamine (butaclamol, domperidone, metoclopramide, spiroperidol). ^{3}H-5-HT was also resistant to displacement by agents known to be active at the two 5-HT receptors that have been identified in the CNS, the S2 (LSD, spiroperidol, ketanserin, mianserin, cyproheptadine) and S1 (LSD, methysergide, trazodone, cinanserin, metergoline, quipazine, 6-nitroquipazine, 5-methoxytryptamine) sites respectively. The enteric ^{3}H-5-HT binding sites are thus specific to 5-HT but unique to the bowel.

Structure-activity requirements for binding to the enteric 5-HT site were studied by analyzing the ability of analogues to compete with ^{3}H-5-HT. Tryptamine, 5-aminotryptamine, and 5-methoxytryptamine all failed to displace ^{3}H-5-HT at 10,000 times the 5-HT concentration, whereas 6-HT, 5,6-dihydroxytryptamine, and 5,6,7-trihydroxytryptamine were nearly as potent as 5-HT itself. Displacement of the hydroxyl group to the 4 or 7 positions decreased activity. A hydroxylated indole ring, preferably at the 5 or 6 position, thus seems to be necessary for binding to the 5-HT site. In contrast, the aliphatic side chain of 5-HT appeared to be relatively unimportant in determining binding to the enteric 5-HT site and could be deleted. These requirements are similar to those needed for pharmacological activation of enteric neurons but dissimilar to those needed for activation of smooth muscle. It is concluded from the effects of antagonists, the correlation of structure-activity requirements for 5-HT binding with the pharmacological activation of enteric neurons by 5-HT, and the radioautographic localization of ^{3}H-5-HT binding, that the enteric ^{3}H-5-HT binding site is a unique neural 5-HT receptor. Supported by NIH grants NS12969 and 5T32 NS07062.

101.2 ^{3}H-TRYPTAMINE BINDING IN THE BRAIN AND SPINAL CORD: IN VITRO AUTORADIOGRAPHY. J.K. McCormack*, A.J. Beitz and A.A. Larson. Dept. of Vet. Biology, Univ. of Minnesota, St. Paul, MN 55108.

Tryptamine, an endogenous trace amine, is currently postulated to be a neuromodulator or neurotransmitter in the mammalian CNS. Both electrophysiological and pharmacological studies have demonstrated differential responses of tryptamine and serotonin (5-HT). Recently, a high affinity binding site has been described for tryptamine in the rat brain homogenate by Kellar and Cascio (Eur. J. Pharmacol. 78:475, 1982). The present study further characterizes tryptamine binding throughout the CNS and delineates its distribution using autoradiographic techniques.

Saturation studies on 20 μ thick brain sections suggest a single class of binding sites (Hill plot coefficient $\simeq$ 1.00) with a high affinity ($K_D \simeq$ 13.00 nM). In a competition study, 5-HT failed to significantly inhibit tryptamine binding (K_I = 1.37 μM). Maximum specific to non-specific binding was achieved using a 30 min incubation period at 25°C in a solution containing 0.1 M Tris citrate (pH = 7.4), 5 mM ascorbic acid, 20 μM pargyline and 2 nM ^{3}H-tryptamine. Tissue sections were processed for in vitro autoradiography using sections from rat brain and brain stem and from dog brain stem and spinal cord. Highest binding occurred in limbic structures: the anterior olfactory nucleus, olfactory tubercle, septal area, amygdaloid complex, anterior cingulate gyrus and hippocampus. Binding was also evident in the nucleus accumbens and medial hypothalamus. In the brain stem, binding was most notable in the interpeduncular nucleus, superficial layer of the superior colliculus, periaqueductal gray, substantia nigral region, spinal trigeminal nucleus, inferior olivary nucleus and the region of the nucleus solitarius. Binding was evident in both the dorsal and ventral horns of the spinal cord. These results demonstrate that tryptamine binding sites are predominant in limbic structures and also are found throughout the spinal cord and selective brain stem sites.

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101.3 GUANINE NUCLEOTIDES MODULATE CORTICAL S_2 SEROTONIN RECEPTORS, M. Titeler, G. Battaglia and M. Shannon* Department of Pharmacology, University of Toronto, Toronto, Ontario M5S 1A8.

S_2 serotonin receptors appear to mediate several important CNS and peripheral effects of serotonergic drugs (1,2). It has been reported that guanine nucleotides do not regulate cortical S_2 serotonin receptors (1).

Contrary to these findings we find a pronounced agonist-specific effect of guanine nucleotides in modulating ^{3}H-ketanserin-labelled cortical S_2 serotonin receptors (see Table). Agonist interactions, in the absence of nucleotides, display high and low affinity components in inhibition of ^{3}H-ketanserin binding. GTP apparently converts the high affinity receptor population to low affinity (3). Antagonist interactions are not affected (see Table).

Computer-assisted analysis of a two-state model for the S_2 serotonin receptor will be presented.

These results are similar to results from radioligand studies of adenylate cyclase coupled receptors, which are modulated by GTP through a guanine nucleotide binding protein. Therefore, we have undertaken studies to determine whether there is an S_2 serotonin receptor coupled to adenylate cyclase activity through a guanine-nucleotide binding protein.

	Control		10^{-4}M GTP	
	Ki(nM)	Hill Coefficient	Ki(nM)	Hill Coefficient
Agonists				
Serotonin	562	0.75	1,818	1.00
5-Methoxytryptamine	686	0.71	2,171	0.98
Antagonists				
Cinanserin	4	0.95	4	0.92
Cyproheptadine	0.7	0.97	0.7	0.97

(1) Peroutka, S.J., and Snyder, S.H., Mol. Pharmacol., 16, 687-714, 1979.
(2) Leysen, J.E., Niemegeers, C.J.E., Van Nueten, J.M., and Laduron, P.M., Mol. Pharmacol., 21, 301-314, 1982.
(3) Titeler, M., Battaglia, G., and Shannon, M., submitted, 1983.

101.4 **^{125}I-LSD: A SELECTIVE, HIGH SENSITIVITY LIGAND FOR SEROTONIN 5Ht$_2$ RECEPTORS.** **P. Hartig, M. Kadan*, A. Krohn*, M. Evans*, and R. Waltz*. Dept. of Biology, Johns Hopkins University, Baltimore, MD 21218.**

We synthesized unlabeled 2I-LSD by the method of Troxler and Hofmann (Helv. Chim. Acta. 60, 2160 (57)). 2I-LSD was over 98% pure by HPLC analysis. This ligand displays a high affinity for serotonin 5Ht$_2$ (S_2) receptor sites in rat frontal cortex (IC_{50} = 6.4 nM for ^{3}H-spiroperidol) and a moderate affinity for dopamine D_2 receptor sites in bovine caudate (IC_{50} = 16 nM for ^{3}H-spiroperidol and 24 nM for ^{3}H-domperidone). In contrast to the parent compound d-LSD, the iodinated ligand is selective for these sites, showing only a weak affinity for 5Ht$_2$ receptor sites (IC_{50} = 99 nM for ^{3}H-5Ht in frontal cortex) or D_2 dopamine receptors (IC_{50} = 110 nM for ^{3}H-ADTN in bovine caudate).

We synthesized ^{125}I-LSD using N-^{125}I-succinimide in a modification of the method of Troxler and Hofmann. It comigrates with 2I-LSD in a high resolution 2D-HPTLC system and has been synthesized carrier free (2175 Ci/mmol). It is stable for over 2 months under the proper conditions.

In rat frontal cortex homogenates, ^{125}I-LSD binding exhibits a linear Scatchard behavior with K_d = 5.6 nM and B_{max} = 37 fmol/mg tissue. The binding is reversible, stereospecific and is destroyed by boiling the membranes. The binding is potently displaced by the 5Ht$_2$ ligands spiroperidol, mianserin and cinanserin (IC_{50} values of 3.1, 15.4 and 19 nM respectively) and serotonin is the most potent neurotransmitter in displacing this binding (IC_{50} = 1.2 µM).

In bovine caudate homogenates, ^{125}I-LSD binds primarily to a single class of sites with K_d = 3.6 nM and B_{max} = 24 fmol/mg tissue. Specific binding represents 75-85% of total binding in both tissues. Displacement of ^{125}I-LSD by a variety of compounds closely parallels that found in rat frontal cortex, indicating that ^{125}I-LSD preferentially labels 5Ht$_2$ receptors in bovine caudate. If this 5Ht$_2$ binding is inhibited by the inclusion of 0.3 µM Mianserin, a second component of ^{125}I-LSD binding is revealed with a K_d = 27 nM and B_{max} = 37 fmol/mg tissue. This secondary binding is most potently inhibited by dopaminergic ligands with a rank order of potency for agonists of: apomorphine > dopamine > epinephrine > norepinephrine, serotonin. The rank order of antagonist inhibition resembles that for ^{3}H-spiroperidol in this tissue. Thus, under the proper conditions ^{125}I-LSD appears to label either serotonergic 5Ht$_2$ or dopaminergic D_2 sites in bovine caudate.

^{125}I-LSD is the first iodinated ligand available for serotonin 5Ht$_2$ receptors. It also may be useful as a dopamine D_2 receptor ligand under the proper conditions. ^{125}I-LSD has been synthesized carrier free which makes it 25-100 fold more sensitive than other available [^{3}H] ligands for these receptor sites. ^{125}I-LSD displays an excellent ratio of specific to nonspecific binding, a high affinity for 5Ht$_2$ receptor sites, and a high degree of selectivity in its binding.
(Supported by NSF grant BNS 81-08080.)

101.5 CHARACTERIZATION OF SEROTONIN BINDING SITES ON HUMAN PLATELETS: METHODS FOR STUDYING SEROTONIN RECEPTOR FUNCTION IN VIVO. P.A. McBride*, J.J. Mann, B. McEwen and A. Biegon. Psychopharmacology Lab., Cornell University Medical College, NY, NY 10021.

In recent years the platelet has been increasingly utilized as a model for the serotonergic neuron. The following study was undertaken to identify the types of serotonin binding sites on the human platelet membrane, and compare their binding characteristics to serotonin receptors found in human brain.

A high affinity, saturable ^{3}H-spiroperidol binding site was demonstrated on the intact human platelet with a K_D of 2.7 ± 0.8 nM and a Bmax of 1.4 ± 0.5 pmoles/10^8 platelets. Binding was preferentially displaced by serotonin-2 (S-2) receptor antagonists such as ketanserin and cinanserin (IC_{50}s approximately 80nM), in contrast to 5-hydroxytryptamine (IC_{50} = 2.3uM), the serotonin uptake inhibitors imipramine (IC_{50} > 10uM) and fluoxetine (IC_{50} = 1.3uM), and the dopamine antagonist sulpiride (IC_{50} 10uM). Comparison of the IC_{50}s of a range of drugs for displacement of ^{3}H-spiroperidol binding to human platelets and frontal cortex showed a correlation of 0.95, $p < 0.001$ (Pearson Product Moment). These studies provide strong evidence that an S-2 receptor is present on the human platelet.

A single population of ^{3}H-serotonin binding sites was also located on the intact human platelet, which appears to represent the 5-hydroxytriptamine uptake site. The K_D of this site was 42 ± 18nM. Serotonin uptake inhibitors were the only effective displacing agents found (IC_{50}s = 10-800nM) and 1.0uM fluoxetine totally abolished detectable specific binding of ^{3}H-serotonin. A highly significant correlation ($r = 0.99$, $p < 0.001$) between the potencies of drugs as ^{3}H-serotonin displacing agents and serotonin uptake inhibitors in intact platelets presents strong evidence that ^{3}H-serotonin is bound to the uptake site. No ^{3}H-serotonin binding site with features of the serotonin-1 (S-1) receptor in brain was located on the platelet. Contrast of the IC_{50}s of multiple agents for displacement of ^{3}H-serotonin binding to platelets and frontal cortex was nonsignificant ($r = 0.31$, $p > 0.2$).

The results described above suggest that specific ^{3}H-spiroperidol binding to the intact human platelet could be used as a model to explore potential defects in central S-2 receptor activity in a number of psychiatric and neurologic illnesses where alterations of serotonergic function have been reported. These include, in particular, depression, autism, and mental retardation. Furthermore, assay of ^{3}H-serotonin binding to the platelet uptake site might supplement studies of the kinetics of altered platelet serotonin uptake reported in depressed patients.

101.6 THE USE OF [^{3}H] RO 11-2465 IN THE SOLUBILIZATION, PARTIAL PURIFICATION AND RECONSTITUTION OF PLATELET IMIPRAMINE BINDING SITES. Alan Davis, Psychopharmacology, Clarke Institute of Psychiatry, 250 College St., Toronto, Ontario, CANADA, M5T 1R8.

[^{3}H] Ro 11-2465 is a structural analogue of chlorimipramine which has high affinity for the imipramine binding site. These binding sites are believed to control serotonin transport into both platelets and CNS serotonergic neurons. In an attempt to elucidate the exact molecular relationship between the binding sites and the transporter protein, I have developed a procedure to solubilize and reconstitute the imipramine binding site.

Outdated human platelets were incubated with [^{3}H] Ro 11-2465 at 4° C. in Tris-ions buffer (50mM Tris, 120mM NaCl, 5mM KCl, pH 7.4). Under these conditions this ligand becomes effectively irreversibly bound. The platelets were solubilized with 2% sodium cholate (containing tracer amounts of ^{14}C detergent). Gel permeation chromatography of cholate-solubilized [^{3}H] Ro 11-2465 bound sites implied a Stokes radius of 4.1nm. Azolectin (Assoc. Conc's) (1 vol at 10 mg/ml) was added to 2 vol of cholate -solubilized material and the mixture dialysed overnight. Essentially no sodium cholate was associated with the cloudy dialysate obtained. Reconstituted [^{3}H] Ro 11-2465 binding sites could be retained on filters (0.45 µm pure size) and showed vesicular-like structure under electron microscopy. Table 1 shows the recoveries of the binding sites and protein into the reconstituted vesicles.

Future studies will focus on whether or not true vesicles are formed and the ability of such vesicles to transport serotonin. By such an experimental approach, it is hoped to show whether the imipramine binding site and the serotonin transporter protein reside on the same molecule, or are separate entities.

Table

	% recovery ligand	% recovery protein
Membrane-bound+	100 ± 12*	100 ± 15**
Solubilized	62 ± 8	35 ± 5
Reconstituted	16 ± 2	16 ± 6

* 2,038 ± 242 fmol
** 2,503 ± 366 µgs
\+ n=8, mean ± s.e.

Supported by the Connaught Foundation and the C.K. Clarke Psychiatric Research Foundation.

101.7 DISCRIMINATIVE CUE PROPERTIES OF QUIPAZINE: MEDIATION BY SEROTONIN-2 BINDING SITES. R.L. Friedman*, R.J. Barrett* and E. Sanders-Bush. Dept. Pharmacol., Vanderbilt University, Tennessee Neuropsychiatric Institute, and Veterans Administration Hospital, Nashville, TN 37232.

Distinct serotonin (5HT) receptor subtypes have been defined in previous radioligand binding studies. Although the value of such in vitro binding studies in identifying and characterizing 5HT-related sites is unquestionable, other approaches are required to determine the relevance of these binding sites to receptors operating in vivo. Our laboratory has focused on the development of behavioral models of 5HT binding sites using the drug discrimination paradigm.

The relative abilities of ten possible 5HT antagonists to block the discrimination of the interoceptive effects of the 5HT agonist, quipazine (Q), were assessed. Male Sprague-Dawley rats were trained to discriminate 2.5 mg/kg of Q from saline. Following acquisition of the discrimination, different antagonists were administered 90 minutes prior to a 5 minute test with 1 mg/kg of Q (which gave 75% Q-lever responding). At least 3 doses of each antagonists were tested. All of the antagonists were able to block completely the Q-cue and none substituted for Q when given alone. ID_{50} values for antagonism of the Q-cue were estimated by linear regression analyses of log-logit plots.

The binding of ^{3}H-5HT (5HT-1 site) and of ^{3}H-spiperone (5HT-2 site) was determined in crude membranes prepared from frontal cortex of naive rats as described previously (Naunyn-Schmeideberg's Arch. Pharmacol. 321: 165-170, 1982). IC_{50} values for the antagonists were determined in competition binding studies. These were compared to the ID_{50} values derived from the Q discrimination studies. A significant correlation ($r = 0.87$; $p < 0.01$) was found between the relative antagonists' affinities for the 5HT-2 site and their inhibition of Q discrimination. In contrast, no correlation existed between the Q discrimination and the 5HT-1 site ($r = 0.15$). These results suggest that the discriminative cue properties of Q may be mediated by central 5HT-2 sites. (Supported by USPHS research grant MH-34007 and a Pharmaceutical Mfg. Assoc. Foundation Predoctoral Fellowship to R.F.).

101.8 VASCULAR PREPARATIONS: USE IN THE DEVELOPMENT OF SEROTONERGIC AGENTS SPECIFIC FOR RECEPTOR SUBTYPES. E.W. Taylor*, S.P.Duckles and D.L.Nelson, Dept. of Pharmacol. & Toxicol., Dept. of Pharmacol., Univ. of Arizona, Tucson, AZ 85721.

Ligand-binding studies using (^{3}H)serotonin ((^{3}H)5HT) have shown that some tryptamine analogs can discriminate between subgroups of 5HT-1 binding sites, suggesting that some of these compounds may be useful in the development of selective agonists and/or antagonists for specific 5HT receptor subtypes. Confirmation of this has been difficult due to the lack of tests of functional 5HT-1 receptors. Recently, however, Peroutka et al. (Brain Res. 259: 327-330, 1983) reported that 5HT-induced contractions of canine basilar artery correlate with 5HT-1 as opposed to 5HT-2 binding sites. Therefore, to test the tryptamines synthesized in our laboratory for activity at functional receptors two systems were examined, the canine basilar artery as a model tissue containing 5HT-1 receptors and the rabbit femoral artery as a tissue containing 5HT-2 receptors. Preliminary experiments compared these tissues using metergoline, an antagonist with high affinity (Ki = 0.3-5.0 nM) for both 5HT-1 and 5HT-2 sites, and ketanserin, a relatively selective 5HT-2 antagonist having low affinity (Ki = 3 μM) for 5HT-1 binding sites. pA_2 measurements indicated that metergoline was essentially equipotent in the two tissues with K_B's of 1-2 nM. Ketanserin, however, showed a nanomolar K_B value in the femoral artery and a micromolar K_B value in the basilar artery. This was consistent with the existence of 5HT-2 receptors in the rabbit femoral artery and 5HT-1 receptors in the canine basilar artery. The canine middle cerebral arteries were also examined and their responses to these antagonists and various tryptamines were found to be essentially identical to those of the basilar, though the maximal contractions were weaker. Thus, in addition to the basilar, other parts of the canine cerebral circulation may also show 5HT-1 receptor pharmacology. Two series of tryptamines were selected for study in the vascular systems because ligand binding studies of the parent compounds, N,N-diisopropyltryptamine (DIPT) and morpholinyltryptamine (4-(2-(3-indolyl)ethyl)-morpholine, MLT), indicated that they could discriminate between two different 5HT-1 binding sites. Computer analysis with a two site model suggested sites with Kd values of approximately 40 nM and 4.5 μM for both compounds. 5-Benzyloxy, 5-methoxy and 5-OH analogues of DIPT and MLT were subsequently synthesized, and they all showed this discriminative ability to varying extents. Compounds from each series were tested in the vascular preparations, and it was found that the DIPT analogues acted as agonists while the MLT series were generally antagonists. 5-MethyoxyMLT and 5-OHMLT were moderately potent antagonists (K_B <1 μM), especially in the basilar artery preparation; however, MLT itself was almost inactive. The ED_{50} of the agonist DIPT in the basilar artery was in the micromolar range, suggesting that the 5HT-1 receptors in this tissue more likely correspond to the lower affinity sites identified using the ligand-binding technique. (Supported by NIH Grant NS16605).

101.9 CHARACTERIZATION OF SEROTONIN RECEPTOR SUBTYPES IN CANINE VASCULATURE. S.J. Peroutka, S.B. Banghart* and G.S. Allen*. Departments of Neurology and Neurosurgery, Johns Hopkins Hospital Baltimore, Maryland 21205.

Serotonin is a potent vasoconstrictor in many vascular smooth muscle systems. However, both drug potencies and the requirement for extracellular calcium vary greatly depending on the specific vessel studied. For example, serotonergic drug interactions with canine basilar arteries are mediated via 5-HT_1 receptors as defined by brain membrane studies (Peroutka et al, Brain Research, 259:327-331, 1983). In addition, serotonin induced contractions of canine basilar artery segments are dependent on the entrance of extracellular calcium via calcium ion channels. Serotonin induced contractions are therefore potently blocked by specific calcium channel antagonists. Analysis of canine basilar artery contractions allows the site of drug antagonism to be localized to either the serotonin receptor or calcium channel binding site.

In marked contrast, significantly higher doses of serotonin are needed to contract the canine femoral artery. Similar contractions are elicited in the presence or absence of extracellular calcium, suggesting that serotonin activates internal stores of calcium that lead to the initiation of the contractile mechanism. As a result, calcium channel antagonists have a minimal effect on serotonin induced contractions of the canine femoral artery. Drug antagonism studies suggest that serotonin induced contractions of the canine femoral artery are mediated via 5-HT_2 receptors.

In summary, 5-HT_1 receptors mediate contraction of canine basilar artery segments via the opening of specific calcium ion channels. By contrast, serotonin induced contractions of the canine femoral artery appear to be mediated by 5-HT_2 receptors and are not dependent on the entrance of extracellular calcium. Canine artery contractions provide a physiologic model for the analysis of 5-HT_1 and 5-HT_2 receptor subtypes that have previously been biochemically identified in brain membranes.

101.10 PHARMACOLOGIC CORRELATES OF 5HT-LESION SYNDROMES. M.Pranzatelli,* G. Rubin,* and S.R. Snodgrass. Neurology Research, Childrens Hospital of Los Angeles, CA 90054.

The effects of 5-HTP, fenfluramine (FF), and p-chloroamphetamine (PCA) on brain regional amine concentration (DA, NE, and 5HT by HPLC), 5HT-S_1 binding (^{3}H-5HT with 5HT as displacer), locomotor activity (photocell activity cage) and videotaped behaviors (rearing, pivoting/circling, hindlimb abduction, forepaw tremor, lateral head weaving, backing up-- as scored by "blind" observer) were evaluated in 45 adult male rats. Prior treatment included multiple intracisternal injections of 5,7-DHT or vehicle, and behavioral testing began two weeks after making the lesions.

5-HTP (65 mg/kg IP, after DHT lesioning) and FF (15 mg/kg), alone or in combination, reduced or eliminated rearing. Backing up behavior was specific to PCA (25 mg/kg) and FF. Forepaw tremor was evoked by all the drugs, but FF had a slight effect. Suppression of locomotor activity decreased in the order of FF, 5-HTP, and PCA. Neither morphine sulfate (8 mg/kg) or naloxone (3 mg/kg) prevented the FF or 5-HTP induced syndromes.

Four DHT injections caused a 90-100% depletion of 5HT concurrently in neocortex, hippocampus, striatum, septum, pons, cerebellum, and cervical spinal cord, yet did not correspondingly deplete midbrain or diencephalon more than single injections. 5HT depletions following PCA were greater in the thalamus and midbrain than those of DHT, but were similar, though lesser in magnitude, in other regions. FF induced the least depletion, and when given after DHT lesioning, did not alter the pattern or degree of 5HT depletion. Significant catecholamine depletion occurred only with FF and PCA in the midbrain.

Although the different drugs resulted in disparate patterns of regional 5HT depletion, they were able to cause similar behavioral syndromes. However, abnormal responses to 5HTP were seen only after previous treatment with DHT. The degree of 5HT depletion correlated directly with behavioral score and inversely with locomotor activity, but did not correlate with K_D or B_{max} of 5HT-S_1 binding, studied two and one-half weeks after lesioning.

101.11 HISTAMINE- AND CIMETIDINE-INDUCED DEPRESSION OF SEROTONERGIC NEURONS IN THE DORSAL RAPHE: INVOLVEMENT OF H_2 AND GABA RECEPTORS J.M. Lakoski, D.W. Gallager and G.K. Aghajanian, Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508

Histamine and the H_2-antagonist cimetidine have potent inhibitory effects when applied iontophoretically onto serotonin (5-HT)-containing cells in the dorsal raphe (DR) nucleus; this response to histamine appears not to be mediated by either a typical H_1 or H_2 receptor type (Lakoski and Aghajanian, Soc. Neurosci. Abstr., 8: 276, 1982). Both the histamine- and cimetidine-induced inhibition of cell firing were found to be selectively attenuated by the iontophoretically applied GABA antagonists bicuculline and picrotoxin, indicating these physiological effects may be mediated directly or indirectly at a GABA receptor complex. Therefore, we investigated the ability of histamine and cimetidine to displace radioligands specific for GABA (^{3}H-muscimol: ^{3}H-MUS) and benzodiazepine (^{3}H-flunitrazepam: ^{3}H-FLU) binding sites in membranes (P2 fraction) prepared from several brain regions in adult male rats.

As in previous studies, histamine was unable to displace ^{3}H-MUS binding (10^{-8}M to 10^{-3}M) in membranes prepared from cortex, hypothalamus, cerebellum and DR; likewise, ^{3}H-FLU binding was not altered by histamine. In contrast, the imidazole-derived H_2 antagonists, including cimetidine, produced GABA agonist-like inhibition of ^{3}H-MUS binding and enhancement of ^{3}H-FLU binding (DR: IC_{50} GABA = 0.20 μm, IC_{50} CIMET = 0.46 μm and EC_{50} GABA = 0.22 μm, EC_{50} CIMET = 2.20 μm, respectively). On the other hand, the non-imidazole-derived H_2-antagonist ranitidine did not significantly alter ^{3}H-MUS or ^{3}H-FLU binding. Since ranitidine did not have GABA-like binding properties we tested its ability to block the histamine-induced depression of 5-HT cell firing in the DR. Iontophoretically applied ranitidine selectively attenuated the histamine-induced but not the cimetidine- or GABA-induced inhibition of cell firing.

These data indicate that the response to histamine in the DR can be blocked by at least one type of H_2-receptor antagonist, ranitidine. The data also suggest that histamine's effect may be mediated by an indirect action at a GABA receptor complex. In contrast, cimetidine acts directly as a GABAmimetic at the GABA receptor complex in DR neurons. Further studies are needed to elucidate the ionic mechanisms underlying the GABA-, histamine- and cimetidine-induced depression of serotonergic DR neurons. (Supported by USPHS Grants MH-17871, MH-14276, Klingenstein Fund, and the State of Connecticut).

101.12 ULTRASTRUCTURAL LOCALIZATION OF HISTAMINE-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN. B.J. Wilcox[§], B. Maley[†] and V.S.Seybold[§]. [§]Department of Anatomy, University of Minnesota Medical School, Minneapolis, MN 55455 and [†]Department of Anatomy, University of Kentucky, Lexington, KY 40506.

Histamine is believed to function as a neurotransmitter in mammalian brain and has been immunohistochemically localized at the light microscopic level in neuronal elements throughout the rat brain (Wilcox and Seybold,Neuroscience Lett.,29:105,1982). In the present study, immunohistochemical methods were employed to visualize histaminergic neuronal components at the ultrastructural level.

Colchicine treated female rats were sacrificed by vascular perfusion with buffered 4.0% paraformaldehyde containing 0.2% glutaraldehyde. Each brain was removed and the hypothalamus was sectioned on a vibratome. Tissue sections one hundred micrometers thick were processed for histamine-like immunoreactivity by the PAP technique of Sternberger using the primary antiserum at a dilution of 1:1000 in phosphate buffered saline containing 0.3% Triton X-100. The secondary and tertiary antisera were both used at a dilution of 1:100 in phosphate buffered saline containing 0.3% Triton X-100. After reacting the tissue with 3,3-diaminobenzidine, the sections were osmicated, dehydrated and embedded in Spurr's resin.

When thin sections were examined under the electron microscope, staining was observed in neuronal elements located adjacent to the dorsal limit of the third ventricle near the paraventricular nucleus. This histamine-like immunoreactivity was located in neuronal structures including unmyelinated fibers, varicosities and terminals containing both small clear vesicles and large granular vesicles. The diaminobenzidine reaction product was distributed over the large granular vesicles and the cytoplasmic space. Apparent synaptic contacts were observed between histamine immunoreactive terminals and unlabeled dendrites.

The localization of histamine-like immunoreactivity in neuronal structures by electron microscopy not only provides further confirmation of a role for histamine in neuronal transmission but also suggests a possible physiological function. Its location in terminals which form apparent synaptic contacts with other neuronal structures is consistent with a possible role for histamine in regulation of some hypothalamic neuroendocrine secretions via neuronal circuitry.
Supported by NS 19312.

MORPHOLOGY OF NEURONS AND GLIA

102.1 EARLY APPEARANCE OF MACROPHAGE-LIKE CELLS IN THE PRESUMPTIVE DORSAL FUNICULUS OF EMBRYONIC MICE. L.E. Wentworth. Department of Anatomy, School of Medicine, University of California, San Francisco, CA 94143 and Basic Science Department, California College of Podiatric Medicine, San Francisco, CA.

Cells are observed in the dorsolateral spinal cord as early as embryonic day 11 (E11, where E0 = vaginal plug) which do not have the appearance of young neurons. The majority of these cells occur in the presumptive dorsal funiculus with only an occasional cell encountered in the dorsal mantle layer. Some of these cells have numerous and extensive projections of various sizes and shapes. The nuclei are often irregular and sometimes quite elongated. The cytoplasm is dense and contains vacuoles of different sizes, the contents of which vary from electron lucent similar to that of extracellular space, to debris ranging from pale amorphous material to electron-dense whorled membranes. Some of these cells are found near the entrance of the incoming dorsal roots. On examining serial sections containing dorsal roots occasionally cells have been observed in the ventral root close to the spinal cord with a process extending into the presumptive dorsal funiculus.

Microglia have been described as originating from neuroectoderm, mesenchymal elements, cells of leptomeninges, vascular adventitia or blood vessels. Cells with the ultrastructural appearance of macrophages have been described within ventral nerve roots in immature rats (Fraher, J.P. and R.D. McDougall, J. Anat., 120:537, 1975). Stensaas and Reichert (Z. Zellforsch. Bd., 119:147, 1971) described round and amoeboid microglial cells in the white matter of neonatal rabbits. Some investigators have suggested that macrophages in the CNS can transform into microglia (Imamoto, K. and C.P. Leblond, J. Comp. Neur., 174:255, 1977) and vice versa (Konigsmark, B.W. and R.L. Sidman, J. Neuropath. exp. Neurol., 22:643, 1963). Blood vessels are not observed in the area in which the macrophage-like cells are found. Additional cells in the presumptive dorsal funiculus and in and around the entering dorsal roots from E11-E14 are being serially reconstructed to see if the cells change shape with age and location and to attempt to shed some light on the origin of these cells.
Supported by NIH NS17268.

102.2 AN ANATOMICAL ANALYSIS OF A CERVICAL DENDRITE BUNDLE IN THE RAT SPINAL CORD. D. Lorton and W.J. Anderson. Indiana Univ. Sch. of Med., Terre Haute Ctr. for Med. Ed., Terre Haute, IN 47809.

In the cervical enlargement of the cat spinal cord, dendritic bundles have been described by the Scheibels (1971), which occur in the ventromedial and ventrolateral aspect of the ventral horn. Recently a survey of the cervical region revealed a similar bundle in the rat which has not been thoroughly described. This dendritic bundle organization appears to be more extensive than that described by the Scheibels. Transverse and longitudinal paraffin sections revealed three discrete dendrite bundles which traversed cervical segments. A midline bundle running from C2-C6 in the ventromedial gray matter had a diameter of 40μ and length of 5.5mm. The central bundle, in the medial aspect of the ventral horn of C3-C5, was 60μ in diameter and 2.5mm in length. The lateral bundle traversed C2-C4 in the ventrolateral gray matter and was 60μ in diameter and 4.0mm long. Alpha and gamma motoneurons, ranging from 20-70μ, were present among the dendrites as was capillaries and glial cells. Using acetylcholinesterase staining techniques, the neurons were acetylcholinesterase-positive staining, indicating these were most probably motoneurons. Golgi analysis revealed that the majority of the dendrites were longitudinally-oriented. However, the central and lateral bundles also contained small dendritic subunits that projected transversely and appeared to connect the central with the lateral and to a lesser extent with the midline bundle. Electron microscopy of these dendrite bundles has shown the presence of dendrodendritic and dendrosomatic contacts of the desmosomal type. It has been hypothesized that the dendrite bundle in the cat is involved in respiratory function (Roney, 1979). In support of this hypothesis, Cameron (1982) has found electrophysiological evidence that this dendrite bundle is highly associated with the phrenic nucleus and nerve in the cat. Perhaps the dendritic bundle organization in the rat is also involved in respiratory function.

102.3 EVIDENCE FOR CONTINUITY OF MICROTUBULES IN DENDRITES. D.E. Hillman and S. Cuccio*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016

A number of studies analyzing microtubules from axons and dendrites by 3-dimensional reconstruction suggest that neurotubules occur as interrupted, overlapping segments from 0.4 to 800 microns in length. One study supports earlier hypotheses that tubules can extend the entire length of processes. An answer to this question is important in understanding the role of microtubules in the cytoskeleton of neuronal processes. Dendrites of pyramidal and Purkinje cells have an orderly arrangement of microtubules and were analyzed by reconstruction for continuity along their course.

Rat brains were rapidly fixed by perfusion with warm, 9% glutaraldehyde in potassium phosphate buffer for chelating calcium. All microtubule appearing structures were followed in serially sectioned segments of dendrites. The results showed that profiles of microtubules begin in or near the soma and only a small percentage end along non-terminal segments. A much higher ending rate was found in segments judged to be terminals. For example in a reconstructed pyramidal cell dendritic segment, 13 µm long, one tubule of 320 was lost distally. In comparison, a small dendrite, having a diameter of 0.3 µm and sectional series extending over 7.5 µm, lost three tubules distally. Microtubules did not start for extension distally along the course of six reconstructed series that ranged in length from 5 - 13 µm.

Occasional structures in cross section had similar diameters to tubules and, when followed for one or two microns in either direction, always enlarged as endoplasmic reticular profiles. Longitudinally cut dendrites confirmed that endoplasmic reticulum frequently acquired the same size as tubules and extended for distances of up to 3 or 4 microns before returning to the larger size. Microtubules passed through sheets of endoplasmic reticulum within a closed perforation but were separated from the membrane by 20 nm. Comparison of reconstructions from conventional fixatives revealed frequent breakdown of tubules into short segments. This most often occurred in the periphery of the cross section. These results show that fixation can result in breakdown of tubules and that endoplasmic reticulum can constrict in diameter to the size of tubules. This study indicates that further analysis is needed in order to determine whether or not microtubules of the axonal cytoskeleton begin at the soma and end at or near terminals. Supported by USPHS Grants NS-13742 and HD-10934.

102.4 MAP2 IS SPECIFICALLY ASSOCIATED WITH DENDRITIC MICROTUBULES IN CEREBELLAR NEURONS. R. Bernhardt* and A. Matus (SPON: W.B. Adams). F. Miescher-Institut, P.O. Box 2543, CH - 4002 Basel, Switzerland.

A number of high molecular weight microtubule-associated proteins (MAPs) have previously been shown to occur only in Purkinje cell dendrites and not in axons (Matus, Bernhardt, Hugh-Jones, PNAS,78:3010,1981).

In the present study we have used immunohistochemical techniques with a rabbit antiserum which reacts specifically with MAP2 (MW 280 000 to 320 000) and some of its degradation products (as shown on microtubule protein gel blots) to study the distribution of the MAP2 antigen in the adult rat cerebellum.

At the light microscopic level MAP2 is detected in Purkinje cell dendrites, extending into the fine dendritic arborization. In addition, there is staining of other processes in the molecular layer which often can be identified as dendrites of basket, stellate and Golgi interneurons. A lower level of immunoreactivity is generally observed in neuronal cell bodies, but in no case is there evidence of axonal staining. For example parallel fiber axons and myelinated tracts are unstained although these fibers do react with a tubulin antiserum. Similarly, an antiserum directed against neurofilament proteins (which stains axons in the white matter and the axons of the basket cells) shows a completely different staining pattern. Non-neuronal cells are unstained by anti-MAP2.

Studies at the electron microscopic level confirm the results of the light microscopic examination. Thus, immunoreactivity is associated with dendritic microtubules of Purkinje, basket, stellate, Golgi and granular cells. There is no staining in any axon, including the readily identified parallel fibers and myelinated axons. Electron microscopic examination of neuronal perikarya shows that the somal staining observed on the light microscope is associated primarily with ribosomes, possibly suggesting that even in the adult animal MAP2 is continually synthesized.

This data suggests a specific role of MAP2 in dendritic maintenance and function.

102.5 MONOCLONAL ANTIBODIES THAT STAIN NEURONAL SOMATA IN THE RAT CEREBRAL CORTEX AND IN PRIMARY CULTURES OF DISSOCIATED CORTEX. J.P. Horn, R. Hofstein* and C.J. Barnstable. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

To study neuronal differentiation in the cerebral cortex, we have initiated a search for monoclonal antibodies against cell-specific markers. Antibody 4G9 was raised against synaptosomal membranes of adult rat brain. Antibody E15.1 was raised against membranes from cortices of embryonic day 15 rats. Antibodies were screened using a solid phase binding assay and indirect immunofluorescent staining of tissue sections.

Cortical cultures were made by mechanically dissociating the cortices of E15 rats. Neurons were defined as cells with phase bright cell bodies(8-25u) and extended processes that stained with anti-neurofilament antibodies. Upon impalement, larger cells having this morphology exhibited action potentials and synaptic potentials.

In adult cortex, 4G9 produced a bright perinuclear staining of all neuronal somata that sometimes extended into large proximal dendrites. In layer 1, which contains mainly axons and glia, faint staining of processes suggested that 4G9 also stains glia. Double labelling with 4G9 and an anti-GFA antibody showed that some of the lighter staining of processes in layer 1 was due to binding of 4G9 to astrocytes. In E15 cortex, 4G9 labelled all cells brightly. In young cultures(< 1 week), in which non-neuronal cells had not reached confluency, 4G9 produced very bright staining of neurons and their processes and weaker staining of all non-neuronal cells. As non-neuronal cells became confluent(1-3weeks), the bright neuronal staining remained and the non-neuronal staining became weaker and in some cells disappeared. Thus it appears that 4G9 recognizes an antigen(s) that is expressed by all cells in embryonic cortex and preferentially by neurons in the adult.

Similar to 4G9, E15.1 stained the perinuclear region of neurons in the adult cortex. However, in addition E15.1 stained radial processes, that grouped into regularly spaced bundles of increasing sizes as they coursed through layers 2 + 3 and then broke up in layer 1. Some of the radial fibers could be traced back to pyramids. In cultures (1-2 weeks) E15.1 stained neurons, their processes and non-neuronal cells. The non-neuronal staining had a fibrillar appearance that was distinct from that seen using 4G9. At present we cannot say how the staining of non-neuronal cells by E15.1 contributes to the staining of adult cortex. Further studies will be targeted to resolving this question and to charactering the antigens recognized by 4G9 and E15.1. (Supported by EY 07042-0551, EY 03735, NS 17309 and the Hereditary Disease Foundation.)

102.6 A SUBSET OF AXONS IN THE CHICK CILIARY GANGLION IDENTIFIED WITH A MONOCLONAL ANTIBODY AGAINST A MICROTUBULE-ASSOCIATED PROTEIN. J.P. Tremblay, C. Gravel* and R. Hawkes* (Spon: L.J. Poirier). Lab. Neurobiology, Laval Univ., Que., Canada, G1J 1Z4.

We are using monoclonal antibodies to investigate synaptic physiology in the chick ciliary ganglion. One antibody we have studied, PS2, was raised in mice against rat brain postsynaptic densities, and has been shown to recognize a microtubule-associated polypeptide antigen (Hawkes, Huber, Matus in preparation). We have used electron microscope immunocytochemistry to study the distribution of the PS2 antigen in the chick ciliary ganglion. The fixative (0.1% glutaraldehyde, 2% formaldehyde in 0.12M cacodylate buffer) was injected in the orbital cavity. The ganglion was dissected out and left in the same fixative for 45 min and transferred overnight to a fixative without glutaraldehyde. The ganglion was cut in 80um thick sections with a tissue chopper. These slices were incubated with the PS2 antibody (undiluted culture medium) for 20h at 20°C, rinsed 3 times for 10 min in buffer, incubated 2h at 20°C with a rabbit antimouse antibody coupled with horseradish peroxidase and rinsed. The peroxidase was revealed by a modified Graham and Karnovsky (J. Histochem. Cytoch. 14, 291, 1966) reaction. The tissue was then dehydrated and embedded in epon.

The antibody reveals a highly selective antigen distribution, with stain deposits restricted to the cytoplasm of a minor subset of axons, comprising less than 10% of the observed axon cross sections. Most of the antigen-positive axons are characteristically narrow and are unmyelinated but a few larger myelinated axons have also been observed. Seen in transverse section, the staining is punctate and distributed throughout the cytosol. Glancing sections through axons reveal the punctate staining to reflect a longitudinally arranged set of long fibers, probably microtubules. The cytoplasm of a few neurons is also labelled by PS2. Since the labelled processes are more abundant in the vicinity of these labelled neurons, they may well be the cells of origin of the labelled axons. We conclude that in the chick ciliary ganglion, as in the rat brain, antigenically-distinct microtubules are present in different cellular populations. (Supported by MRC awards DG 285, DG 286, DG 287 (RH) and MT5977 (JT).

102.7 IMMUNOCYTOCHEMICAL LOCALIZATION OF NEUROFILAMENTS AND RELATED PROTEINS IN THE CENTRAL NERVOUS SYSTEM. R. Hofstein[1]*, R.E. Majocha[2]*, C.J. Barnstable[1] and C.A. Marotta[2]. Departments of [1]Neurobiology and [2]Psychiatry, Harvard Medical School, Boston, MA 02115, and [2]Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Intermediate filaments are characteristic marker molecules of various cell types in the mammalian nervous system. In order to extend our understanding of both the molecular heterogeneity of these proteins and their role in cytoskeletal architecture, we have produced monoclonal antibodies against the 200,000 dalton and 145,000 dalton neurofilament polypeptides (NF 200 and NF 145). A subcellular fraction enriched for those proteins was prepared from white matter of rat or human brain. The proteins were purified by polyacrylamide gel electrophoresis (PAGE), and gel strips corresponding to 200K and 145K protein bands were excised, crushed, mixed with Freund's adjuvant and used for immunization of mice. A final injection with proteins eluted from corresponding gels was performed four days prior to fusion of the spleen cells with P3-NS1 myeloma cells. Hybrid cultures derived from the fusions were screened by a solid phase radioactive binding assay using NF fractions as the target, by immunoblots of PAGE-separated NF proteins attached to nitrocellulose membranes, and by indirect immunofluorescence on 10μ fixed frozen sections of retina and brain. Fusion of spleen cells sensitized with NF 200 gave rise to two classes of monoclonal antibodies. The first was the expected one against NF 200 which did not cross-react with the other NF proteins on blots. These gave a characteristic staining of neurofilaments in fibers of brain and retina. The second class of antibodies reacted with two proteins of approximately 240K and 160K and in retina stained primarily astrocytes and Muller cells, particularly the endfeet in the ganglion cell layer. Whether these antibodies arose as a result of some cross-reaction with NF 200 or because of a contaminating protein in the immunogen is currently being investigated. Both antisera and antibodies against NF 145 showed staining of retinal sections similar to that of NF 200 except that ganglion cell bodies and dendritic processes were clearly labeled. In addition, two unexpected classes of antibodies were produced in these fusions. The first reacted with a 160K protein and stained all nuclei in retina and also gave weak labeling of optic nerve fibers. The second reacted with a 240K protein and stained what appeared to be a membrane-associated antigen of all retinal cells.

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102.8 NEURONS MODIFY THE SHAPE OF CEREBELLAR ASTROGLIA IN VITRO M.E. Hatten Dept. Pharmacology, New York Univ. Sch. of Med., New York, N.Y. 10016

In the embryonic mouse cerebellum, proliferating precursors of granule neurons move across the rhombic lip and form the external granular layer (EGL); radial glia transform into Bergmann glia beneath the layer of proliferating young neurons. During the first two postnatal weeks, postmitotic granule neurons leave the EGL and descend into the cortex by associating with the processes of Bergmann astroglia. The present study explores whether granule neurons influence the expression of the astroglial cell shape required for neuronal migration and positioning.

Cerebellar cells harvested from C57Bl/6J mouse cerebellum at postnatal day 3 were dissociated into a single cell suspension with trypsin, passed through a 33 μm filter to remove cellular aggregates, applied to a step gradient of Percoll and enriched glial (G) and neuronal (N) fractions were collected at the three resulting interfaces. Complete separation was achieved by preplating the fractions on a polylysine-coated substratum.

In the absence of cerebellar neurons, astroglia assumed a flattened morphology and underwent cell division. Their identity was confirmed by staining with antiserum raised against purified glial filament protein (AbGF). In the absence of cerebellar astroglia, granule neurons formed cellular reaggregates which did not attach to a culture substratum coated with 10-25 μg/ml polylysine. Their identity was confirmed by staining with antisera raised against the NILE glycoprotein and by electron microscopy.

When purified astroglia were plated in the presence of increasing numbers of granule neurons (0-90% of the total cell population), the flattened shape expressed in the absence of neurons transformed into "Bergmann-like" or "astrocyte-like" shapes and specific glial-neuronal interactions were observed.

These results raise the possibility that neurons influence the differentiation of astroglia.

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102.9 RADIAL GLIA OF MATURE TURTLE CEREBELLUM: MORPHOLOGY AND PHYSIOLOGY. C. Nicholson, M. Chesler, P. Thompson* and R. Do Carmo*. Dept. Physiol., New York Univ. Med. Ctr., 550 First Avenue, New York, N.Y. 10016

Golgi studies show that the adult turtle cerebellum possesses no true astrocytes (Stensaas & Stensaas: Z Zellforsch 91: 315, 1968) but instead has a system of radial glia (RG) with cell bodies at the ventricular surface and processes that reach to the pia.

Using fine micropipettes and an isolated, superfused whole cerebellar preparation, we iontophoresed HRP into the cell bodies of RG (typical diameter 10 um). We confirmed the radial structure of the cells and showed that the processes usually branch several times. This classifies the cells as ependymal astrocytes (Tennyson & Pappas; Z Zellforsch 56: 595, 1962). The cell processes were adorned with numerous leaf-like appendages that made them similar to those of Bergmann fibers found in mammals. Using cobalt intensification, we showed also that HRP injected into one RG sometimes transfered to Purkinje, and other, cells close to the RG processes.

As with other glia, RG depolarized when $[K^+]_o$ was increased. We changed $[K^+]_o$ in 3 ways: a) superfusing the preparation with physiological salines containing different $[K^+]$ and sampling many cells at each concentration; b) changing $[K^+]_o$ while recording from one cell; c) recording the membrane potential during a spreading depression, while $[K^+]_o$ was monitored with a K^+ ion-selective microelectrode immediately outside the cell. In all cases we found that the slope (range 41-47 mV/decade) of the membrane potential versus log ($[K^+]_o$) was below that predicted by the Nernst equation. This may show that these RG accumulate K^+ when $[K^+]_o$ is elevated (Kettenmann et al: J. Neurosci. 3: 500, 1983). Comparison of the transient glial depolarization during spreading depression with the change in $[K^+]_o$ showed that the RG act as a very localized K^+ electrodes. Evidence for the continuity of the radial glial cables was obtained by passing current across the cerebellum and measuring laminar profiles of $[K^+]_o$ (Gardner-Medwin & Nicholson; J. Physiol. 335: 375, 1983). The profiles showed a complete reversal of the current-induced changes in $[K^+]_o$ from one cerebellar surface to the other.

We conclude that the radial glia of the turtle cerebellum have many of the morphological and physiological properties presently associated with astrocytes in other species.

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102.10 MONOCLONAL ANTIBODIES REVEAL THE CYTOARCHITECTURE OF THE BERGMANN GLIA FIBERS IN THE CEREBELLUM, Angel L. De Blas and Holly M. Cherwinski*. Department of Neurobiology and Behavior, SUNY at Stony Brook, NY 11794.

The cytoarchitecture of the cerebellar Bergmann glia fibers in the adult rat was investigated. Two monoclonal antibodies, one specific for the Bergmann fibers and astrocyte processes and the other specific for the cell bodies and dendrites of the Purkinje cells, as well as an antiserum to the glial fibrillary acidic protein (GFA), were used in immunocytochemical peroxidase-anti-peroxidase assays.

The Bergmann fibers are revealed as columns organized in long vertical palisades parallel to the longitudinal plane of the folium. The palisades are not continuous; instead they are formed by sets of two to six aligned Bergmann fibers. Each of these sets of Bergmann fibers is separated from its longitudinally aligned neighbors by gaps. Each Bergmann fiber is formed by a bundle of two to four Bergmann glia strands which frequently show a helical organization.

These results help to reconcile the different views on the organization of the Bergmann fibers derived from the studies done with the light microscope versus the electron microscope. The Bergmann glia may play a fundamental role in directing the geometrical organization of the cerebellar constituents. Supported by NIH grant NS-17708 and by a Basil O'Connor starter research grant from the National Foundation - March of Dimes.

102.11 A CHARACTERIZATION OF HYPERTROPHIED,MULTIVACUOLATED ASTROCYTES FOLLOWING A CEREBRAL CORTICAL LESION. James R. Connor. Dept. of Anatomy, Boston Univ. Sch. of Med. Boston MA 02118

Following a stab wound to the parietal cortex of 3 month old rats, a large (12 um diameter) astrocyte appears by 8-days later. This large cell is seen by reacting the tissue with glial filament acidic protein antisera (generously provided by Dr. L.F. Eng) and visualization of the binding sites by the peroxidase-anti-peroxidase method of Sternberger. From the light micrographs, this cell appears to contain many nonimmunoreacted areas or vacuoles. These vacuoles range in size from one to four microns. After reconstruction from one micron sections and counterstaining with thionin, the vacuoles have a basophilic nature, staining similarly to the nuclei of neighboring astrocytes. Electron microscopic examination of the immunohistochemically prepared material reveals that the multivacuolated cell is an astrocyte, frequently containing large lipid droplets. The nucleus of this cell is so grossly lobulated that as many as five distinct nuclear profiles are seen in a given section. As many as three of these profiles can contain nucleoli. Serial reconstruction at the EM level shows strands of nucleoplasm can bridge between each lobulated nuclear portion. Thus rather than portions of the nucleus budding off, perhaps in an aborted attempt to divide, it is apparent the nucleus is grossly lobulated and the many nuclear regions result in the vacuolar appearance of this cell at the light microscopic level.

Rats have been injected with ^{3}H-thymidine to determine if the convoluted nucleus of this cell is synthesizing DNA. Also tissue from lesioned animals that have been perfused for straight electron microscopy will be used to compare the hypertrophied astrocytes with the astrocytes that have multilobulated nuclei.

Whether or not this multivacuolated astrocyte is functional or in an agonal state is presently unclear. It is of interest that the multivacuolated cell is preferentially located in layer VI, the white matter, and the hippocampal sulcus. It has not been observed contacting the lesion site. In addition, the multivacuolated astrocyte does not appear until after at least 2-days post-lesion. It is present by 8-days post-lesion in 3 month old rats, but it is not seen until 16-days post-lesion in 2 year old rats. In conclusion, the multivacuolated astrocyte may be the Monsterzellen described by Penfield to occur following "chronic pathological conditions" and if so, it is also present in high numbers in multiform glioblastoma.

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102.12 A LIGHT AND ELECTRON MICROSCOPIC ANALYSIS OF THE DEVELOPMENT OF RADIAL GLIAL CELLS IN THE RAT DENTATE GYRUS. M. Rickmann* and D.G. Amaral, (SPON: JAMES W. PATRICK) The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

To determine the possible role of radial glial cells in guiding dentate granule cell precursors as they migrate from the ventricular zone to their secondary proliferative zone, we have examined the development and distribution of glial cells using standard immunocytochemical procedures for localization of the glial fibrillary acidic protein (GFAP) and routine EM preparations. At the EM level the first indication of radial glial cells (as judged by the presence of numerous glycogen particles) was on day E14. GFAP-positive radial glial fibers were first observed in the dentate gyrus on day E17. At these stages, the cells extend from the neuroepithelium lining the ventricular surface of the fimbria to the pia overlying the presumptive dentate gyrus. The proximal processes of the cells are radially oriented but they become increasingly tangential in orientation as they approach the pia. At later stages, when the dentate gyrus begins to enlarge and take on its characteristic C-shape, the glial cells retain their radial orientation with respect to the granule cell layer. It is likely, therefore, that the radial glial cells serve to guide the precursors from the ventricular layer to the secondary proliferative zone in which most of the granule neurons are generated. In support of this notion we have observed a class of immature cells that are closely apposed to the radial glial processes from day E17 onwards which are characterized by their electron dense cytoplasm and their high content of free ribosomes and polyribosomes.

By day P1 a second form of GFAP-positive cell is observed. These cells do not have a ventricular process and their perikarya are preferentially located just beneath the developing granule cell layer of the suprapyramidal blade. Both forms of radial glial cells coexist until about day P7, but thereafter only those within the hilar zone persist into adulthood. Although it is possible that the original radial glial cells become transformed into the persistent radial glia by withdrawing their ventricular processes, it seems more likely that most of the secondary cells are generated de novo, since we have observed numerous GFAP-labeled mitotic figures during the late fetal and early neonatal periods.

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102.13 TRANSITIONS IN FORM AND CYTOSKELETAL CONTENT OF CEREBELLAR ASTROGLIA. P. Bovolenta*, R.K.H. Liem*, C.A. Mason, Dept. of Pharmacology, New York Univ. Sch. of Med., New York, NY 10016.

There is increasing evidence that glial cells form templates for neuronal migration and axon outgrowth, but the identity and transitional forms of glia during normal development are not well understood. We have been studying the morphology of developing astroglia in embryonic and postnatal mouse cerebellum, particularly in relation to axon outgrowth. Monospecific antibodies to intermediate filaments found in glia - glial filament protein (GF, 51 K) and Vimentin (Vim, 58 K) - and in neurons (the 150 K component of the neurofilament triplet, NF) were applied to fixed cryostat and vibratome sections of embryos and postnatal cerebellum and visualized by peroxidase-anti-peroxidase (PAP) and fluorescence techniques at the light microscope level. Antisera to NF reveal that axons are present in the cerebellar anlage as early as embryonic day (E)15. From E15 to postnatal day (P)2 Vim-positive cells are ubiquitous and include radial forms, cells with amorphous shapes and 2-3 irregular processes, and thick longitudinal elements oriented parallel to axons within axon tracts. GF-positive cells do not appear in the cerebellum until P3-4 and by P7 there is a sudden and dramatic increase of GF-positive cells in the developing white matter with stellate shapes and long processes. These cells are more numerous and more darkly stained than at any time thereafter. Bergmann fibers begin to stain with GF by P7. Between P7 and P14 Vim and GF coexist in both astrocytes and Bergmann fibers but after this age Vim expression is progressively rarer in all astrocytes except for Bergmann fibers. From P10 onwards, GF-containing cells become more common in the granule cell layer and fewer are found in the developing white matter. This trend continues until P21 when the distribution of GF-positive astrocytes is more even throughout the cerebellum, but more sparse than at P7.

These results demonstrate that the form, disposition and cytoskeletal components of astroglia change considerably during embryonic and postnatal development. Two issues are raised: (1) The lineage and relationship of cells expressing Vim and GF. This is currently under investigation by means of double-labelling experiments with ^{3}H-Thymidine and antisera to intermediate filaments. (2) Since the appearance of GF-positive cells corresponds to the cessation of axon ingrowth and onset of myelination, axon outgrowth must occur in a terrain comprised of Vim-positive cells, thus having different cytoskeletal composition and shape than mature astrocytes, or the GF-rich astrocytes that proliferate after injury. (Supported by NIH Grants NS-16951 (CAM) and NS15182 (RKHL).

102.14 GLIAL-SPECIFIC MARKERS IN THE HORIZONTAL NEURONS OF TELEOST NEURAL RETINA. P. J. Linser* (SPON: D. Price). C. V. Whitney Laboratory of the University of Florida, St. Augustine, FL 32084

The horizontal cells of the vertebrate retina are a morphologically diverse and perhaps functionally unique class of neuron. Early histological studies of lower vertebrates raised questions concerning whether these cells are neuronal or glial in character. However, synaptic activity of horizontal cells in most vertebrates has been demonstrated and it is generally accepted that these cells are true neurons.

The focus of attention in our lab is the expression and compartmentalization of the proteins glutamine synthetase (GS), carbonic anhydrase-C (CA-C) and glial fibrilary acidic protein (GFA), all of which have been demonstrated to be glial-specific in higher vertebrate nervous tissues (Roots, B. I. J. Exp. Biol. 95: 167, 1982). Recent immunohistochemical analyses of teleost retina show that horizontal cells do indeed share certain characteristics with neuroglia. Histological sections of formaldehyde-fixed retinas from the southern flounder (Paralichthys lethostigma) were immunohistochemically stained to localize GS, CA-C and GFA. We have previously shown that GS and CA-C are found in high concentration only in the Müller glial cells of chicken retina (Linser, P. J. and Moscona, A. P.N.A.S. 78: 7190, 1981). This observation was mimicked in the flounder except that in addition to the glial cells, intense staining for CA-C was also evident in the horizontal neurons. In addition, antisera to GFA obtained from two independant sources gave marginally detectable staining of Müller cell end feet, particularly near the ciliary margin of the retina, mimicking previous observations in other vertebrate retinas (Bignami, A. and Dahl, D. Exp. Eye Res. 28: 63, 1979). However, very intense staining with anti-GFA sera was seen in the horizontal cells and their processes in the flounder retina. The presence of GFA in synaptically active neurons has not been previously described. The specificity of all antisera used in this study was verified by standard tests including immunodiffusion, immunoabsorption and immunostaining of Western blots of SDS polyacrylamide gel electrophoretograms of retina extracts. Further ongoing studies indicate that glial characteristics are likewise found in horizontal cells of other teleost and elasmobranch retinas.

Our observations thus serve to enforce the tantalizing suggestion that horizontal cells may represent a bifunctional cell type combining both neuronal and glial characteristics. Indeed, it is tempting to speculate that perhaps the horizontal cells of lower vertebrates actually represent a transitional element in the evolution of the neuronal-glial dichotomy.

102.15 HETEROGENEITY OF 50kd IMMUNOREACTIVE SITES IN THE GLIAL CYTOSKELETON* REVEALED BY MONOCLONAL ANTIBODIES A. L. Gard, J. L. Eastburn* and G. R. Dutton. Department of Pharmacology, University of Iowa , Iowa City, IA 52242.

Using immunocytochemical methods we have reported that rabbit antisera to glial fibrillary acidic protein (GFAP) specifically label rat liver Kupffer cells (Soc. Neurosci. Absts. 8: 240, 1982, in press). To authenticate the localization of GFAP to these cells, a panel of monoclonal antibodies (MCA's) was raised using C3H/OuJ mice immunized with GFAP isolated and partially purified from normal human brain (gift of Dr. L. Lapham). Hybridoma supernatants were screened by an immunoperoxidase dot binding assay on 80 ng of antigen spotted onto nitrocellulose. Positive supernatants were then incubated on frozen parasagittal sections of rat cerebellar vermis and their binding specificities visualized by indirect immunoperoxidase staining. Those MCA's that specifically bound Bergmann glial fibers and/or astrocytes in the granule layer and white matter were then incubated on Western blots of fresh cerebellar homogenate proteins electrotransfered from 10% SDS-PAGE slab gels. Three categories of antibodies were identified that label a single band at 49-50 kd which co-migrated with authentic GFAP. Group I MCA's stained all $GFAP^+$ cells in sections and in astrocyte-enriched primary dissociated cell cultures. Rat liver Kupffer cells were also stained. Group II MCA's differ from Group I in that these MCA's appeared to stain fewer astrocytes in the granule layer of the cerebellum. The medial portion of Bergmann glial fibers was not stained. In astrocyte-enriched cultures, Group II MCA's stained appproximately 75% of the $GFAP^+$ cells, but within a given cell, the pattern of staining was more restricted than that observed with a polyclonal rabbit antisera made against GFAP (gifts of Dr. R. Pruss and L. Eng). Kupffer cells were positive, but staining was apparent only in small segments of Kupffer cell processes. The cellular distribution of Group III MCA specificities was similar to those of Group II, except that the glial limitans and pial surface were not stained. In addition, the peroxidase reaction product was more finely distributed along delicate astrocyte processes than was observed with rabbit anti-GFAP antisera or the MCA's of Groups I and II. In cultures, Group II and III MCA's showed similar staining of astrocytes except that the latter also stained a small population of $GFAP^-$ flat cells. When tested by indirect immunofluorescence, MCA's in Groups I, II and III and rabbit anti-GFAP did not stain the cytoskeleton of 3T6 fibroblasts, which are known to be vimentin-positive. This work was supported by USPHS grant NS 16518 and training grant GM 07069.

102.16 DORSAL-VENTRAL DIFFERENCES IN GLIA LIMITANS IN THE IRRADIATED DEVELOPING RAT SPINAL CORD. S. A. Gilmore and T. J. Sims. Dept. of Anatomy, Univ. of Arkansas Med. Sci., Little Rock, AR 72205 and Dept. of Neurology, Stanford Sch. of Med. and V. A. Med. Ctr., Palo Alto, CA 94304.

Schwann cells develop in spinal cords of immature rats following exposure to radiation. These intraspinal Schwann cells occur consistently in the dorsum of the spinal cord and develop only occasionally in the ventral region. The intraspinal conditions which facilitate the development of these Schwann cells are not totally clear, either from studies in this laboratory or from attempts of others to graft or transplant peripheral nervous elements into the central nervous system. Recent data from this laboratory showed that the glia limitans on the dorsum of the spinal cord was markedly damaged following irradiation. The present study examines possible differences in the dorsal and ventral aspects of the spinal cord (1) at the time of irradiation and (2) during the interval between irradiation and the development of intraspinal Schwann cells.

A beam of soft x-rays (total dose, 4000R) was administered to a 5mm length of lumbosacral spinal cord in 3-day-old Charles River rats. For light microscopy, the animals were perfused-fixed with 2% paraformaldehyde and the spinal cords were embedded in JB4. For ultrastructure, the rats were perfused with a modified Karnovsky's fixative and prepared in the usual fashion. Normal 3-day-old rats and rats killed at 5 and 10 days post-irradiation were examined.

At 3 days of age the glia limitans on the dorsal surface of the spinal cord consists of 1 or 2 layers of glial processes. The axons are mostly small-caliber, are interdigitated by glial processes and are rarely myelinated. Ventrally, subpial glial cells appear more numerous, and the glia limitans is multilayered, often composed of 2 to 4 glial processes flattened against one another. More axons are myelinated ventrally than dorsally. By 10 days following irradiation a decrease in number of glia is evident light microscopically throughout the white matter. Ventrally the glia limitans shows some thinning in both irradiated and controls, but these two groups cannot be distinguished on this characteristic. In contrast, on the dorsal surface the glia limitans is markedly thinned and breaks are present. These data suggest that the glia at the ventral surface are either more mature than those dorsally or that the greater number ventrally is sufficient to maintain an intact glia limitans in the irradiated rat.

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102.17 CYTOPLASMIC ORGANIZATION IN SYNAPTIC TERMINALS ON LIZARD MUSCLE. J. P. Walrond* and T. S. Reese. NINCDS, NIH, Bethesda, MD 20205.

Axonal cytoplasm is known to contain interlocking domains defined by neurofilaments or microtubules, but little is known about how these domains end at the synapse, or how they interface with the microfilament domains containing the synaptic vesicles. Furthermore, neither the exchange of organelles between the axon and the synapse nor the fate of the filamentous systems constantly moving into the synapse is well understood. The low concentration of synapses in muscle and their complex and extensive cytoplasmic domains limits the productivity of conventional rapid freezing and deep etching for studying their cytoplasmic structure. Here we describe a new technique which provides better access to pertinent structural details in motor terminals from lizard intercostal muscles. Rapid-frozen Anolis intercostal muscles were freeze-substituted at -80°C, then warmed to 0°C and stained in 20% uranyl acetate in methanol, 0.5% hafnium tetrachloride in acetone, and 0.25% lead octoate in methanol, and finally embedded in Araldite for serial thin sectioning. The uniformity of staining and contrast were adequate to form detailed stereo images of the entire thickness of the section (~120 nm) without staining the individual sections; series of 10-15 stereo pairs permitted small cubes of presynaptic cytoplasm to be reconstructed in fine detail. Synaptic vesicles reside in distinct cytoplasmic domains which are always contiguous to regions of the cell membrane contacted by basal lamina. This invariant juxtaposition of the synaptic vesicle domains to the basal lamina leads us to suggest that the basal lamina specifies the positions of synaptic vesicle domains, perhaps by transmembrane attachments. A ring of mitochondria and membrane sacs resides at the border between the vesicle-rich cytoplasm and a central core of axon-like cytoplasm. Serial longitudinal and transverse sections through the bouton-shaped terminals have revealed that neurofilaments end in specific regions of the core cytoplasm. Individual filaments in the bundle terminate simultaneously on or near disk-like structures (discontinuity plaques). A filament bundle (though not the same filaments) resumes a similar course on the distal side of the plaque. The presence of a discontinuity plaque in several consecutive longitudinal sections indicates that the filaments do not leave the plane of section on either side of it; the neurofilaments are discontinuous at the plaques. It is therefore clear that the plaque transects the neurofilament bundle and that the neurofilaments terminate nearby. Discontinuity plaques would be obvious candidates for the sites of filament degradation in the synaptic terminal, but it is unknown yet whether they are found in the axon.

102.18 SPATIAL ANALYSIS OF THE DENDRITIC TREES OF TRICEPS SURAE α-MOTONEURONS. S. Cullheim*, R.E. Burke, J.W. Fleshman and I. Segev*. Lab. of Neural Control, NINCDS. NIH. Bethesda, MD 20205.

Complete dendritic trees of six triceps surae (TS) α-motoneurons(two each of types FF, FR and S) were reconstructed from LM serial sections after intracellular staining with HRP. Soma dimensions and the lengths of all dendritic branches were measured from the original sections. Branches were then broken into segments, depending on total branch length, and the diameters of all segments were measured. The length, diameter and 3-space coordinates of the proximal and distal ends of each segment were input to computer files, along with code names specifying position in the dendritic tree. We then examined the three-dimensional distribution of dendritic branches and of membrane area within the space centered on the motoneuron soma and orthogonal to the axes of the spinal cord. Analysis of the dendritic tree of each cell in terms of vectors based on the coordinates of branch points and/or terminations showed little evidence for strong dendritic directivity in any cell. However, when the perisomatic space was divided into 24 equal radiating pyramids, the distribution of dendritic branch points, terminations and membrane areas in these pyramids was non-random for each cell studied (χ^2 analysis). This non-randomness was most prominent in the transverse (cross-section) plane and was less strong in the horizontal and sagittal planes. Each motoneuron studied showed a different pattern of dendritic predominance, particularly in the transverse plane, and pooling the results from the six TS cells studied disclosed no consistent departure from a basically radial organization and no apparent differences correlated with motor unit type. In all TS cells studied, the membrane area present in concentric shells centered on the soma increased with radial distance to reach a maximum (mean about 12 percent of total cell area) between 700 to 800 μm from the soma, beyond which the area increments declined rapidly. All dendrites terminated within 2000 μm from the soma. Using earlier data on the spatial distribution of HRP-identified group Ia synaptic contacts on TS motoneurons (Glenn et al., Neurosci. Abstr. 8:995, 1982), we estimate that about 60 percent of TS motoneuron membrane area overlaps with the territory of Ia afferent collaterals and is available to receive Ia synapses. Within the region of overlap between the box-like territory occupied by Ia collaterals within the ventral horn (which is centered on the TS cell somata) and the radiating dendrites of TS motoneurons, there appears to be a random pattern of Ia connectivity, with no preference for proximal versus distal locations.

102.19 MATCHING ELECTROPHYSIOLOGICAL WITH MORPHOLOGICAL MEASUREMENTS IN CAT α-MOTONEURONS. J.W. Fleshman, I. Segev*, S. Cullheim* and R.E. Burke. Lab. of Neural Control, NINCDS, NIH, Bethesda, MD.

In pentobarbital-anesthetized cats, the input resistance (R_N) of triceps surae (TS) α-motoneurons was measured (spike height and pulse methods), using HRP-filled micropipettes. Averaged records of voltage decay transients following short de- and hyperpolarizing current pulses were also obtained, permitting estimates (by "peeling" exponentials) of the effective electronic length of the cell (L_{peel}) and of membrane time constant (τ_0) (Rall, Biophys. J. 9:1483, 1969). Six HRP-filled motoneurons (2 each of types FF, FR and S) were completely reconstructed from serial LM sections, the lengths and diameters of dendritic branches were measured, and data representing the soma and dendritic tree of individual cells were stored in computer files (see Cullheim et al., this meeting; Fleshman et al., Neurosci. Abstr. 8:414, 1982). Using established cable theory (Rall, Exp. Neurol. 1:491, 1959), the R_N implied by the morphology of each cell was calculated for cytoplasmic resistivity of 70 ohm-cm^2 and various values of specific membrane resistivity (R_m). The electrotonic lengths (L_i) of the paths from terminal dendrites to soma were also calculated from the morphological data. The effective overall electrotonic length of the entire dendritic tree (L_{est}) was calculated for each of cell morphology as the simple mean of the L_i's or as a conductance-weighted function of the L_i's, with and without correction for the soma-to-dendritic conductance ratio, ρ. These results were compared with L_{peel} obtained from the experimentally observed voltage transients. With the assumption that R_m is uniform, values of R_m that matched R_N gave L_{est} values much longer than L_{peel}. Furthermore, these uniform R_m values implied specific membrane capacitance (C_m) values as high as 5 - 6 μF/cm^2 in order to match the physiologically determined τ_0's. It was possible, however, to reconcile observed and calculated values for R_N and electrotonic length by assuming that R_m was non-uniform and increased with distance from the motoneuron soma. Monotonically increasing R_m also allowed us to match experimental and calculated values of τ_0 with C_m near the conventional value of 1 μF/cm^2. The presence of dendritic paths with different L_i greatly complicates evaluation of the results of voltage transient analysis (see Segev and Rall, this meeting). Constraints on the ranges of spatial variation in R_m, and the influence of possible artifactual "leak" conductances introduced by micropipette penetration, are being studied by calculating voltage transients in dynamic compartmental models based on the morphology of the reconstructed cells and comparing these with the data obtained experimentally.

102.20 THEORETICAL ANALYSIS OF NEURON MODELS WITH DENDRITES OF UNEQUAL ELECTRICAL LENGTHS. Idan Segev* and Wilfrid Rall. Lab. Neural Control, NINCDS and Math. Res. Br., NIADDK, NIH, Bethesda, MD.

A neuron's dendritic tree often includes paths from soma to dendrite terminals of different anatomical lengths. If the electrical lengths of the paths are also different, the dendritic trees of such cells cannot be strictly represented as a single equivalent cylinder. We have examined three cases analytically and with computer models: (I) two cylinders of equal diameters but different electrical lengths, L, coupled at a point of common origin (very small "soma"); (II) two cylinders of different diameters and L's, coupled at a "soma"; and (III) four cylinders with either equal or unequal diameters and different L's , coupled at a "soma". For all the cases, the passive decay of potential after a voltage perturbation anywhere in the system can be expressed as a sum of exponential decays: $V(t) = \sum C_i \exp(-t/\tau_i)$, where $i=0, ..,\infty$. The values of the time constants that can be recovered by analysis of such decay transients are sensitive to the relative values of the coefficients, C_i. If $C_1 << C_2$, we can recover τ_0 and τ_2, but not τ_1; then the method (Rall, Biophys. J. 9:1483) of peeling the exponential decay to estimate L (L_{peel}) from a time constant ratio requires re-evaluation. We addressed the question how the value of L_{peel} compares with the L values specified for each of the three cases above, for current injection and voltage decay at the "soma". In case (I) we find that when the L of each cylinder does not differ by more than 20 percent from the mean electrical length (i.e., $A = |(L_1-L_2)/(L_1+L_2)| < 0.2$), $L_{peel} \cong (L_1+L_2)/2$ (as is strictly true when $L_1 = L_2$). When $A > 0.2$, L_{peel} is increasingly dominated by the L value of the longer cylinder. In case (II), when the thicker cylinder (L_A) is longer than the thinner one (L_B), $L_A < L_{peel} < L_A+L_B$. When $L_A=L_B$, $L_{peel} = L_A = L_B$, as expected. However when $L_A<L_B$, L_{peel} is, paradoxically, longer than L_A+L_B. The latter phenomenon should be observed experimentally only in extreme cases when the ratio between the diameters of the two cylinders is bigger than 5, for very brief current and very small soma. We examined many variations of case (III). One general conclusion is, again, that if all the cylinders have the same diameters and their L values do not differ by more than 20 percent from the mean value, the L_{peel} of the system is close to the mean value. For other situations of case (III), L_{peel} is better approximated by the sum of the L values, each weighted by the cylinder input conductance (as seen from the soma), rather than by the simple average obtained by equal weighting of all L values. These results highlight the importance of weighting branches and dendrites in order to estimate L_{peel} for a whole neuron (see Fleshman et al., this meeting).

102.21 IDENTIFICATION OF INTERNODES IN THE GOLDFISH MAUTHNER AXON WITH INTRA-SHEATH INJECTION OF LUCIFER YELLOW OR HRP. Malcolm R. Wood, Paul G. Funch & Donald S. Faber. Div. Neurobiology; Dept. Physiology; SUNYAB; Buffalo, NY 14214.

The Mauthner (M-) axon of the goldfish apparently lacks the node of Ranvier characteristic of myelinated vertebrate axons. However, electrophysiological evidence has demonstrated active sites at 2.0-2.8 mm intervals along the axon in the medulla (Funch & Faber, J. Neurophysiol. 47:1214-1231, 1982). The experiments described here were desiged to determine the limits of single internodes.

In 2 series of experiments, Lucifer Yellow (LY) (31 axons) or HRP (16 axons) were pressure injected into the myelin sheath of the M-axon in the medulla. Microelectrode recordings from the sheath display a maximal voltage ≥ 50 mV produced by the M-axon action potential; ≥ 20 mV was used as the criterion for sheath penetration. HRP injections labelled the cytoplasmic belts, incisures and Schmidt-Lantermann clefts forming a complex interlacing network. Nine oligodendrocyte somata have been identified with processes in continuity with the sheaths and are located either just dorsal to the sheath or close to the midline. The broad, flattened cytoplasmic process from the soma wraps around the M-axon forming a tight spiral of 5-7 loops and then spirals more loosely. A single glial cell may contribute more than 1 process to a single M-axon sheath. Cleared wholemounts of LY and reconstructions of 30 μm vibratome sections of HRP preparations showed that dye diffusion stopped at specific loci (3.8 and 1.4 mm anterior to and 1.1 and 3.2 mm posterior to the posterior margin of the vagal lobes). The particular internodal segment stained was predictable on the basis of the rostral-caudal location of the site of dye injection, and the rostral and caudal limits of dye diffusion were unrelated to the precise location and the number of injections within any particular internode. Although single HRP injections resulted in staining of segments as long as 2.45 mm, multiple injections along a single internode never resulted in staining over a distance greater than 2.75 mm (mean=1.94 mm; SD=0.53; n=16); thus HRP diffusion was restricted to single internodes. In contrast, in 31 single injections of LY, staining of less than 2.14 mm occurred only once; in 18 injections, dye coupling was observed between adjacent internodes. The resulting bimodal distribution (m_1=2.5-3.0 mm; m_2=4.0-4.5 mm) of LY-stained segments suggests a diffusion barrier exists between adjacent internodes. These data show that a) the M-axon sheath is divided into internodes and b) their termination points coincide with the physiologically identified nodes. (Supported by NIH Grant NS 17063.)

102.PO TANYCYTES OF THE SPINAL CORD OF THE ADULT RAT: A COMBINED GOLGI-GOLD TONING STUDY. J.A. Rafols and H.G. Goshgarian Dept. Anat., Wayne State Univ., Sch. of Med., Detroit, MI 48201.

In the course of a Golgi study of the cervical spinal cord of the adult rat, numerous tanycytes were observed radiating from the ependyma into the gray matter that surrounds the central canal. In 100 μm thick Golgi sections, ependymal (E) and subependymal (S) types of tanycytes were distinguished by virtue of cell body position in relation to the ependymal lining. The cell body of both types of tanycytes has an apical pole that reaches the surface of the central canal and a basal pole that gives rise to a tail process. The tail process extends into the adjacent gray matter and sometimes into the more distal neighboring white matter. The tail ends as a foot-like process which surrounds blood vessels with cross sectional lumina measuring 6-15 μm.

After deimpregnation and gold-toning, the same tissue was embedded in plastic and sectioned for light or electron microscopy. At the ultrastructure level, gold-toned tanycytes contained fine clusters of gold particles underlining the plasma membrane of the cell body and coarse clusters of gold particles throughout the tail and foot processes. The apical surfaces of tanycytes is characterized by numerous microvilli and large, bleb-like protrusions containing mitochondria, microtubules, bundles of fibrils, ribosomes and vesicles. Cilia are found in association with the non-tanycytic ependyma. At the luminal surfaces, adjacent tanycytes are joined laterally by junctional complexes with punctate tight junctions and zonulae adherentes associated with microtubules and fibrils. Other types of junctions are found between apposing tail and foot processes of tanycytes. The foot process abutts the basement membrane of endothelial cells, pericytes, and/or smooth muscle cells of blood vessels. In addition thin astroglial sheets may interpose between the membrane of the foot process and the perivascular space. At their basal ends the lateral surfaces of adjacent foot processes form extensive infoldings which are filled with electron dense material. These basal membrane labyrinths are continuous with the basement membrane of the blood vessel and may provide an extensive surface relation for the exchange of substances between the cerebrospinal fluid and the neighboring vasculature of the spinal cord. Supported by U.S. Public Health Service grant NS-14705.

103.1 SYNTHESIS OF THE TRANSFERRIN RECEPTOR BY CULTURES OF EMBRYONIC CHICKEN SPINAL NEURONS. G.J. Markelonis, T.H. Oh, C.Y. Cha*, J.W. Kim* and P. Azari*. Dept. Anatomy, Univ. Maryland Sch. Medicine, Baltimore, Maryland, 21201.

We have purified a glycoprotein from chicken sciatic nerves, sciatin, which has pronounced trophic effects on avian skeletal muscle cells in culture. Recent studies have shown that sciatin is identical to the iron transport protein transferrin in terms of its physical properties and biological activity. In order to determine whether transferrin is synthesized and released by neuronal tissue, we incubated cultures of dissociated chicken spinal neurons in a medium free of L-leucine containing either ^{3}H-L-amino acids or ^{14}C-L-leucine and immunoprecipitated transferrin with highly specific antibodies. The radiolabeled protein precipitated by rabbit heteroclonal, goat heteroclonal or mouse monoclonal antitransferrin antibodies increased in specific activity in a linear manner for at least 30 min. Synthesis of this protein was abolished by the presence of puromycin (20 µg/ml) or cycloheximide (10^{-5} M). The disappearance of the radiolabeled protein from cells was linear with a half-life ($t_{\frac{1}{2}}$) of 8-10 h, although only about 10-12% was eventually released into the culture medium. When immunoprecipitates were separated by SDS gel electrophoresis, a prominent band corresponding to transferrin (M_r:84k) was visualized by staining with Coomassie blue. However, when such gels were fluorographed, no radioactivity was apparent in the transferrin region of the gel although a prominent radioactive band was visualized at an M_r of 56k. The protein of M_r 56k was not simply a degradation product of transferrin since this particular protein band was not generated by incubating radiolabeled transferrin with unlabeled neuronal homogenates. This protein of M_r 56k was purified from embryonic chicken brain and spinal cord by immunoabsorption chromatography on mouse monoclonal antitransferrin IgG conjugated to Sepharose 4B. As expected, the purified protein failed to crossreact with antitransferrin antibodies. From these data, we conclude that transferrin is not synthesized by cultures of chicken spinal cord neurons but that the receptor for transferrin is synthesized by this tissue and is precipitated by antitransferrin antibodies as an antigen-receptor complex. Furthermore, we suggest that the abundance of transferrin found in peripheral nerves in the chicken is due to the selective receptor-mediated uptake of this protein from the surrounding environment.

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103.2 MONOCLONAL ANTIBODIES TO CHICK CILIARY GANGLION ISOLATE A NEURAL CREST SUBPOPULATION BY FLUORESCENCE ACTIVATED CELL SORTING. K.F. Barald. Neuroscience Program, Dept. Anatomy and Cell Biology, University of Michigan Med. School, Ann Arbor, Michigan 48109.

Two highly specific monoclonal antibodies have been isolated that identify neuronal cell surface antigens present on ciliary ganglion neurones but not other neuronal or non-neuronal cells of embryonic chick and quail. The antibodies, designated CG-1 and CG-4, also label a small (≤ 5%) population of mesencephalic neural crest cells as early as 31 hrs. after fertilization. This population is believed to be the precursor of the ciliary ganglion. The antibodies are cytotoxic for the neural crest cells or the neurones in the presence of complement. Neural crest cells can be isolated from embryonic chick at 31 hrs. after fertilization, lightly trypsin-treated and grown in suspension culture. After 4 hrs., cell surface elements removed by trypsin treatment are restored. The cells can then be labeled with either antibody CG-1 or CG-4 and subsequently with a goat or rabbit anti-mouse second step antibody conjugated with a fluorescent label. Cells from at least 100 embryos are necessary for the isolation of the subpopulation by sorting labeled from unlabled cells on a fluorescence activated cell sorter (FACS).

These cells can then be placed in microwells of a 96-well tissue culture plate. Mini-assays for high affinity choline uptake, choline acetyltransferase (CAT) activity, and acetylcholinesterase (AChE) activity have been developed to characterize both the sorted and the residual population.

The antibody-positive neural crest population (APNC) possesses a high affinity choline uptake mechanism that is inhibitable by hemicholinium-3, a specific inhibitor of high affinity choline uptake. The Michaelis constant (apparent) Km for the uptake is 0.5 µM. The high affinity choline uptake Km for isolated ciliary ganglion neurones in tissue culture is 0.3 µM. Neither the general (unsorted) mesencephalic crest population nor the antibody-negative neural crest (ANNC) expresses a high affinity choline uptake mechanism. Instead, both populations express only a low affinity apparent Km of 50-75 µM. In addition, the APNC population possesses CAT activity, and autoradiography of the cultures using a technique for the identification of cells with high affinity choline uptake shows that all cells in the APNC but none in the ANNC cultures are labeled.

This result indicates that the isolated population possesses some of the properties of cholinergic neurones without expressing a neuronal morphology. The presence of both cell surface antigens and cholinergic properties supports the possibility that the FACS-isolated crest cells contribute to the ciliary ganglion. (Supported by USPHS NS-17262 and the Dysautonomia Foundation).

103.3 SELECTIVE STAINING OF PURKINJE CELLS, PYRAMIDAL CELLS, AND OTHER NEURONS WITH MONOCLONAL Ab AGAINST A GLYCOPROTEIN OF PC12 CELLS. P. Sajovic, Ety Moraru*, L. A. Greene*, and M.L. Shelanski*. Dept. of Pharmacology, N.Y.U. Medical Center, New York, NY 10016.

In order to study surface glycoproteins of the mature and developing nervous system, we have used the NGF-responsive PC12 pheochromocytoma cell line, a model neuronal system, as a source of material. Monoclonal antibodies were raised against high molecular weight glycoproteins of PC12 cells with an immunogen made by extracting the cells in 1% buffered deoxycholate, removing nucleic acids, running the extract over a wheat-germ agglutinin affinity column, and eluting with buffered N-acetylglucosamine in 0.002% deoxycholate. The eluate was then concentrated and fractionated on an HPLC sizing column. The first protein peak to come through contained three major glycoproteins at 230, 180, and 140 kd, as well as minor bands at 300 and about 80 kd (all molecular weights are SDS-PAGE estimates). This mixture was used to immunize mice, and hybridomas that produce monoclonal antibodies were made in the usual way. Hybridoma supernatants were screened by their ability to stain PC12 cells via second-antibody immunofluorescence and to recognize immobilized immunogen in a radio-binding assay. We have developed three stable clones of interest thus far, as well as control clones.

One of the clones produces an IgG which specifically immunoprecipitates a single glycoprotein of 180 kd from PC12 cells labelled with radioactive fucose or methionine and extracted in Triton-SDS. When used for immunofluorescence microscopy on PC12 cells, the antibody stains a bright rim at the perimeter of the cell. Membranous sheets which form "spikes" and partial "skirts" around attached cells can be seen; the neurites of NGF-treated PC12 cells, including growth cones, are also stained. In fixed and frozen sections of adult mouse brain, this anti-180 antibody stains some but not all neurons. Within the cerebellum, only Purkinje cells are stained. Fluorescence is strong over cell bodies and the trunks of dendrites. In hippocampus, pyramidal cell bodies stain while cell bodies in adjacent layers do not. In neocortex, pyramidal cells are brightly stained on the soma and trunk portions of the apical dendrite. We have not seen any evidence of glial staining. Further studies of the distribution of the 180 kd protein in adult brain and other tissues and in development are under way, as well as studies of its biochemistry and possible functions.

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103.4 GANGLIOSIDE LOCALIZATION AT NODES OF RANVIER AND INTERNODAL SCHWANN CELL SURFACES BY CHOLERA AND TETANUS TOXIN BINDING. A. L. Ganser and D. A. Kirschner*. Dept. of Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115.

Cholera and tetanus toxin receptors have been localized on the surfaces of myelinated, peripheral nerve fibers by indirect immunofluorescence. The toxins have high affinities for G_{M1} and G_{1b} series gangliosides, respectively. To expose the most superficial, extracellular surfaces to the toxins, mouse sciatic nerves (unfixed) were teased into individual, intact fibers.

Cholera toxin binding sites were abundant at all nodes of Ranvier; they were scarce on the internodal Schwann cell surfaces. The nodal receptors were resistant to various degradative enzymes, including trypsin and proteinase K. The enzymes did not unmask receptors on the internodal surfaces. Exogenous G_{M1} successfully competed for the toxin binding sites on the fibers. From this evidence and the specificity of cholera toxin binding, we conclude that G_{M1} ganglioside is abundantly present on the membrane surfaces of peripheral nodes of Ranvier and is not present on the internodal Schwann cell surfaces in an appreciable amount. The pattern of fluorescence within the node suggests that both Schwann cell structures and axolemma are sites where G_{M1} is localized.

Cholera toxin binding to the internodal Schwann cell surface resulted from treatment of the teased fibers with V. cholerae neuraminidase which is known to reduce polysialogangliosides to the monosialoganglioside G_{M1}. The induced receptors, as well as their precursors, were resistant to trypsin and proteinase K. We conclude that the internodal Schwann cell surface is rich in an unidentified polysialoganglioside(s) that can be converted to G_{M1} by neuraminidase.

Tetanus toxin also bound to the teased nerve fibers. Binding sites were localized exclusively to the nodes of Ranvier, i.e. no binding was apparent on the internodal Schwann cell surfaces. Proteinase K degradation of the fiber surface did not eliminate the toxin receptors at the node and did not unmask receptors on the internodal Schwann cell surface. Exogenous G_{T1b} was able to compete with the receptors at the nodes of Ranvier. These results and the pattern of nodal fluorescence with tetanus toxin suggest that the axolemma, and not Schwann cell structures, is the only site where the polysialogangliosides that bind tetanus toxin are localized.

Supported by NINCDS grant NS 14326.

103.5 A MONOCLONAL ANTIBODY (mAb) PRODUCED BY IN VITRO IMMUNIZATION AGAINST CLONAL PITUITARY CELLS RECOGNIZES A SURFACE PROTEIN ON SPECIFIC POPULATIONS OF NEURONS IN THE MOUSE CNS. G. Kapatos*, M.T. Caserta and J.L. Barker, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

The GH3 clonal pituitary cell line expresses a wide variety of membrane receptors for hypothalamic hormones. These cells are also excitable, exhibiting several classes of ion channel mechanisms. The surface of GH3 cells may thus serve as a rich and ready source of the membrane components comprising receptor and ion channel mechanisms important in neurohormonal communication. These properties have led us to use GH3 cells as immunogens in the hope of generating a panel of immunoreagents that would bind with, and identify specific receptor and ion channel determinants.

A subclone of GH3 cells grown on polylysine-treated flasks were fixed in situ with 4% paraformaldehyde. To each flask was added freshly prepared Balb/c spleen cells and media conditioned by a 48 hour incubation of a mixed culture of Balb/c and C57bL thymocytes. After 4-7 days in culture responsive spleen cells were harvested and fused 1:1 with SP2/0-Ag14 myeloma cells. Hybridoma culture supernatants were screened on fixed GH3/6 cells using an ELISA technique. From this in vitro immunization, hybridization and screening protocol a number of clones secreting mAbs were isolated. The first mAb studied, an IgM designated G1, bound to living, as well as fixed GH3/6 cells. Vibratome sections taken from fixed adult mouse spinal cord showed that G1 stained an anatomically distinct cluster of neurons. Analysis of mAb binding to fixed or living cells derived from embryonic day 13 mouse spinal cord demonstrated that G1 labelled a subpopulation of small cells that exhibited neuronal morphology and that were also stained by A2B5, an mAb which has previously been shown to bind a neuronal surface marker. Cell surface labelling by G1 in many cases was localized to synaptic boutons and that portion of the soma from which a process emanated. Flow cytometry of acutely dispersed embryonic SC elements demonstrated that the majority of the viable cells were labelled by A2B5. In contrast, only 5% of these cells were labelled by G1 at fluorescent intensity equal to A2B5. Immunoblot analysis revealed that G1 binds to the same 41 Kdalton protein extracted from pituitary and SC cell membranes.

Further immunohistochemical and immunoblot analyses have shown that the 41 Kdalton protein bound by G1 is also present on certain neurons in primary cultures derived from rodent hypothalamus, hippocampus, and cortex, as well as neurons present in adult brainstem and diencephalon. The expression of this 41 Kdalton protein in vitro and in vivo may allow us not only to study what function, if any, this protein plays in excitability, but also the biological properties of the neurons that express it.

103.6 GFA- AND NF-LIKE IMMUNOREACTIVITY IN RODENT IRIS AND ENTERIC NERVOUS SYSTEM. H. Bjorklund*, D. Dahl[1], L. Olson* and A. Seiger.* Department of Histology, Karolinska Institutet, Stockholm, Sweden, and [1]West Roxbury VA Medical Center and Harvard Medical School, Boston, MA, USA.

We have studied the appearance and distribution of cells and fibers showing GFA- or neurofilament (NF)-like immunoreactivity in whole mounts of rodent iris and in the small intestine myenteric nervous system using immunofluorescence. In adult rodents we found dense plexuses of GFA- and NF-positive fibers organized in thicker bundles as well as in thinner fibers evenly distributed over the iris. GFA- but not NF-positive cells bodies were found. Grafting of irides to the anterior eye chamber caused a total disappearance of the NF-positive fibers but left the GFA-positive plexus intact. However, in grafted as well as in host irides strongly fluorescent spider-like cells with short branching processes not seen in normal irides were visualized with GFA antiserum. Sensory denervation of the eye caused a degeneration of most NF-positive fibers. Sympathetic and parasympathetic denervations, however, caused no observable degeneration of NF-positive fibers suggesting that most, if not all, such fibers emanate from cell bodies in the trigeminal ganglion. Prenatally, NF- and GFA-positive fibers were visualized at embryonic day 18 in the rat in a gradually increasing system of fibers. In whole mounts of rat and mouse myenteric plexus GFA- and NF-positive cell bodies and fibers were observed in the ganglia of Auerbach's plexus as well as in the interconnecting strands, although most NF-positive perikarya were present within the ganglia. The NF-positive cell bodies were comparatively large with an excentric nucleus. Most of the cells seemed to be unipolar with a large axon-like fluorescent process. The GFA-positive cell bodies were more numerous, clearly smaller and had several short branching processes. Double labelling experiments showed that the GFA- and NF-positive cells represented separate populations with GFA-positive processes surrounding the NF-positive perikarya. Furthermore, cryostat sections of rodent small intestine showed that GFA- and NF-positive fibers were present in most layers of the gut wall including the intestinal villi. The ontogeny of GFA- and NF-positive fibers were studied in rat pups and embryos. We conclude that immunofluorescence with NF-antiserum can be used to study the distribution and appearance of neuronal populations in whole mounts of different peripheral tissues. Similarly, GFA-immunohistochemistry reveals a system of fibers and cells in peripheral organs likely to be peripheral glia. Supported by the Swedish MRC (14X-03185, 14P-5867, 14X-06555, 25P-6326), Magn. Bergvalls Stiftelse, Karolinska Institutets fonder, The "Expressen" Prenatal Research Foundation and the Veterans Administration.

103.7 CHARACTERIZATION AND SUBCELLULAR DISTRIBUTION OF A CEREBELLAR GLYCOPROTEIN RELATED TO FODRIN. Douglas E. Groswald and Paul T. Kelly. Div. of Biol., Kansas State Univ., Manhattan, KS 66506.

Subcellular fractions from rat cerebellum(CBLM), forebrain(FB) and non-neural tissues were examined for the presence of a 240kd glycoprotein, designated GP-A. GP-A was enriched in CBLM synaptic junction(SJ) fractions when compared to synaptic plasma membrane(SPM) fractions and was not found in FB subcellular fractions. GP-A was not detected in myelin, mitochondria, purified nuclei, or cytosolic fractions from CBLM but was present in microsomal fractions. GP-A was absent from liver, kidney and lens plasma membranes. GP-A was sparingly soluble in Triton, however, it was completely solubilized when CBLM SPMs were extracted with the ionic detergent N-lauryl sarcosinate(NLS). The extent of solubilization of GP-A from CBLM membranes in Triton was dependent on calcium at concentrations in the uM range.

Comparisons between affinity purified fodrin (Levine and Willard, JCB, 90:631-643, 1981) and the 235kd/230kd doublet in CBLM and FB synaptic fractions by 2-D peptide mapping indicated that they were identical. Peptide mapping comparisons between Con A-agarose affinity purified GP-A and GP-A in SPM and SJ fractions indicated that GP-A was apparently homogeneous in synaptic fractions. Peptide mapping of GP-A and the 235kd fodrin polypeptide indicated that these two proteins are highly related (50% of their peptides were common). GP-A is the major Con A binding glycoprotein in CBLM SJ fractions and migrates on SDS-gels with a slower relative mobility than the 235kd/230kd fodrin doublet. Fodrin polypeptides did not bind dectectable amounts of Con A. To examine the possibility that GP-A may be a highly mannosylated form of fodrin, GP-A was digested with endoglycosidase H(endo-H). Endo-H treatment removed greater than 95% of GP-A's Con A binding activity. However, on the basis of apparent molecular weight, endo-H treatment did not convert GP-A to fodrin. Both the 235kd fodrin polypeptide in SJs and affinity purified fodrin reacted reacted on Western blots with anti-fodrin antisera. However, GP-A failed to react with anti-fodrin antibodies. These observations indicate that GP-A is a CBLM-specific, membrane glycoprotein that is enriched in SJs and related to fodrin. The role of calcium in synaptic transmission, together with GP-A's decreased solubility in Triton in the presence of micromolar concentrations of calcium, suggests that GP-A may play a role in stabilizing cerebellar synapses. (Supported by NIH grant NS-15554(to P.K.) and RCDA NS 00605).

103.8 EVOLUTIONARY PATTERNS OF MOLECULAR CONSERVATION AND VARIATION IN NEUROFILAMENT PROTEIN. L. L. Phillips, R. J. Lasek and L. Autilio-Gambetti. Dept. of Anat., and Inst. of Path., Case-Western Reserve Univ., Cleveland, OH 44106.

The high affinity of the Bodian silver stain for neurofilament (NF) polypeptides, originally described in rat brain (Gambetti, et al., Sci. 213: 1521), is now known to be a property of NFs in a wide variety of species (Phillips, et al., Anat. Rec. 202: 149A). Our initial comparative study showed that the NF binding of silver stain was conserved over a long span of evolution and demonstrated the potential for variability in both NF molecular weight and subunit number. In order to better determine the NF variability among vertebrates we expanded the comparative approach using Bodian silver and immunomethods as molecular markers.

Nineteen additional species were chosen from both unexamined and previously selected vertebrate classes (mammalia, avia, reptilia, amphibia, osteoichthyes, and chondroichthyes). Half of the sampled species represented the two classes of fish, whose NFs showed the most variable pattern of molecular weights in our initial study. Samples of CNS tissue, concentrated in cytoskeleton, were separated by one-dimensional SDS-PAGE and stained with both the modified Bodian method and antibodies to NF polypeptides.

Bodian silver selectively stained a subset of polypeptides in all samples, each of which was confirmed as NF protein by immunochemistry. All of the species studied had at least one NF subunit with a molecular weight between 60-80 thousand and one or more subunits of molecular weight 130-200 thousand. Consistent with previous studies, all mammals had a NF triplet. However, non-mammalian vertebrates showed great variation in their NF subunit composition. In some of these species the NF subunit pattern consisted of only two major proteins. Other species had a triplet or even more complex patterns. This variation in NF subunit composition was observed in all of the major vertebrate classes. Selected examples of these differences are listed as follows: chicken (180,160,70), finch (180,145,68); Anolis (lizard) (160,68), turtle (140,68); Xenopus (frog) (200,145,68), newt (135,80); pufferfish (200,145,68), flounder (182,130,86,68), silver hake (145,68); dogfish (147,130,68), hammerhead shark (180,68) and lamprey (170). These observations suggest that all vertebrate NFs contain a lower molecular weight subunit which may be relatively conserved in evolution and may be particularly important in forming the basic structure of the NF. Vertebrate NFs also contain higher molecular weight components that exhibit a great deal of evolutionary variation in their apparent molecular weight. These high molecular weight subunits may provide the potential for evolutionary changes in NF structure and function among species.

103.9 ISOLATION OF ACETYLCHOLINE RECEPTOR CLUSTERS FROM CULTURED RAT MYOTUBES USING SAPONIN. W.G. Resneck* and R.J. Bloch (Spon: L. Goldman) Department of Physiology, University of Maryland School of Medicine, Baltimore, Md. 21201.

Clustering of acetylcholine receptors (AChRs) in muscle membrane is an important step in neuromuscular junction formation. Because it occurs in muscle cells cultured without nerve, it can be easily studied, to learn more about events and structures involved in postsynaptic differentiation. Such studies would be facilitated if isolated preparations of muscle membrane enriched in AChR clusters could be achieved. We have developed such a preparation.

Cultured rat myotubes are washed in buffered saline containing EGTA, $MgCl_2$ and bovine serum albumin. They are then treated for 20 min in the same solution supplemented with 0.2% saponin, with gentle shaking. After a brief lag, most (95-97%) of the cellular material detaches, but large sheets of muscle membrane remain attached to the culture substrate. Scanning electron microscopy shows that such sheets of membrane are left associated with the substrate as the rest of the myotube retracts. If cultures are prelabeled with fluorescent α-bungarotoxin (R-αBT) to visualize AChRs, the membrane sheets are found to contain essentially all (>85%) of the large, substrate-apposed AChR clusters originally in the culture. Prelabeling with ^{125}I-BT reveals that ~15% of the total AChRs in the cultures are recovered in the cluster-rich fraction. As only 3-5% of the total protein is recovered, purification of AChR clusters is >20 fold.

By following individual AChR clusters before and after isolation, we found that the isolated clusters are essentially identical to the clusters in unextracted cells. The contact domains of isolated clusters are clearly visible using interference reflection microscopy, suggesting that they are still closely apposed to the culture substrate. However, vinculin, a cytoskeletal protein closely associated with contact domains, is extracted from clusters within 5 min of addition of saponin. No other cytoskeletal proteins have been found at contact domains, suggesting that vinculin and associated structures detach from the cluster during the isolation procedure. However, actin and myosin appear to be associated with AChR domains in isolated clusters, and at least actin seems to be involved in maintaining AChR organization.

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103.10 BIOSYNTHESIS AND EXTRACELLULAR TRANSPORT OF ACETYLCHOLINESTERASE IN PRIMARY MUSCLE CULTURES. M.W.Klymkowsky*, R.I.Jappin* and L.L.Rubin. (SPON: W.V.Bleisch)The Rockefeller University 1230 York Avenue, New York, New York 10021

The appearance of acetylcholinesterase (AChE) at newly formed chick nerve-muscle synapses in tissue culture is dependent upon synaptic transmission, specifically on muscle contraction. To study the effects of muscle contraction on synaptic AChE, we have begun an examination of the synthesis of collagen-tailed (A12) AChE, the presumptive synaptic form, in primary cultures of chick, mouse, rat and Xenopus skeletal muscle. All of these types of cells synthesize the A12 form, although in the case of chick muscle its appearance is inhibited by the presence of an acid-stable, high molecular weight factor(s) present in horse serum.

When chick muscle cells were grown in a simple defined medium the monomeric (G1) and dimeric (G2) forms appeared rapidly, within 5 to 10 minutes after inhibition of total cellular AChE. They were followed by tetrameric (G4) (1-2 hrs.) and A12 (6-8 hrs.) forms. Both G2 and G4 were secreted in soluble form into the culture media; their S-values were not signficantly different from the corresponding cellular forms. Secretion of AChE was inhibited by lysosomotropic agents. A12 was not secreted into the medium. By using the membrane-impremeable AChE inhibitor echothiophate, we found that both A12 and G4 were located preferentially on the cell surface, although there was a substantial intracellular pool of both forms. The proportion of A12 and surface AChE was increased by treatment with the sodium channel activator veratridine (VER), which concomitantly decreased the amount of surface acetylcholine receptor. The sodium channel blocker tetrodotoxin (TTX) decreased the percentage of A12 and surface AChE while it increased the amount of surface acetylcholine receptor. VER and TTX had little effect on the level of total cellular AChE in cultured chick muscle.

In rat cultures, TTX decreased the total level of AChE and decreased the percentage of A12 form. In mouse, TTX decreased total levels of AChE and the percentage of A12 form transported to the cell surface, although the level of A12 as a percentage of total cellular AChE was not always decreased.

Our results indicate that the secretion of soluble AChE is activity-independent, while the accumulation of the A12, synaptic form on the surface of muscle cells is dependent on activity.

103.11 SYNTHESIS, ASSEMBLY, AND ACTIVATION OF ACETYLCHOLINESTERASE IN CULTURED MUSCLE CELLS. Richard L. Rotundo. Carnegie Institution of Washington, Dept. of Embryology, Baltimore, MD. 21210

We have studied the synthesis and processing of acetylcholinesterase (AChE) in muscle using monoclonal and polyclonal antibodies to immunoprecipitate isotopically labeled enzyme molecules. We find that chicken muscle AChE consists of at least two distinct polypeptide chains, α and β, with apparent Mr of 105,000 and 100,000. Each polypeptide chain includes approximately 12,000 daltons of asparagine-linked oligosaccharides. Immunoblotting of AChE with monoclonal and polyclonal antibodies shows that the two polypeptides share antigenic determinants. The dimeric and tetrameric forms of AChE, isolated by velocity sedimentation, appear to consist of equal numbers of α and β subunits, however the structural relationships are not yet understood.

Pulse labeling studies using ^{35}S-methionine indicate that the α and β AChE subunits are synthesized concurrently and do not interconvert. A portion of these subunits are rapidly assembled in the RER to dimeric and tetrameric forms. Comparisons of newly synthesized catalytically active AChE molecules with ^{35}S-methionine labeled AChE molecules suggest that this enzyme is synthesized as a catalytically inactive precursor. The predominant enzymatically active forms of AChE in cultured muscle cells are dimers and tetramers, whereas the major ^{35}S-metnionine labeled species are monomers and dimers. The vast majority of newly synthesized AChE molecules do not become activated but rather are degraded intracellularly with a half-life of approximately one hour. On the other hand, once activated, the AChE molecules are stable. Using a variety of pulse-labeling procedures, irreversible AChE inhibitors, immunoprecipitation, treatment with endoglycosidase-H, and lectin binding, we can show that all the catalytically active AChE molecules pass through the Golgi where further processing of the oligosaccharides occurs. However, only multimeric forms of AChE appear to follow this pathway and are exported by the cells whereas the monomers remain intracellular. In the presence of tunicamycin, a drug which inhibits the addition of asparagine-linked oligosaccharides, AChE is synthesized at least at half the control rate, yet none of these molecules are catalytically active nor are they exported by the cell. Thus glycosylation is necessary but not sufficient for activation and subsequent transport to occur. These studies suggest that post-translational controls of enzyme assembly and activation may play a role in the regulation of muscle AChE.

This work was supported by grants from the NSF, Muscular Dystrophy Association, and the Sloan Foundation.

103.12 ADULT MAMMALIAN MUSCLE FIBERS CULTURED IN THE ABSENCE AND PRESENCE OF NEURONS. D.S.Grega & J.J.Jay. Dept. Anatomy & Cell Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109.

In order to study the factors necessary for the formation and maintenance of synapses, we have begun to examine the components of the basal lamina (BL) and the distribution of rhodamine-conjugated α-bungarotoxin binding sites (R-BTX BS, putative acetylcholine(ACh) receptors as physiological confirmation is necessary), in cultures of single fibers of adult rat flexor digitorum brevis (FDB) muscle. Single muscle fibers were obtained by collagenase treatment and trituration. The effect of neurons was examined by the addition of pieces of embryonic ventral spinal cord. Fiber viability was assessed using trypan blue and/or acridine orange/ethidium bromide. In the BL,the distribution of laminin was determined using fluorescein-conjugated antilaminin and that of acetylcholinesterase (AChE) was assessed histochemically.

The continuity of the BL,as assessed by laminin distribution,is maintained after dissociation on most but not all fibers. The R-BTX BS at the endplate are clearly visible on FDB muscle ranging from freshly dissected muscle bundles to individual fibers in culture up to several days. Thus,the endplate retains R-BTX BS (and presumed ACh receptor)integrity for several days after in vitro denervation (being placed in culture). In contrast, the AChE staining of fibers changes with time in culture. At the time of culturing,the AChE reaction product is observed only at the endplate,either as a discrete endplate alone or surrounded by diffuse stain. Generally within the first day in culture only a diffuse endplate area staining is seen. By 2 days in culture,very few,if any,viable fibers demonstrate any AChE stain; note that during this same time period R-BTX BS at the endplate can still be detected . By 7 to 11 days in culture a diffuse AChE stain is observed over entire fibers often with areas of concentrated reaction product which may include the old endplate region. Thus,the time course for changes in the distribution of AChE differs than that for R-BTX BS (putative ACh receptors) for muscle fibers denervated in vitro.

Preliminary experiments with nerve-muscle cocultures indicate that there is not a directed growth of neurites to any particular region (e.g. old endplate) of the muscle fibers. Laminin distribution does not correlate significantly with areas of neurite-muscle contact. Further experiments will be done to assess ACh receptor and AChE distribution in these nerve-muscle cocultures. (Supported by grants to K.F.Barald USPHS NS-17262, NS-17017 and the Muscular Dystrophy Association. DSG is a fellow of the MDA).

103.13 A Possible Molecular Mechanism For Modification of Dendritic Spine Shape. R. Siman, M. Baudry and G. Lynch. Department of Psychobiology, Univ. of Calif. Irvine, Ca. 92717.

Although several kinds of experimental manipulations have been found to modify the number and shape of dendritic spines (for example, Lee et al., J. Neurophys. 44, 1980), the molecular mechanisms underlying these changes are not yet understood. Fodrin is a likely candidate to play a major role in regulating dendritic spine shape. This actin-binding protein lines the cortical cytoplasm of neurons (Levine and Willard, JCB 90, 1981) and is present in postsynaptic densities (Carlin et al., JCB 92, 1983). Furthermore, fodrin closely resembles erythrocyte spectrin, the principal component of the cytoskeleton that controls erythrocyte shape. We report here that a calcium-activated thiol-protease, calpain I, degrades neuronal fodrin and erythrocyte spectrin, and that proteolytic degradation of spectrin profoundly and irreversibly alters erythrocyte shape.

The calcium-activated thiol-protease calpain I was purified from cytosolic fractions from rat erythrocytes or brain and was tested for its capacity to degrade fodrin and spectrin. In the presence of micromolar calcium concentrations purified calpain I degraded both purified fodrin and the fodrin present in hippocampal synaptic membranes. Both effects were blocked by the thiol-protease inhibitor leupeptin. Addition of calcium to hippocampal membranes also caused a leupeptin-sensitive decrease in fodrin content, suggesting that endogenous calpain I can degrade fodrin. Similarly, calpain I degraded both purified spectrin and the spectrin present in erythrocyte membranes. Using erythrocyte ghosts as a model system, we tested the possibility that calpain-mediated degradation of spectrin-like proteins could be involved in the regulation of cell shape. Resealed ghosts uniformly exhibited the concave disc shape characteristic of intact erythrocytes. In contrast, ghosts that were resealed to contain micromolar concentrations of calcium were spherical with no central concavity. The calcium-induced shape change was blocked by leupeptin in concentrations that prevented spectrin degradation, and was not reversed by lysis, removal of calcium, and subsequent resealing. We propose that in dendrites, as in erythrocytes, calpain-mediated degradation of spectrin-like proteins provides a mechanism by which brief increases in intracellular calcium levels irreversibly modify cell shape.

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103.14 **CHARACTERIZATION OF A 100,000 M_r PHOSPHOPROTEIN FROM THE NERVOUS SYSTEM OF APLYSIA CALIFORNICA WHICH IS RECOGNIZED BY AN ANTIBODY TO MUSCLE PARAMYOSIN** **Flora Katz*, Tsunao Saitoh, Leland Ellis+, and James H. Schwartz. (SPON: D.J. Goldberg) Center for Neurobiology & Behavior; The New York State Psychiatric Insitute; and +Dept. of Anatomy & Cell Biology, Columbia College of Physicians & Surgeons, New York, N.Y. 10032.**

Serotonin and cAMP are known to play a role in synaptic events in Aplysia. We are currently studying neuronal proteins phosphorylated in response to these modulators. In in vitro phosphorylation reactions, we detected a major cAMP-stimulated phosphoprotein with the molecular weight of paramyosin, a contractile protein which forms the core of the thick filament of invertebrate muscle. Because, additionally, the phosphorylation of paramyosin is known to be modulated in certain molluscan muscles by serotonin and cAMP, we wanted to determine (1) if the 100K-phosphoprotein is paramyosin and (2) if the state of this molecule can be modified in response to these physiological modulators. Therefore, we have characterized paramyosin from Aplysia muscle. Using antiserum against this molecule, we identified a phosphoprotein in neurons which is recognized by this antiserum.

Paramyosin, purified from Aplysia muscle by differential ethanol and salt extractions, migrated on two-dimensional gels with M_r 100K and pI 6, and formed characteristic 72.5 nm paracrystals. Paracrystals were purified further by preparative gel electrophoresis. The paramyosin band was excised, electroeluted, and injected into rabbits. The antiserum raised precipitated a 100K M_r phosphoprotein from muscle.

Neuronal cell bodies were isolated from desheathed ganglia. Contamination by muscle proteins was undetectable. On immunoblots of homogenates of these cells, the antiserum recognizes a protein that comigrates with muscle paramyosin and whose in vitro phosphorylation is stimulated by cAMP. Moreover, this phosphoprotein comigrates with the phosphorylated muscle protein on two-dimensional gels. Together, these results suggest that paramyosin might be present in nerve cells of Aplysia. Structural studies of this neuronal molecule and its localization in nerve cells by immunofluorescence techniques are in progress.

To investigate the functional role of this paramyosin-like molecule in the nervous system, we are now studying the actions of serotonin and cAMP on this protein's state of phosphorylation and on its subcellular distribution in intact ganglia.

103.15 GANGLIOSIDES SUPPORT SPECIFIC ADHESION OF EMBRYONIC NEURAL RETINA CELLS. P. Swank-Hill*, C.C. Blackburn*, and R.L. Schnaar. Dept. of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Balto. MD 21205

Cell surface carbohydrates and carbohydrate-specific recognition proteins may mediate cell-cell recognition during neuronal development. We have recently reported an experimental system for testing the ability of gangliosides, an important class of cell surface glycoconjugates in neuronal tissue, to support specific cell recognition and adhesion in vitro (Blackburn, C.C. and Schnaar, R.L. (1983) J.Biol.Chem. 258, 1180-1188). In the present report, we have used this system to test the ability of cells dissociated from embryonic chick neural retina to adhere specifically to gangliosides purified from bovine brain or extracted from embryonic chick neural retina itself.

Neural retinae were dissected from 10-day chick embryos, radiolabelled by incubating the tissue in [^{32}P]orthophosphate, and dissociated by incubation with trypsin. The cells were resuspended in HEPES-buffered medium and aliquots were placed in polyvinyl chloride microwells which had been adsorbed with the desired lipids. The cells were centrifuged onto the lipid-adsorbed surface (60 x g, 1 min), then incubated at the desired temperature (0-37°C) and time (15-60 min). After the incubation, non-adherent cells were removed by immersing and sealing the wells in medium, inverting, and centrifuging at 500 x g. The well bottom (with adherent cells) was removed, and cell associated radiolabel was measured.

The results indicate a rapid and specific adhesion of neural retina cells to surfaces adsorbed with gangliosides. The most effective gangliosides were those extracted from the embryonic neural retina. Surfaces adsorbed with as little as 175 pmol/well of these gangliosides supported adhesion of 35-40% of the input cell population, compared to background adhesion of approx. 15%. Purified bovine gangliosides including GM1, GD1a, GD1b, and GT1b were typically less effective than neural retina gangliosides, while several lipids did not support adhesion above background levels including ceramide, the complex glycosphingolipid globoside, and the negatively charged phospholipids phosphatidyl glycerol and phosphatidic acid at concentrations up to 700 pmol/well. We conclude that neural retina cell adhesion is specific for the carbohydrate moiety of the adsorbed ganglioside and is not due to non-specific ionic or hydrophobic interactions. The ganglioside-specific adhesion was completely eliminated when adhesion was performed at 20°C or below, and was not dependent on the presence of calcium in the adhesion medium. The above results may indicate a key role for gangliosides in cell-cell recognition in the developing nervous system. Supported by NIH HD14010, GM07626 (CCB) and March of Dimes Grant 5-302.

103.16 A RETINOL-BINDING GLYCOPROTEIN SYNTHESIZED AND SECRETED BY MAMMALIAN NEURAL RETINA. C.D.B. Bridges*, S.-L. Fong*, G.I. Liou*, R.A. Landers*, F. Gonzalez-Fernandez*, P. Glazebrook*, and D.M.K. Lam. (SPON: S.J. Fliesler). Dept. of Ophthalmology, Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030

Exchange of nutrients and metabolites between the retina and pigment epithelium (RPE) entails passage across the space between these tissues. This space is filled with interphotoreceptor matrix. We have demonstrated that the interphotoreceptor matrix of bovine eyes contains a glycoprotein that has all-trans and some 11-cis retinol as its endogenous ligands (Liou et al., Vision Res., 22:1457-1467, 1982). By gel-filtration, the M_r = 260K. By SDS-PAGE the M_r = 144K. We have referred to it as interstitial retinol-binding protein (IRBP). The protein has 2-3 binding sites for retinol ($K_D \cong 10^{-6}$M) and also binds α-tocopherol, retinoic acid, cholesterol and retinal. All are displaced by retinol.

^{125}I-lectins were used to achieve a tentative determination of the type of carbohydrate chain in IRBP (Irimura and Nicolson, Carbohydrate Res., 115:209-220, 1983). Wheat-germ agglutinin (WGA) bound to IRBP, but removal of sialic acid resulted in loss of binding. Ricinus communis agglutinin I (RCA_I) bound to IRBP only after removal of sialic acid. These findings suggest the presence of terminal sialic acid residues (WGA) followed by galactose residues (RCA_I). Lens culinaris hemagglutinin (LCH) bound to IRBP, suggesting a biantennary complex structure. Experiments described below suggest that fucose is present.

Similar retinol-binding glycoproteins are present in human (M_r = 135K), rat, rabbit and frog. Since rabbit anti-bovine IRBP cross-reacts with human and rat IRBP, we have visualized IRBP in these eyes by immunocytochemistry. The results demonstrate that IRBP is confined to the space between the external limiting membrane and the apical surfaces of the RPE. It was not detected intracellularly.

Isolated rat, human and bovine retinas were incubated in media containing [^{3}H]-leucine, -fucose or -glucosamine. After 3 hrs, the medium contained labeled IRBP. Virtually no other labeled proteins were present. Medium from parallel incubations with RPE did not contain labeled IRBP. In retinas from rats of the RCS strain of retinal dystrophy, we could not detect IRBP synthesis or localization after the photoreceptors had degenerated. We conclude that IRBP is synthesized and secreted by cells of the neural retina, possibly the photoreceptors.

The function of IRBP has not been determined. It may have a role in the transport of retinol and other hydrophobic compounds between the retina and RPE.

Supported by the Retina Research Foundation of Houston and NIH/NEI. We thank T. Irimura for providing the ^{125}I-lectins.

103.17 THE INFLUENCE OF CLONED INTERFERON-γ ON THE EXPRESSION OF SURFACE MAJOR HISTOCOMPATIBILITY ANTIGENS ON MURINE NEURAL CELLS. Perry F. Bartlett* and Grace H.W. Wong* (SPON: P. Jeffrey). Laboratories of Neuroimmunology and Immunoregulation, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia 3054.

Mouse neural cells are unique in that they express very low levels of class I (H-2) and class II (Ia) major histocompatibility complex (MHC) antigens. Less than 5% of neural cells dissociated from 14 day old neonatal CBA mice have detectable levels of cell surface MHC antigens when examined using monoclonal anti-MHC antibodies on the fluorescent activated cell sorter (FACS) and this level of expression only increases marginally in isolated adult brain tissue. The paucity of MHC antigen expression probably accounts for the immunologically privileged nature of brain tissue as these antigens are essential for the initiation and regulation of immune responses. It has recently been demonstrated that interferon- γ (IFN-γ) can increase the expression of class I and class II MHC antigens on a variety of cell types (Wong et al. Proc.Nat.Acad.Sci.USA 79:6989, 1982) and we have examined the effect on neural cells. Single cell suspensions from whole brain of 2 day old neonatal mice were prepared and cultured in the presence of IFN-γ obtained from monkey cells (COS) transfected with the cloned murine IFN-γ gene. (Provided by Dr. P.Gray of Genentech Inc.). It was found that within 24 hrs of exposure to IFN-γ at the level of 1-2U/ml of antiviral activity high levels of H-2 antigens were expressed on more than 95% of viable cells. These included, astroglia, oligodendroglia and microglia as identified by double antibody labelling. In addition it was found that astroglia, identified by the presence of glial fibrillary acidic protein, would express surface Ia antigens when incubated with IFN-γ. By contrast, sensory neurons obtained from the dorsal root ganglion were not able to express MHC antigens when exposed to γ-interferon. Thus it appears that cells within the brain are capable of expressing large amounts of MHC antigens when exposed to a T-lymphocyte product γ-IFN and that this may be essential if appropriate antigen presentation and subsequent immune responses are to be initiated in the brain. The lack of MHC expression in neurons may account, in part, for the ineffectiveness of immune mechanism to combat viral infection of neurons.

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103.18 NEURONS AND MYELIN ASSOCIATED GLYCOPROTEIN SHARE A COMMON DETERMINANT. R.C. McGarry*, R.J. Riopelle and J.C. Roder*, Departments of Microbiology and Immunology, and Medicine (Neurology), Queen's University, Kingston, Canada. (SPON: C. Romero-Sierra)

We have recently demonstrated that a well characterized monoclonal antibody HNK-1 (Leu 7), directed against a surface determinant of a subpopulation of human lymphocytes called natural killer (NK) cells recognizes an antigenic determinant of myelin associated glycoprotein (MAG). HNK-1 also recognizes a surface determinant expressed on central and peripheral chick embryo neurons in vitro.

Dissociated cultures of 8 day embryo dorsal root ganglia (DRG), spinal cord, and optic lobe were enriched for tetanus toxin positive process-bearing cells by differential adhesion, defined medium and cytosine arabinoside. At various times, in vitro cells were labelled with HNK-1 using immunofluorescence or PAP techniques. HNK-1 labels both cell bodies and processes of neurons from all regions of the nervous system examined. Processes visualized at the light microscopy level by HNK-1 ramify extensively on the poly-D-lysine substrate of the culture dish.

Neurons will attach to, and extend processes on, MAG-coated culture plastic only if HNK-1 is present in the serum-free defined culture medium. Other monoclonal antibodies of the same class that do not recognize determinants on MAG or on neurons do not enhance neuron performance. These bridging data provide further evidence that the monoclonal antibody HNK-1 is recognizing a determinant shared between MAG and the neuronal cell surface.

Supported by the Multiple Sclerosis Society of Canada (RCM & JCR) and the Medical Research Council of Canada (RJR).

103.19 IMMUNOCYTOCHEMICAL LOCALIZATION OF (Na^++K^+) ATPase ALONG AXONS FROM SPINAL ROOTS OF NORMAL AND DYSTROPHIC MICE. R.G. Ariyasu, M.H. Ellisman, and J.A. Nichol*. Laboratory for Neurocytology, Department of Neurosciences, School of Medicine, University of California at San Diego, La Jolla, CA 92093.

Schwann cells in the PNS of dystrophic mice (Bar Harbor, 129 ReJ, Dy/Dy) apparently have a genetic defect in their ability to myelinate axons adequately in both ventral and dorsal spinal roots. The result is the presence of heminodes and "amyelinated" axons in regions of hypomyelination. Polyclonal antibodies raised against rat renal (Na^++K^+) ATPase were used in immunocytochemical studies to examine the distribution of the enzyme along myelinated axons from peripheral nerves. These immmunoelectron microscopic studies revealed a concentration of the $_+$ enzyme along the nodal axolemma, with an absence of (Na^++K^+) ATPase along the paranodal region. Staining of the internodal axolemma has not been observed, however, the possibility of limited accessability of antibody into this region has not been fully discounted. These antibodies were also employed to determine the distribution of (Na^++K^+) ATPase along the partially myelinated axons of dystrophic mice. Amyelinated axons were recognized by the absence of intervening Schwann cells and basal lamina and demonstrated punctate staining along the axolemma. Amyelinated axolemma in the region of heminodes revealed a similar distribution of the enzyme. In areas adjacent to paranodal loops, corresponding to the nodal axolemma of normal nodes, the distribution of (Na^++K^+) ATPase resembled the distribution in amyelinated axolemma. Occassionally larger patches of increased reaction product were observed in these regions. Enzyme distribution at complete nodes of Ranvier resembles the $_+$ distribution at nodes in normal mice. The absence of (Na^++K^+) ATPase along the paranodal axolemma of normally myelinated axons, along with the data presented above suggests that the myelination process influences the distribution of the enzyme along the axolemma of nerves. (Supported by NIH grant PHSGM 07198 to R.G.A. and NIH NS14718, MDA and NMSS grants to M.H.E.)

103.20 EXPRESSION AND DISTRIBUTION OF ENTACTIN IN EXTRACELLULAR MATRIX OF THE ADULT RAT PERIPHERAL NERVE AND PERIPHERAL NERVE CELLS IN CULTURE. C. Cornbrooks, Dept. of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

The synthesis and organization of extracellular matrix (ECM) occupies an important role in the development and regeneration of the PNS. Previous studies have demonstrated that the differentiating Schwann cell produces a number of ECM molecules and that the nature and distribution of these components varies with the development of the neuron-glial relationship. As a first step in understanding these relationships, I have begun to correlate appearance of individual ECM molecules with known events in the formation of the mature PNS. This study characterizes the location, temporal expression and cellular origin of a novel sulfated glycoprotein, entactin (JBC 256:5209, 1981) in the adult peripheral nerve and peripheral nerve cells in culture. Affinity-purified antibodies, directed against entactin were used as immunological reagents to visualize the expression of the antigen at light microscopic resolutions. Indirect immunohistochemical staining of unfixed, frozen 8 μm cross sections of adult rat sciatic nerve revealed a pattern characterized by discrete rings around each Schwann cell-neuron unit in the endoneurium and concentric lines in the perineurium. In order to correlate the expression of entactin with cell-cell interactions in the PNS, neurons, Schwann cells and fibroblasts (in various combinations) were established in culture. Pure populations of intact neurons (derived from either dorsal root or superior cervical ganglia), fibroblasts or Schwann cells were not stained regardless of the time in culture. However, Schwann cell cultures containing fibroblasts demonstrated glial membrane fluorescence and a random, fibrillar staining pattern in areas where both cell types co-localized. Schwann cells in contact with neurons in defined medium (which proliferate but do not differentiate to ensheath axons) were not stained unless permeabilized with 95% ethanol. Schwann cell-neuron cultures (maintained in serum/embryo extract supplemented medium) which differentiate and form an organized, mature ECM were intensely stained in a linear pattern identical to the distribution of basal lamina (BL). This linear pattern was maintained in similar cultures which were allowed to form a mature ECM, denervated and immunohistochemically examined one month later.

These results indicate that entactin is synthesized by Schwann cells regardless of neuronal contact and is organized onto intact tissue where fibroblasts are present and/or when mature BL are organized around ensheathed or myelinated axons. Furthermore, entactin once added to the mature BL, remains as a stable component even though the integrity of the neuron-glial relationship is compromised.

104.1 CULTURED CHICK MYOTUBES INFECTED WITH A TEMPERATURE-SENSITIVE ROUS SARCOMA VIRUS: DEFECT IN ACETYLCHOLINE RECEPTOR CLUSTERING, Donna T. Anthony* and Lee L. Rubin. The Rockefeller University, New York, NY 10021.

Motor innervation of embryonic skeletal muscle fibers causes acetylcholine receptors (AChRs) to accumulate at synaptic sites. The process of receptor aggregation on cultured embryonic myotubes has been studied extensively, but is still not well understood. Normal chick myotubes cultured at 37° or 42° show some AChR clusters, and the number of clusters can be increased several-fold by treatment with a high salt extract of Torpedo californica organ or with a soluble factor derived from chick brain.

Transformation of myoblasts by temperature sensitive (ts) mutants of Rous Sarcoma Virus (RSV) has been used in the past to study events occurring during cellular differentiation. Chick myoblasts transformed by tsNY68 fuse to form multinucleated myotubes at 42°, the nonpermissive temperature for transformation. At 42° both normal and transformed muscle cells produce similar levels of AChR and acetylcholinesterase. The AChR channels of transformed myotubes show kinetics characteristic of embryonic chick muscle AChR channels. However, tsNY68-infected myotubes do not cluster AChRs at 42°, even in the presence of active clustering agents. This phenomenon is not merely a result of viral infection, since myotubes infected with a transformation-deficient RSV — td-107A which lacks the src gene — behave like non-infected myotubes with respect to receptor-clustering; thus, the effects of tsNY68 on the clustering process must be mediated by the src gene product. It is known that viral transformation produces cytoskeletal alterations; our observation in tsNY68-infected myotubes suggests that residual $pp60^{src}$ kinase activity at 42° may phosphorylate cytoskeletal factors necessary for the formation or maintenance of AChR clusters.

104.2 MONOCLONAL ANTIBODIES TO THE α SUBUNIT OF ELECTROPHORUS ACETYLCHOLINE RECEPTOR CROSS REACT WITH A COMPONENT IN THE SYNAPTIC MEMBRANE OF CHICK CILIARY GANGLIA. Michele H. Jacob, Darwin K. Berg, and Jon M. Lindstrom. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093; and The Salk Institute, San Diego, CA 92138.

Chick ciliary ganglion neurons receive cholinergic transmission from preganglionic terminals. To identify markers for nicotinic acetylcholine (ACh) receptors on the neurons, we have tested monoclonal antibodies (mAbs) raised against purified ACh receptors from other sources for cross reactivity with membrane components on the neurons. We have found that mAbs which recognize the "main immunogenic region" (MIR) of the α subunit of skeletal muscle and electric organ ACh receptors (Tzartos et al., J. Biol. Chem. 256:8635 (1981)), and which cross react with components in the lateral spiriform nucleus of chick brain (Swanson et al., Proc. Natl. Acad. Sci.: in press), also recognize a component present at chemical synapses on chick ciliary ganglion neurons.

Monoclonal antibody 35 (mAb 35), which was raised against Electrophorus ACh receptor and recognizes the MIR, was conjugated with horseradish peroxidase (HRP-35) and used to label 16-d embryonic chick ciliary ganglia. Ultrastructural examination of labeled ganglia revealed HRP reaction product associated predominantly with synaptic membranes, often filling the synaptic cleft. Synapses were identified by relatively straight pre- and postsynaptic membranes, a wide synaptic cleft, the accumulation of synaptic vesicles along the presynaptic membrane, and a fibrous postsynaptic density. Reaction product was also present to varying extents along the surfaces of small processes which project out from the postsynaptic cell in the vicinity of the presynaptic terminals. Labeling of the processes was usually light and no reaction product was observed elsewhere on the neuron surface membrane. Synaptic and process labeling were specific in that omission of HRP-35 or co-incubation of the ganglion with HRP-35 and excess unlabeled mAb 35, mAb 203, or mAb 6 (mAbs specific for the same region of the MIR) resulted in no significant HRP reaction product. Competition with non-immune serum had no effect.

The distribution of HRP-35 binding is different from HRP-labeled α-bungarotoxin binding. HRP-α-bungarotoxin binding results in heavy labeling of the processes but no labeling of synaptic membranes on ciliary ganglion neurons (Jacob & Berg, J. Neurosci. 3:260 (1983)). Whether the antibody binding at synapses reflects the ACh receptor or some other synaptic component remains to be determined. (Supported by NIH grant NS12601, the Muscular Dystrophy Association, & the American Heart Association.)

104.3 SUBCELLULAR LOCALIZATION OF [^{3}H]-FLUNITRAZEPAM BINDING SITES IN THE RAT HIPPOCAMPUS: AN E.M. AUTORADIOGRAPHIC ANALYSIS. M.B. Laskowski, B.L. Roth*, A.J. Lechner*, D.B. Bennett* and C.J. Coscia. Department of Physiology and E.A. Doisy Department of Biochemistry, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Binding studies on subcellular fractions from brain have revealed that receptor enrichment occurs in both synaptic plasma membranes (SPMs) and microsomes for several transmitters including acetylcholine, dopamine, norepinephrine, serotonin and enkephalins. To determine whether benzodiazepine receptors were also localized in these two fractions of adult rat hippocampi, binding assays were performed with the agonist, [^{3}H]-flunitrazepam. SPMs and smooth microsomes were isolated by sucrose density centrifugation (Roth et al., J. Biol. Chem. 256, 10117, 1981). Benzodiazepine receptors were photoaffinity labeled by UV irradiation in the presence of 3 nM [^{3}H]-flunitrazepam with or without 10 μM unlabeled diazepam. Specific binding was determined by glass fiber filter, centrifugation and electrophoretic assays. Upon SDS gel electrophoresis of photoaffinity-labeled membranes, a single protein band was resolved that contained 2000 dpm (nonspecific binding = 100 dpm). By filter assay specific binding was 332 fmol/-tube (0.5-1 mg protein) for SPMs and 175 fmol/tube (0.2-0.4 mg protein) for microsomes. Membranes photoaffinity labeled in this manner were also fixed and processed for EM autoradiography. Sections were placed on gold grids, coated with emulsion and incubated at 0°C for three months. Autoradiographs were processed with gold-latensification followed by fine grain development. The resultant grain density was 3.5 grains/1000 μm^2. Each grain was photographed at 27,000x and an area 1.35 μm to the left (equivalent to 10 half-distances) was similarly photographed. Final prints were analyzed while remaining blind to the fraction and treatment identities. Over each grain a mask was placed consisting of the 50% probability circle (0.467 μm diameter) containing 30 points for volume density analysis. A similar volume density analysis was made on the area ten half-distances from the grain. The data reveal that in microsomal fractions exposed to [^{3}H]-flunitrazepam, more grains were associated with flattened saccules than would be expected by chance alone. No significant differences in grain distribution were found in microsomes exposed to [^{3}H]-flunitrazepam in the presence of 10 μM diazepam. SPM fractions contained synaptosomes as well as vesicles that were larger (0.29 μm) than those in microsomal fractions (0.19 μm). Grains in SPM fractions were not preferentially associated with vesicles of any specific size. These results provide the first evidence for the localization of brain microsomal receptors on a specific organelle and suggest a preferential association of benzodiazepine receptors to Golgi-like elements in microsomes. (This work was supported by NSF Grant BNS-81-14947.)

104.4 THE PRESENCE OF INSULIN RECEPTORS IN PRIMARY CULTURED BRAIN CELLS J. A. Weyhenmeyer, I. Reynolds* and A. Killian*. College of Medicine, University of Illinois, Urbana, IL 61801.

Recent evidence has indicated the presence of high affinity binding sites for insulin on cultured brain cells from the fetal rat that are associated with the incorporation of uridine and thymidine into macromolecules, suggesting that these receptors are involved in the biological responses of insulin in CNS tissues. In this study, we report the localization of insulin receptors in primary cultured brain cells with the use of a specific insulin receptor antibody.

Dissociated cells from the fetal brain (E.D. 20) of the Sprague Dawley rat were cultured as previously described (Neurosci. Lett. 16: 41). Aliquots of 2.8 x 10^6 cells in DMEM containing 10% fetal calf serum were inoculated onto 35 mm tissue culture dishes containing 25 mm glass coverslips. Medium was removed on the third day and replaced with either N1 or N2 medium. The medium was changed on days 5 and 7, and the cells were used for experiments on day 8. Cells were stained for the presence of insulin receptors according to the peroxidase anti-peroxidase method. Insulin receptor antibodies (obtained from Dr. S. Jacobs, Wellcome Research Labs) were raised by the polyclonal method using purified (insulin-agarose affinity chromatography) insulin receptors from rat liver membranes.

Insulin receptors were localized on approximately 5% of the neuronal population. In addition, the majority of the cells in the background monolayer, which are predominantly of glial origin, stained positively. When cultured brain cells were fixed prior to incubation with the antibody, the staining was relatively homogenous over the neuronal cell body and along its fiber profiles. When insulin receptor antibodies were incubated with the brain cells for 30 min prior to fixation, microaggregates or clusters of labeled insulin receptors were observed on the cell surface.

In this study, we report the immunocytochemical localization of insulin receptors on neuronal and non-neuronal cells in primary cultures from fetal rat brain. Insulin receptors were found to be either uniformly distributed or clustered on the cell surface in relation to the pre- or postfixation of the cells prior to incubation with specific insulin receptor antibody.

This work was supported by NIH grant HL 27757 to J.A.W.

104.5 EFFECTS OF DIVALENT CATIONS, CHELATORS AND NUCLEOTIDE TRIPHOSPHATES ON THE IN VITRO STABILITY OF GLUCOCORTICOID RECEPTORS IN BRAIN. C.L. Densmore*, W.G. Luttge and S.M. Emadian*. Dept. of Neuroscience, Univ. of Florida Coll. of Medicine, Gainesville, FL 32610.

In vitro studies in a variety of tissues and cell types suggest that glucocorticoid receptor binding capacity is not static and that binding sites are subject to up- and down-regulatory mechanisms. Studies reported here investigate effects of divalent cations, heavy metal chelators and nucleotide triphosphates on these potential regulatory mechanisms in brain.

All studies used CD-1 mice adrenalectomized and ovariectomized 5-8 days before perfusion with buffered saline, decapitation and removal of brain and liver. In our standard buffer (10 mM 4[2-hydroxyethyl]-1-piperazineethanesulphonic acid [HEPES], 1 mM dithiothreitol, 10% glycerol [w:v], pH 7.6), brain cytosol exhibited a temperature-dependent loss of [^{3}H]dexamethsone binding capacity when incubated without steroid. Incubation at 12°C resulted in a 50% loss of binding capacity after 180 min (t/2). Addition of 5 mM $MgCl_2$ resulted in a 15% decrease in the t/2 whereas addition of 5 mM $CaCl_2$ resulted in a 70% decrease in t/2. Dose-response studies revealed that reducing the $CaCl_2$ to even 0.25 mM still produced a significant 20% reduction in the t/2. Addition of ethylenediaminetetraacetate (EDTA) resulted in complex, dose-dependent effects on receptor stability. In brain cytosol containing 750 µM EDTA the t/2 was reduced by 65%, whereas an EDTA concentration of 16 mM resulted in a 90% increase in t/2. Similar results were obtained in liver with EDTA. Simultaneous addition of 5 mM $CaCl_2$ and 5 mM EDTA resulted in a 15-fold increase in t/2 whereas simultaneous addition of 5 mM $MgCl_2$ and 5 mM EDTA resulted in a 9-fold increase in t/2 over control. When 16 mM EDTA was added to cytosol incubated previously for 2 hr at 12°C, stabilization again resulted but no recovery of lost binding was observed. Ethyleneglycol-bis-N,N'-tetraacetate (EGTA) and O-phenanthroline provided some stabilization, but were less effective than EDTA. An adenosine triphosphate (ATP)-dependent (1 mM) increase (2 to 4-fold) in t/2 resulted from the incubation of crude homogenate or low speed (1000-17000 x g) supernatants although ATP had no significant effect on high speed (109000 x g) cytosol. The ATP-dependent increase in stabilization was recovered upon adding the particulate "microsomal" fraction isolated from 17000 x g supernatant back to cytosol. Other nucleotide triphosphates proved to be ineffective at stabilizing the receptor. Although a clear demonstration of in vitro up-regulation of binding sites was not provided by these studies, the significant degree of stabilization afforded these receptors by the above conditions is consistent with the proposal that glucocorticoid receptor binding may be modulated in vivo by a complexity of factors present in both soluble and particulate fractions.

104.6 BINDING OF ^{125}I-TETANUS TOXIN TO NEURONAL CELL LINES IN CULTURE, T.B. Rogers. Department of Biological Chemistry, University of Maryland, School of Medicine, Baltimore, MD 21201.

Many neuroblastoma cell lines do not display high affinity tetanus toxin receptors. However we have characterized tetanus toxin receptors in several cell lines including the pheochromocytoma cells, PC12, and a retinal ganglion hybrid, N18 RE105. When ^{125}I-tetanus toxin (0.1 - 0.3 nM) was incubated with these cells specific binding was observed. The binding was specific in that addition of mixed brain gangliosides (1 µM), tetanus antitoxin or unlabeled tetanus toxin (2 µg/ml) nearly completely inhibited binding. Tetanus toxoid (5 µg/ml) and tetanus toxin that had been incubated in boiling water for one hour (2 µg/ml) did not compete for ^{125}I-tetanus binding. When ^{125}I-tetanus toxin (0.22 nM) was incubated with cells in suspension the binding reached a maximum in 1.5 hr at 0°. Under these conditions PC-12 cells bound 1.6 pmol of ^{125}I-toxin/mg of protein and the N18 RE105 cells bound about 5-fold more toxin relative to total cell protein. Preliminary data suggest that PC-12 cells grown in the presence of nerve growth factor bind about 30% more ^{125}I-toxin than the untreated cultures, relative to total cell protein. The affinity of the receptors was measured in competition binding assays using unlabeled tetanus toxin. The following IC_{50} values for tetanus toxin were measured: rat synaptic plasma membranes, 6 nM; PC12 cells, 65 nM; N18 RE105 cells, 60 nM. There was no indication of toxin degradation during the incubation. The properties of the N18 RE105 receptor-toxin interactions were studied in more detail and were compared to those of rat cortical synaptic plasma membranes. Both systems showed similar dependencies on pH, cation concentration and temperature. The binding decreased with increasing pH; there was a 70% decrease in binding when the pH was increased from pH 6 to pH 9. NaCl (120 mM) inhibited binding by 80% and there was a 30% decrease in specific ^{125}I-tetanus toxin bound when the incubation temperature was increased from 4° C to 37° C. The reversability of ^{125}I-tetanus toxin bound to N18 RE105 cells was found to be temperature dependent. When the cells were incubated at 4° C for 1.5 hours nearly 80% of the bound toxin could be removed from the cells by the addition of 1 µM mixed brain gangliosides. If the incubations were done at 37° less than 10% of the bound ^{125}I toxin could be removed by 1 µM gangliosides. These data show that several cell lines contain receptors of tetanus toxin with similar properties as those that have been characterized in brain membranes. (Supported in part by USARMDC grant DAMD17-83-C-3114)

104.7 THROMBIN BINDING TO CELLS IN MURINE SPINAL CORD CULTURES. E.D. Means, D.K. Anderson, M. Fitzgerald*, C. Price*, J.W. Fenton II*, & J.L. Lessard*. Spinal Cord Lab, VAMC and Depts. of Neuro. & Physiol., Univ. of Cinti. Col. of Med., Cinti., OH. 45220 and Cent. for Lab & Res., N.Y. State Dept. of Health, Albany, N.Y. 12208.

Alpha-thrombin a serine protease involved in hemostasis is known to bind to cell surface receptors of platelets and certain cell types. Snider and colleagues (Fed Proc, 42:883, 1983) recently described thrombin binding to neuroblastoma cells and proposed that thrombin may be involved in certain pathological processes such as stroke or CNS trauma.

A polyclonal antibody was developed to alpha-thrombin in rabbits and subsequently affinity purified. The antibody was tested for reactivity with the ELISA System. One nM alpha-thrombin was reacted to cells in dissociated murine spinal cord cultures (12-14 day embryos). The cells were fixed in acetone and reacted with antibody (1/500) overnight after treatment with goat serum (1/100). This was followed by biotinylated goat antirabbit IgG (1/400) and finally, avidin-FITC (1/1000) or avidin-peroxidase (1/160). Cells identified as neurons and some glial stained heavily, while other cells, probably fibroblasts, stained weakly or not at all. Hirudin, a potent thrombin inhibitor, actually enhanced staining but produced significant clustering. Hirudin plus DIP-thrombin decreased staining. Negative controls including the absence of primary and linking antibodies eliminated most staining. The clustering produced by hirudin and alpha-thrombin was possibly attributed to an enzymic function of thrombin on the cell, leading to an irreversible event rendering thrombin inaccessible to hirudin.

Alpha-thrombin is known to produce alterations in the platelet membrane by activating phospholipase A_2, which releases fatty acids (arachidonic) from membrane phospholipids. Arachidonate may be important in the pathogenesis of several CNS diseases. Thrombin is extravasated from the blood vessel into CNS parenchyma following trauma or other hemorrhagic injuries. We propose that thrombin, by altering the neuronal membrane, may play a role in cell death following hemorrhage into the CNS.

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104.8 NGF RECEPTORS ON CENTRAL AND PERIPHERAL AXONS OF DORSAL ROOT GANGLION NEURONS IN VIVO. R.J. Riopelle and P.M. Richardson Division of Neurology, Queen's Univ., Kingston, Canada and Division of Neurosurgery, McGill Univ., Montreal, Canada

Previous studies of retrograde transport of NGF have indicated the presence of receptors at or near peripheral terminals of sensory axons. To investigate the distribution of NGF receptors along the course of peripheral and central axons of primary sensory neurons, ^{125}I-NGF was injected into the spinal cord or sciatic nerve of adult rats. For most studies 0.1-40 ng ^{125}I-NGF (30-60 microCi/microg) was injected in a volume of 1 microl intraneurally or 2-4 microl intraspinally. Uptake and retrograde transport were monitored by gamma emission counting and radioautography of lumbar DRG.

^{125}I-NGF injected into the sciatic nerve was taken up by a saturable high affinity process with K_M 0.2 pmoles and Vmax 1.0 fmoles. Receptor density was estimated to be at least 10^5 binding sites per cm segment of axon. The specificity of uptake of NGF was indicated by the observations that negligible activity accumulated in DRG after comparable sciatic injection of ^{125}I-cytochrome C or ^{125}I-oxidized NGF. To study transport to DRG in central axons, ^{125}I-NGF was injected into the cervical, thoracic or lumbar regions of the spinal cord. ^{125}I-NGF injected into the conus medullaris was transported to lumbar DRG at a rate not more than 5mm/h. K_M and Vmax were 0.5 pmoles and 0.5 fmoles respectively. In radioautographs, label was concentrated in large neurons. Following cervical or mid-thoracic injections of ^{125}I-NGF, significant gamma activity was detected in lumbar DRG compatible with retrograde axonal transport of 0.02-0.03 fmoles NGF/DRG. Activity in DRG was not above background following cervical or lumbar injection of ^{125}I-cytochrome C. Labelled neurons were not found in the brainstem or lumbar grey matter after cervical injection of ^{125}I-NGF. Thus, ^{125}I-NGF was taken up specifically and selectively by spinal axons of DRG neurons.

NGF receptors on primary sensory neurons in the adult rat are not limited to peripheral axon terminals but are extensively distributed along both peripheral and central axons. Axonal receptors provide a mechanism whereby NGF of neuroglial origin could influence neuronal maintenance and axonal regeneration.

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104.9 ANALYSIS OF ASTROGLIAL BETA-ADRENERGIC RECEPTOR (β-AR) AND βAR-REGULATED GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) PHOSPHORYLATION FOLLOWING TREATMENT WITH BRAIN EXTRACT AND DIBUTYRYL CYCLIC AMP. Teven A. Harmon* and Ken D. McCarthy* (SPON: Michael J. Rosner). Dept. of Pharm., Univ. of N. Carolina, Chapel Hill, NC 27514.

Primary cultures of purified astroglia contain at least two morphologically distinct cell populations. The predominant cell type (polygonal, GFAP positive) expresses β-AR and is mitotic. The second population (multipolar, GFAP positive) expresses very few β-AR and appears to be postmitotic. Studies in progress suggest that process-bearing astroglia are derived from polygonal astroglia.

Treatment of polygonal astroglia with either brain extract (BE), dibutyryl cyclic AMP (dbcAMP) or the β-AR agonist isoproterenol has been shown to convert polygonal astroglia to process-bearing astroglia. These changes often have been interpreted to reflect astroglial cell maturation. However, it is not known whether treatment with BE or dbcAMP influences the expression of β-AR on cultured astroglia. To examine this question, ^{125}I-cyanopindolol was used to label β-AR on astroglia treated with BE or dbcAMP. While treatment with these substances induced a morphological change in the polygonal astroglia, they had only a marginal effect on the number of β-AR expressed by the cells.

Our laboratory has shown that treatment of polygonal astroglia with isoproterenol results in the enhanced phosphorylation of GFAP. Experiments were carried out to determine if astroglia treated with BE or dbcAMP remained responsive with respect to the β-AR regulation of GFAP phosphorylation. Astroglia were treated for 1-3 days with 1 mM dbcAMP or 20% BE. After treatment, the medium was replaced with low-phosphate medium containing $^{32}P_i$, and the cells incubated for 2 hours. Isoproterenol was added to the medium during the final 30 min. of labelling to stimulate GFAP phosphorylation. Under these conditions treatment with either BE or dbcAMP have little, if any, effect on the regulation of GFAP phosphorylation by β-AR agonists.

The results of both the β-AR binding and phosphorylation experiments may reflect the presence of polygonal astroglia unchanged by BE or dbcAMP. To address this possibility, β-AR were analyzed via autoradiography on single, GFAP positive cells. The number of silver grains associated with untreated polygonal astroglia were compared to those from BE or dbcAMP-treated astroglia that had undergone morphological transformation. Treatment with BE or dbcAMP did not markedly alter the expression of β-AR on astroglia.

These results suggest that astroglia treated with BE or dbcAMP undergo morphological changes but continue to resemble polygonal astroglia with respect to their expression of β-AR and receptor regulation of GFAP phosphorylation. Supported by NS 16992.

104.10 AGE RELATED CHANGES IN THE EXPRESSION OF BETA ADRENERGIC RECEPTORS BY IMMUNOCYTOCHEMICALLY-DEFINED ASTROGLIA IN VITRO. Patricia A. Trimmer* and Ken D. McCarthy* (SPON: Ronald C. Bohn). Dept. of Pharmacology, Univ. of North Carolina, School of Medicine, Chapel Hill, NC 27514.

The expression of beta adrenergic receptors (βAR) by astroglial cells has been firmly established using purified primary cultures prepared from newborn rat cerebral cortex. However, it has not been possible to determine if the expression of βAR by astroglia varies during development. This is primarily due to the difficulty in preparing purified astroglial cultures from fetal or older postnatal cortical tissue in the quantity necessary for standard radioligand receptor binding assays. Using a new method (McCarthy, 1983), however, the distribution of βAR on individual, immunocytochemically-defined astroglia can now be assessed via quantitative receptor autoradiography. Therefore, in order to examine the influence of in vivo maturation on the expression of βAR, primary cerebral cortical cultures were simultaneously prepared from fetuses 16 days in utero as well as rats 1,7,14,21 and 28 days postnatal and plated into 6 well culture dishes. At various times thereafter (4-30 days), cells from each age were harvested and replated at low density onto polylysine-coated glass chamber slides. Two days later the cells were fixed, permeabilized and double stained with antibody to glial fibrillary acidic protein (GFAP) and fibronectin. The βAR were labelled with iodinated cyanopindolol. Following the binding procedure, the cells were dried and opposed to emulsion coated coverslips. Seven to fourteen days later the autoradiograms were developed. The distribution of silver grains over immunocytochemically-defined GFAP or fibronectin positive cells was quantified using a microcomputer-based video digitizing system. Using this methodology we have detected the presence of βAR on polygonal astroglia from all ages examined. Preliminary results furthermore suggest that the levels of βAR did vary as a function of age in vivo. The maximum density of βAR was found on astroglia from newborn rats. Astroglia from fetal cerebral cortex consistently expressed lower levels of βAR. Furthermore, βAR continued to be expressed by astroglia prepared from older animals, although the levels were generally less than that expressed by astroglia derived from one-day-old animals. In contrast, process-bearing astroglia and fibronectin-positive fibroblasts consistently expressed low levels of βAR which did not appear to vary as a function of in vivo age. These observations infer that the expression of βAR on polygonal astroglia is subject to modification by maturation in vivo. Supported by NS 16992.

104.PO PERINATAL MATURATION OF THE Fc IgG RECEPTOR OF RABBIT CILIARY PROCESSES. N. S. Peress, V. Mederios-Roxburgh* and M. C. Gelfand* Lab. of Neuropathology, V. A. Medical Center, Northport, NY 11768.

Using immunoglobulin-G (IgG) coated erythrocytes as a probe for Fc-IgG binding sites, we studied the location, development and blocking characteristics of the rabbit ciliary body Fc-IgG receptor in frozen sections of rabbit eyes obtained from 22 rabbits varying in age from 22 weeks of gestation to 4 weeks after birth. We compared the findings in the perinatal rabbits to those in adult eyes studied simultaneously. Adult eyes showed an intense, diffuse, selective adherence of the IgG coated erythrocytes to the epithelial layer of the posterior portion of the ciliary processes. The iridal portion was free of adhering erythrocytes. There was no significant adherence in other eye tissues studied, viz, iris, cornea, sclera or retina. The degree of binding of the IgG coated erythrocytes to the ciliary processes depended upon age-related changes in the first 2 weeks of life being minimal before birth and within the first 24 hours (4% of the adult level), then rising to a mean of 18% of the adult level by days 3-5 and increasing sharply to full maturation by 2 weeks of age. When adherence was minimal the pattern was of scattered clusters of adhering cells. With increasing adherence a more diffuse pattern emerged. At all ages, binding was confined to the epithelial layer of the posterior, non-iridal group of processes, and it was blocked (95% or greater) by 5.0 mg/ml solutions of non-aggregated and aggregated IgG but not by albumin, IgM or $F(ab')_2$. This selective pattern within the ciliary body is consistent with other morphological and biochemical data which suggest differences between the iridal and posterior processes. These studies have suggested that the iridal region is the main secretory area while our data suggest an immune function for the posterior portion of the ciliary processes.

105.1 DOCOSAHEXAENOYL-COENZYME A SYNTHETASE AND ARACHIDONOYL-COENZYME A SYNTHETASE OF BRAIN MICROSOMES ARE INHIBITED BY CATIONIC AMPHIPHILIC DRUGS. T. Sanjeeva Reddy*, and Nicolas G. Bazan. LSU Eye Center, LSU Medical Center School of Medicine, New Orleans, LA 70112

The mechanism by which local anesthetics act on the neural membranes is not properly understood although some mechanisms have been proposed, e.g., interaction with the lipid bilayer causing increased membrane thickness and fluidity. Previous studies from our laboratory have shown an alteration in the biosynthesis of glycerolipids in retina incubated with propranolol in vitro (See reference in J. Neurochem. 40:260-266, 1983). In the present investigation, we tested a series of local anesthetics on rat brain microsomal arachidonoyl CoA synthetase in vitro. All the drugs were found to inhibit the activity of arachidonoyl CoA synthetase, but with differing potencies. The order of inhibitory potency was: propranolol > tetracaine > chloroquine > procaine > lidocaine. The ID_{50} values for propranolol, tetracaine, and chloroquine were 37, 68, and 125 μm, respectively. The inhibition by lidocaine and procaine was very low (20-25%) and no additional effect was obtained by increasing the concentration of the drug from 100 μm to 800 μm. Since propranolol seemed to be a potent inhibitor of arachidonoyl CoA synthetase, additional studies were carried out on docosahexaenoyl CoA synthetase. The results showed similar but more profound effects, with an ID_{50} value of 7 μm. Thus, the results show that cationic amphiphilic drugs inhibit the activation of the polyenoic fatty acids found in large quantities in phospholipids of excitable membranes.

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105.2 INTRAMEMBRANOUS PARTICLE DISTRIBUTION IN THE JUXTAPARANODAL AXOLEMMA OF FROG OPTIC NERVES. J. H. Tao-Cheng and J. Rosenbluth. Depts. of Physiology and Rehab. Medicine, N.Y.U. Medical Center, New York, N.Y. 10016.

In the freeze-fractured axolemma of myelinated fibers, high concentrations of large (~10nm) E face particles (1000-1500/μ^2) have been shown at the node of Ranvier, and it has been proposed that these represent sodium channels. Accumulations of similar E face particles are sometimes seen, especially in the CNS, in the juxtaparanodal portion of the internode (JPI) immediately adjacent to the paranodal junctions. Each node is flanked by a proximal and a distal paranode and JPI with respect to the nerve cell body. The distribution of particles at the respective JPI's was examined in the present study.

Optic nerves of adult frogs (Rana pipiens) were cut into 3-4 segments and prepared for freeze-fracturing with the proximal to distal orientation monitored throughout the process. E face particle accumulations are present in almost all examples of JPI's (90%), and they occur with equal frequency in proximal and distal JPI's. The concentration of these particles is usually highest (200-500/μ^2) immediately adjacent to the last strip of the paranodal junction and then decreases over ~1-5μ to the background level (~100/μ^2). It appears that the higher the concentration, the more gradual the decrease. No consistent relationship was found between the particle concentrations in the JPI's and the number of myelin lamellae. However, all examples of JPI's with high concentrations over considerable lengths along the axons are from fibers with more than 10 myelin lamellae. No apparent difference was seen between optic nerve segments adjacent to or distant from the retina.

The last few rows of paranodal junctional strips near the internode are usually more loosely packed than the strips closer to the node. Accumulation of large E face particles can often be found in the wider and sometimes irregularly shaped axolemmal spaces ("lakes") between these junctional strips. The particle concentration in such "lakes" can be as high as ~600/μ^2 and appears to be in direct proportion to the particle concentration in the nearby JPI. Again, "lakes" with particle accumulations are found with equal frequency in proximal and distal paranodal regions.

The number of E face particles in the paranodal "lakes" and JPI's flanking each node is considerable, compared with that at the node itself, and the total of all these particles is, therefore, much greater than the number at the node alone.

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105.3 WEAK EXPRESSION OF HLA-A,B,C MOLECULES ON HUMAN NEURONS, NEUROBLASTOMA TUMOR, AND NEURONAL CELL LINES. James P. Whelan*, William F. Hickey*, Nicholas K. Gonatas and Lois A. Lampson. Univ. of PA, Depts. of Anatomy and Neuropathology, Phila., PA 19104

HLA-A,B,C molecules have been demonstrated on many types of human tissue and play an important role in the immune response. In many cases, the recognition of tumor specific or other "foreign" antigens by effector phase cytotoxic T cells is dependent on the co-recognition of HLA-A,B,C molecules on the target cell ("restriction phenomenon"). We have used a panel of monoclonal antibodies to different determinants on the HLA-A,B,C molecules (including polymorphic, non-polymorphic, conformational and sequential determinants) to assess the expression of HLA-A,B,C in human neural tissue. (1) Utilizing a variety of assays, we find that cell lines derived from neuroblastoma, a tumor of the sympathetic neuroblast, express less than 1% of the HLA-A,B,C activity seen in lymphoid or glial cell lines (Lampson, L. A. et al., J. IMMUNOL., 130:2471, 1983). (2) In a microscopic PAP procedure, <50% of single cells from bone marrow infiltrated with metastatic neuroblastoma tumor are HLA-A,B,C positive, as compared to ≥ 96% in normal bone marrow. Additionally, cell aggregates in neuroblastoma bone marrow, identifiable morphologically as tumor, are HLA-A,B,C negative. (3) In normal brain, the strongest stain was seen in the blood vessel endothelium. No specific stain was seen in the cell bodies of neurons or glia, or in myelin.

Possible Implications: (1) Low levels of HLA-A,B,C expression may protect infected, damaged, or transformed neural tissue from T cell-mediated surveillance. (2) From a beneficial standpoint, weak HLA-A,B,C expression, and the consequent protection from T cell-mediated immunity, may favor the acceptance of neural transplants.

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105.4 IN VITRO MEMBRANE SUBSTRATES FOR PROTEIN CARBOXYMETHYLASE. B.A. Johnson* and D.W. Aswad* (SPON: J. Koerner). Dept. of Psychobiology, Univ. of California, Irvine, Irvine, CA 92717.

Protein carboxymethylase (PCM) transfers methyl groups from S-adenosylmethionine to side chain carboxyl groups of its substrate proteins. The enzyme is abundant in brain and has been implicated in several neuron-specific processes, including the development of excitability in neuroblasts (Kloog et al., J. Neurochem. 40: 522, 1983), the regulation of neurotransmitter release in the striatum (Billingsley and Roth, J.Pharmacol.Exp. Ther. 233:681, 1982), and the regulation of acetylcholine receptor sensitivity (Kloog et al.,Biochem.Biophys.Res.Commun. 97:1474, 1980). Another hypothesis is that PCM selectively methylates D-aspartyl residues of age-racemized proteins as a step in their repair (McFadden and Clarke, Proc.Natl.Acad.Sci,USA 79:2460,1982). It should be possible to discriminate between these hypotheses by identifying and characterizing the endogenous protein substrates for PCM.

We have studied the in vitro protein methylation patterns of various particulate fractions from brain and other tissues using an acidic disc-gel electrophoresis system which preserves the labile methyl esters while providing a good separation of protein bands. All of the methylation we have observed has required the addition of purified PCM.

Crude particulate fractions from rat brain, heart, skeletal muscle, lung, and liver all contain methyl accepting proteins. Of these tissues, brain has the greatest number of substrates and the greatest amount of total methyl incorporation. When particulate fractions from bovine brain are divided further, substrates are found in both the synaptic membrane and the myelin fractions, whereas none are found in the mitochondrial fraction.

The synaptic membrane substrates appear as lightly labeled bands, the most predominant of which migrate at apparent molecular weights of 50K and 94K. The reaction is specific, as there are bands with dark protein staining which fail to incorporate methyl groups.

Myelin contains one heavily methylated band of very low molecular weight. Judging from methyl incorporation relative to protein staining, this is the best substrate for PCM that we have found in brain, although even this band was apparently methylated with a low stoichiometry. It seems unlikely that this band is an in vivo substrate, because little PCM activity is found in myelin.

Our results suggest several candidates for endogenous synaptic membrane substrates for PCM. However, the presence of a good methyl acceptor in myelin and the low stoichiometry of the methylation of all substrates are more consistent with the aging-repair hypothesis. (Supported by NIH NS-17269.)

105.5 ENHANCEMENT OF Na^+-K^+-ATPase ACTIVITY & PREVENTION OF Fe^{++} INITIATED FREE RADICAL INDUCED LIPID PEROXIDATION IN SPINAL CORD BY METHYLPREDNISOLONE. D.K. Anderson, E.D. Means, E.S. Green*, & T.R. Waters*. Spinal Cord Injury Lab, VAMC & Univ. of Cinti. Col. of Med., Cincinnati, Ohio 45220.

Demopoulos, et al (Acta Physiol Scand Suppl, 492:91-119, 1980) have proposed that trauma initiated & Fe^{++} & Cu^{++} (from extravasated blood) catalyzed free radical induced lipid peroxidation of cellular membranes contributes to the autodestruction of spinal cord (SC) tissue subsequent to injury. We have demonstrated (J Neurosurg, 55:200-8, 1981) that the synthetic glucocorticoid, methylprednisolone sodium succinate (MPSS) in high doses is effective in reducing the neurological deficit & preserving SC tissue following compression trauma to the feline SC. The purpose of this study was to assess the antioxidant capabilities of MPSS in an in vivo model known to damage cellular membranes by peroxidative mechanisms (i.e. microinjection of Fe^{++} into the SC of cats). Lipid peroxidation & membrane integrity were evaluated by measuring the activity of Na^+-K^+-ATPase, a plasma membrane bound enzyme & the levels of malonaldehyde (MDA), a product of lipid peroxidation. In pentobarbital anesthetized cats, a 29-gauge needle was stereotaxically placed in each anterior horn of the L_4 SC segment. Five microliters of 100mM $FeCl_2$ was infused into each anterior horn. Controls were infused with similar volumes of 0.9% NaCl. Normal values for SC ATPase activity & MDA levels were obtained from uninfused cats. 30mg/kg of MPSS was injected I.V. 30min after either $FeCl_2$ or NaCl infusion. The infused segment of SC (in both untreated & MPSS treated cats) was frozen in situ with liquid nitrogen at 1 & 2hrs after SC infusion & removed. Na^+-K^+-ATPase activity was unchanged at 1hr post-$FeCl_2$ infusion but fell to 30% of normal by 2hrs. MDA levels were elevated almost 2-fold in $FeCl_2$ injected SC's at 2hrs. MDA levels & Na^+-K^+-ATPase activity were not different from normal in NaCl injected SC's. Treatment with MPSS resulted in a significant 1.8 & 1.6-fold increase in Na^+-K^+-ATPase activity at 1 & 2hrs post-NaCl injection, respectively, concurrent with halving of MDA levels at 2hrs. MDA levels & Na^+-K^+-ATPase activity in $FeCl_2$ injected SC's were normalized at both 1 & 2hrs after treatment with MPSS. The extent of lipid peroxidation was inversely related to Na^+-K^+-ATPase activity suggesting a causal relationship between these 2 neurochemical events. This data shows that large I.V. doses of MPSS attenuates lipid peroxidation & enhances Na^+-K^+-ATPase activity, demonstrating the antioxidant properties of this agent. Since lipid peroxidation is a consequence of trauma to the SC, MPSS likely exerts its protective effect, in part, by quenching these peroxidative reactions in cellular membranes. (Supported by the Veterans Administration.)

105.6 CALCIUM PARADOX IN SKELETAL MUSCLE. S. Carpenter*, G. Karpati and M. Armani* (SPON: L.S. Wolfe). Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A-2B4.

Calcium paradox was discovered in rat cardiac muscle by Zimmerman et al. 1967. Perfusion of the isolated heart for 3 minutes with a calcium-free, but otherwise physiological, solution destroys the cells' ability to control calcium fluxes through their sarcolemma. At this point they are normal by EM except for separation of basal lamina from plasma membrane. Addition of physiological concentrations of calcium to the perfusate then results in rapid influx of calcium, egress of creatine kinase, and hypercontraction. We are describing calcium paradox for the first time in skeletal muscle in vivo and in vitro using EDTA solution to chelate interstitial calcium. Exposed muscle of anesthetized rats was bathed in EDTA solution for 20 minutes. For in vitro experiments, the soleus muscle was suspended in oxygenated, glucose-enriched, calcium-free Tyrode solution with EDTA. The only immediate effects of EDTA were to separate basal lamina from plasma membrane and increase space between muscle fibers. These changes were visible after exposure to 5% EDTA on semithin sections. After 0.5% EDTA plasma membrane separation could only be seen by electron microscopy. Return of normal interstitial calcium resulted in necrosis in vivo and hypercontraction in vitro, presumably from excessive entry of calcium into muscle cells. EDTA saturated with calcium had no effect. An analogy may be drawn with Duchenne dystrophy, where one finds hypercontraction and necrosis, as well as separation of basal lamina from the plasma membrane of non-necrotic fibers. The analogy suggests that a fundamental abnormality in Duchenne dystrophy might involve calcium binding proteins in the plasma membrane.

105.7 CELL CONTACT-MEDIATED REGULATION OF TYROSINE HYDROXYLASE IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. H. Thoenen and A. Acheson. Max-Planck-Institute for Psychiatry, Dept. of Neurochemistry, 8033 Martinsried, FRG.

A number of factors are known to influence adrenal tyrosine hydroxylase (TH) levels, such as splanchnic nerve activity and glucocorticoids. Cell contact may also play a regulatory role, since the specific activity of TH varies with cell density in primary cultures of bovine adrenal chromaffin cells (Naujoks et al. Dev. Biol. 92: 365, 1982). In order to further examine this effect, cells were cultured at a low density (.03 X 10^6 cells/cm^3) for 24 hrs, during which time specific TH activity decreased to a value well below that found in vivo. This low level was maintained if cells were kept at this low density. After this initial 24 hr period, cells were harvested using .01% trypsin and .5 mM EDTA in Ca^{++}-Mg^{++}-free PBS at 37°C. The enzymatic reaction was stopped by the addition of trypsin inhibitor and 1% BSA. The yield of this procedure was 65-75% of originally plated cells. Cells harvested in this manner were then replated at the original low density or at a 10-fold higher density and maintained in culture for up to 1 week. Cells which were replated at low density showed no change in TH activity as compared to unreplated low density cells. However, there was a 6-fold rise in TH activity in cells replated at high density over 48 hrs. Conditioned medium from high density replated cells did not mimic the effect of direct cell contact. Immunotitration showed that the increased TH activity was due to an increased number of molecules, and cycloheximide blocked the effect. These data suggest that increased TH synthesis, rather than decreased degradation, is responsible for the rise in TH. The effect was also blocked by both α-amanitin and 9-ß-arabinofuranosyladenine, inhibitors of mRNA synthesis and polyadenylation respectively, consistent with a transcriptional level of regulation. Finally, the specific activity of acetylcholinesterase (which is induced together with TH by NGF in these cells, see Acheson and Thoenen, this volume), was not affected by cell density.

106.1 A BASIC MECHANISM OF CENTRAL NERVOUS SYSTEM (CNS) FUNCTION. C. Torda. Res. Dpt. N. Y. Center PA Training. (Curr. Adr. P. O. B. 4866, Stanford University, Stanford, Calif., 94305).

Several functions of mind and brain escape currently available measuring techniques and cannot be explained by the concepts of classical physics (e.g. the automatically performed processing (including memory, recall, imagination), several time relationships of biological processes, etc.). Therefore, the possibility of involvement of submolecular processes in the function of the CNS has been here addressed. The nature of submolecular processes have been studied with quantum mechanical concepts based mainly on the theoretical rules and mathematics of David Bohm and Geoffrey F. Chew. Bohm's theory is based on conservation of energy. Everything in the universe is formed from the same energy. Each cm^2 contains 10^{32} joules of energy that assumes implicit and explicit forms. The consequences are, in part, the human potential capacity for insight into every manifestation of the universe and for intellectual growth. Bohm's philosophical concept of quantum mechanics led to the holonomic principle: every part contains the whole. Theories based on the experimental data of the particle physics offer a more scientific explanation of the processes of the universe, mainly the "bootstrap" theory based on S-Matrix mathematics of Chew. Everything is formed by interactions of basic processes. The socalled elementary particles are formed from more basic processes related not only to conservation of energy, but also the several other conserved functions, e.g. momentum, electric charge, spin, parity, Baryon and Lepton numbers, etc. Due to intervention of soft photon energy, elementary particles and also classically observable objects are formed through interaction of these basic processes. This quantum mechanical theory implies limitless potential for change, regulated by soft photons. It can also accommodate far more processes than the functions of human mind and brain require. The immense number of possibilities is reduced by inherited species specific control and organizing systems that permit comparable functions and concept formation by members of the same species. The organizer control mechanisms are transmitted by DNA and consist, at least in part, of the complex ubiquitous inhibitory processes, e.g. the human DNA is responsible for our current inability to imagine anything beyond four dimensions. The submolecular events are translated to electromagnetic waves measurable by classical physical means following Fourier mathematics. This seems to be responsible for the oscillatory processes of living organisms, including the electromagnetic wave systems used by neurons to transmit information. This quantum mechanical theory of particle physics already led to insight into the nature of some biological mysteries. Large part originated by Adey.

106.2 THE INTRAPARIETAL SULCUS OF THE RHESUS MONKEY: INTRINSIC CONNECTIONS AND ARCHITECTONICS. Benjamin Seltzer and Deepak N. Pandya. V.A. Hospital, Bedford, MA 01730

A previous study of the intrinsic connections of the parietal lobe in the rhesus monkey showed projections to the intraparietal sulcus (IPS) from both the superior and inferior parietal lobules (Pandya and Seltzer, '82). In addition, projections from visual-related cortex to a distinct architectonic sector (area POa) in the lower bank of the sulcus have also been demonstrated (Seltzer and Pandya, '80).

The present study combines a reappraisal of architectonics with a study of the intrinsic parietal connections of the IPS. Architectonically, the rostral part of the sulcus is a continuation of area 2. Caudally, its cortex merges with that of area OA. Between these two extremes, the upper and lower lips of the IPS belong, respectively, to area PE of the superior parietal lobule and area PG of the inferior parietal lobule. Deeper within the sulcus, in the upper bank is area PEa and in the lower bank area POa. Finally, a distinct architectonic region is identified in depth of the IPS and designated "area IPd."

The afferent and efferent connections of these IPS regions were studied in 19 rhesus monkeys using autoradiographic techniques. Each architectonic zone has a specific set of connections. Rostrally, area 2-type cortex receives projections from the superior parietal lobule (area PE) and itself projects to area PEa. Area PEa also receives projections, in a topographical manner, from the ventral part of area PE in the upper lip of the IPS as well as the cortex at the apex of the superior parietal lobule (area PEc) and on the medial surface of the parietal lobe (area PGm). Area POa, however, is the recipient of a projection from the rostral inferior parietal lobule (area PF) and from area POa itself. Area IPd, by contrast, receives projections from both the medial parietal cortex (area PGm) and the caudal inferior parietal lobule (areas PG and Opt). The contribution from the latter is the more substantial. Thus, area IPd represents a zone of topographically-convergent somatic sensory input, from both the superior and inferior parietal lobules.

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106.3 IMAGING OF CORTICAL SULCI IN MONKEYS WITH CONTRAST RADIOGRAPHY. P. E. Haenny*, J. H. R. Maunsell and W. K. Abend*. Department of Psychology, M.I.T., Cambridge, MA 02139 and Department of Neurology, Harvard Medical School, Boston, MA 02115.

Individual variability in the position of cortical sulci limits the accuracy of stereotactic procedures for locating structures in the cortex. It is possible to determine the position of sulci from gyral bone impressions (Wolpaw 1979 Brain Res. 160: 505-508), but this technique requires a substantial trephination, which may be undesirable if chronically prepared animals are used, or when multiple determinations are necessary. We report a method of determining the location of sulci over large regions of individual hemispheres.

Sulcal images were obtained by taking skull X-rays after subarachnoid injections of a water-soluble contrast medium. Rhesus monkeys were anesthetized with ketamine and xylazine. Eight intrathecal injections of the contrast substance (metrizamide, 625mg in 2.5ml) were made in 5 rhesus monkeys by lumbar or suboccipital punctures. The animal was positioned so that the substance would distribute over the lateral surface of one hemisphere. At a series of times following the injection, skull X-rays were taken using a variety of X-ray beam angles.

Images of sulci on the lateral convexity of the hemisphere were obtained. The best results were obtained 6 to 20 minutes after the injection, when the contrast substance was well distributed, but remained in the subarachnoid space. Projections which were roughly lateral gave the best results. With other views, bony structures obscured many of the sulci. The suboccipital approach provided greater ease of subarachnoid injection than did the lumbar procedure. When the head is properly aligned, X-rays can be used to establish sterotactic coordinates for the sagittal plane, providing an accurate approach to specific cortical loci.

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106.4 A COMPUTER SYSTEM FOR THREE-DIMENSIONAL ANALYSIS OF REGIONAL BRAIN ACTIVITY USING SERIAL 2-DEOXYGLUCOSE AUTORADIOGRAPHS. D.S. Schlusselberg, W.K. Smith, D.L. McEachron*, D.J. Woodward, Dept. of Cell Biology, Univ. Texas Health Science Ctr., Dallas, Texas 75235.

This laboratory has been involved in the development of general purpose computer imaging for three-dimensional reconstruction of neuroanatomical information from serial sections. The system is designed for obtaining quantitative measurements, as well as delineating subtle differences in neuronal connectivity and regional neuronal distributions. In this report we describe an adaptation of this system for analysis of serial 2DG autoradiographs to provide similar three-dimensional information about relative metabolic activity in different brain regions.

Brains from rats injected with 2DG during an experiment are cut into serial sections which are then placed on radiographic film. The autoradiographic images for each section are digitized using a light box and video digitizer, and stored in an image memory buffer. Variations in lighting conditions are corrected by subtracting a pre-digitized background image. Pixel values in the image memory are then normalized to yield relative optical density values, using methods described by Gallistel et al (Neuroscience & Behavioral Rev, 6(4):409-420, 1982) to correct for differences caused by overall exposure of the radiographic image. Color coded windowing techniques can be used to enhance subtle variations in radiographic densities.

Perimeters which delineate neuroanatomical boundaries are generated by tracing histological images superimposed over the autoradiographs. A raster-scan conversion algorithm is used to read normalized pixel values within the regions bounded by the perimeters. The algorithm allows segments of pictures within specified regions to be stored in a three-dimensional anatomical database for further analysis. The system capabilities permit sequential section alignment and identification of neuroanatomical structures which appear in several sequential sections.

The perimeters and relative optical density data are used to create three-dimensional graphical images, showing regional brain activity per volume element in relation to reconstructed neuroanatomical structures. (Support from NIAAA 1F32-5198 to DSS, NIAAA 3901, and Biol. Humanics Foundation)

106.5 A DATABASE STRUCTURE FOR THREE-DIMENSIONAL RECONSTRUCTION OF NEUROANATOMICAL OBJECTS. W.K. Smith, D.S. Schlusselberg, B.G. Culter* and D.J. Woodward, Dept. Cell Biology, Univ. Texas Health Science Ctr., Dallas, TX, 75235.

Ongoing studies in this laboratory have explored the application of computer graphics techniques for three-dimensional reconstruction of biological structures from serial sections. The design of a database for storage, manipulation and display of the serial section data has proven to be the dominant task. In comparison, software for graphical input-output has been relatively straightforward.

Data can be collected from a variety of sources: light microscopy, electron micrographs, enlarger, photographs and tracings. Points along the perimeters of biological structures and cell positions make up the largest part of the input data. A hierarchical database has been designed to store this biological information. The 'C' programming language utilized for this task has proven useful for manipulating complex data structures. Data records are organized into nodes which contain information about the type of data stored and address pointers to lists of graphical data and other nodes. The hierarchical node relationships follow the Knuth transform of an n-ary tree.

Different levels in the hierarchy are represented by different types of nodes. The first node in a database file is the ROOT node, which contains global information about the file and pointers to a group of FAMILY nodes. Included among the FAMILY nodes are SECTIONS and OBJECTs. Data acquisition routines must first create SECTION nodes for each section to be analyzed. Data is entered for each section as SEGMENT nodes, which are the children of SECTION nodes. Once section and segment data are entered, segments from each section which form part of biological objects must be identified. The biological object is identified by an OBJECT node which points to a hierarchy of BRANCH nodes. Each BRANCH node points to a family of STRIP nodes, which represent the surface definition of that branch.

Virtual memory emulation software eases the task of handling large amounts of information. A package of powerful data analysis and graphical display routines exists to access this database. This software provides such operations as file editing, interactive alignment, object building, surface triangulation and image generation on a variety of graphical output devices. Applications include: analysis of catecholamine cell distribution in the substantia nigra and locus coeruleus in normal and aging brain, examination of cortico-cortical connections in the rat and cortical-subcortical connectivity studies in the thalamus, the raphe nucleus and the inferior olivary nuclei. (Support from the Biological Humanics Foundation to DJW and the NIAAA 1F32AA05198-01 to DSS.)

106.6 COLLATERAL AXONAL BRANCHING OF VENTRAL TEGMENTAL AND RAPHE PROJECTIONS TO THE MEDIAL PREFRONTAL AND SULCAL CORTICES IN THE RAT: A RETROGRADE FLUORESCENCE DOUBLE LABELING STUDY. Erik C. Sobel* and Dale Corbett*. (SPON: J.R. Stellar). Dept. of Psych. & Soc. Rel., Harvard Univ., Cambridge, MA 02138.

One striking characteristic of monoamine neurons is the dichotomy between extensive distribution of monoamine axons in the central nervous system and the relatively small number of monoamine neurons within each cell group (Fallon and Loughlin, 1982). This dichotomy has led investigators to suggest the possibility that monoamine neurons possess highly collateralized divergent axonal branches innervating their terminal fields. Within the A9-A10 regions there is anatomically demonstrated overlap of the origins of dopamine projections to functionally different parts of the forebrain (Fallon and Moore, 1978; Fallon, 1981). Fallon and Loughlin (1982) previously reported findings of the relative degree of collateralization to the forebrain from the three major monoamine cell groups. Their findings show that while the pars compacta (SNC) and VTA appear to contain less collateralized cells than the dorsal and median raphe nuclei (DR-MR) the cells of each nuclei sustain both highly branched and poorly branched axons. The question remains as to the specific projections of collateralized and non-collateralized neurons within the monoamine nuclei.

The collateralization of SNC-VTA and raphe projections to two distinct areas of the frontal cortex was examined using a retrograde fluorescent double labeling technique (Kuypers et al., 1980). 50 nl pressure injections of 3% propidium iodide (PI) and 10% bisbenzimide (Bb) were made into the medial prefrontal cortex (MFC) and sulcal cortex of 10 animals. Five animals received Bb in the MFC with PI in the sulcal cortex and five received each dye in the other cortical area in a balanced design. To compare the relative degree of labeling between the two dyes two additional animals received Bb in the MFC with PI in the homotopic MFC.

Double labeled cells were found in the VTA and medial portion of the SNC as well as in the MR and throughout the DR. Double labeled cells were found ipsilaterally located in the SNC-VTA and bilaterally in the DR-MR. Comparing the two dyes revealed that while they both label the same areas PI labels more cells than Bb.

The findings clearly indicate the collateralization of neurons in the VTA, SNC, MR and DR to the MFC and sulcal cortex. The apparent greater sensitivity of propidium iodide in labeling the cells of the SNC-VTA and the careful study of one specific collateral terminal field system made possible the visualization of the previously unreported collateralization from the VTA to the frontal cortex. Further study combining retrograde double labeling fluorescence and catecholamine or immunohistochemical fluorescence is necessary to characterize the dopaminergic nature of these collateral projections to the prefrontal cortex.

106.7 THALAMIC CONNECTIVITY OF THE SOMATIC MOTOR CORTEX (SMI) IN THE RAT. L.D. Aldes*. Department of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, Texas 77025. (SPON: J.F. DeFrance)

The aim of this study was to investigate the organization of thalamocortical and corticothalamic projections of the somatic motor cortex (SMI) in the rodent, and thereby, further define the SMI on the basis of thalamic connectivity. This was accomplished by microiontophoretically applying horseradish peroxidase (HRP) or tritiated amino acids into low threshold cortical foci within the leg, arm and head representations of SMI as defined by intracortical microstimulation.

Injections at each cortical site produced consistent patterns of retrograde and anterograde labeling in several specific and non-specific thalamic nuclei. The former included the ventrolateral (VL), ventromedial (VM), and posterior (PO) nuclei, and the latter the centrolateral (CL), paracentral (PC), central medial (CeM) and parafascicular (PF) nuclei. The heaviest labeling occurred in the VL nucleus and, secondarily, in the PO nucleus, while labeling of specific nuclei was more extensive than in non-specific groups. The ventrobasal (VB) and lateral posterior (LP) nuclei were labeled after injection of the leg representation of SMI, and terminal fields, whose position varied with that of the injection site, were found in the reticular (Ret) nucleus.

A topographic organization was indicated by the varied position of labeling following injections at different locations. Injection of the leg representation produced labeling in rostral, lateral and more dorsal regions of VL, in the dorsal, lateral and more rostral PO and ventrolateral VM. In contrast, labeling occurred in the caudal, medial and more ventral regions of VL, in the ventral, medial and more caudal PO and dorsolateral VM following injection of the head representation. Characteristic labeled patterns that occupied an intermediate position were found after injections into the arm representation of SMI. Finally, rostrolateral injections of SMI labeled more caudal regions of CL, PC and CeM, whereas dorsomedial injections labeled more rostral areas of these nuclei.

The results from this study indicate that, in the rodent: 1)the VL nucleus provides the principal source of input to the SMI; 2) there is convergence of multiple input from several specific and non-specific thalamic nuclei onto each functional area of the SMI; 3)the projections are reciprically organized and topographically arranged; and 4)the SMI may be further defined on the basis of thalamic connectivity.

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106.8 CORTICO-THALAMIC FIBERS ARE DISTRIBUTED BILATERALLY IN RAT. M. Molinari*, D. Minciacchi*, M. Bentivoglio*, G. Macchi* (SPON: European Neuroscience Association). Inst. of Neurology, Catholic University, Rome, Italy.

The anterograde transport of lectin-conjugated horseradish peroxidase (WGA-HRP) was employed in order to investigate the organization of corticothalamic fibers efferent from the sensorimotor cortex in rat. Cortical injections ranging from 0.1 μl to 0.4 μl of 5% WGA-HRP were made unilaterally in rats. The survival time was 48 hours, except for one case which survived 24 hours. The animals were perfused with 2.5% glutaraldehyde. The material was incubated with tetramethylbenzidine. Retrogradely labeled cells and anterogradely labeled terminals were seen in the ipsilateral thalamus. In addition, a consistent anterograde labeling was seen in the contralateral thalamus in the intralaminar nuclei and in the ventral nuclear complex. This finding could either indicate the existence of a crossed cortico-thalamic component or represent a false positive result . Control experiments were made in order to exclude this latter possibility. It was hypothesized that false positive results could have derived either from callosal cell bodies, labeled in the contralateral cortex, or from the ipsilateral labeled thalamo-cortical cells, due to two mechanisms: i) transport through axonal branches of the labeled cell populations; ii) transneuronal transfer. Three sets of control experiments were made: A) unilateral kainic acid injections in the sensorimotor cortex followed by WGA-HRP injections in the same place. This procedure abolished both the ipsilateral and contralateral anterograde labeling in the thalamus, whereas labeled cells were still seen in the ipsilateral thalamus. These experiments indicated that the contralateral anterograde labeling, as well as the ipsilateral one, derived from cortical cell bodies. B) Kainic acid injections in the thalamus, followed by WGA-HRP injections in the ipsilateral cortex. This procedure abolished the ipsilateral thalamic retrograde labeling, whereas anterograde labeling was still seen in both thalami. These experiments indicated that the contralateral anterograde labeling could not derive from labeled thalamo-cortical cells. C) Split of the corpus callosum followed by WGA-HRP injections in the cortex. In these experiments anterograde labeling was still seen bilaterally in the thalami and this confirmed that the contralateral thalamic anterograde labeling could not derive from labeled callosal cell bodies. Altogether these experimental findings indicate that cortico-thalamic fibers are distributed bilaterally in the rat , as it was previously suggested in cat (Rinvik, E., Exp. Brain Res. 5: 129, 1968) and monkey (Goldman, P.S., Brain Res. 166: 166,1979), and that the crossed cortico-thalamic fibers do not travel via the corpus callosum.

106.9 PROJECTIONS TO THALAMIC ASSOCIATION NUCLEI FROM WIDESPREAD PARTS OF THE LIMBIC CORTICES IN THE RHESUS MONKEY. E. H. Yeterian, Department of Psychology, Colby College, Waterville, ME 04901.

Recent autoradiographic studies of corticothalamic connections in macaque monkeys have revealed more extensive and intricate interrelationships than had been observed using older anterograde methods. Within this area of research, thalamic projections of limbic cortices, in particular, projections from limbic cortices to thalamic association nuclei, have been less completely investigated than those of other cerebral cortical areas. The present study examined the distribution of projections from various limbic cortical areas to the dorsomedial (DM), lateral dorsal (LD), lateral posterior (LP), and medial pulvinar (PM) nuclei. In 15 monkeys, tritiated amino acids were injected into various architectonic subdivisions of the limbic cortices as delineated by Brodmann, and Bonin and Bailey. Each case was examined for evidence of terminal label over thalamic association nuclei as demarcated by Olszewski. Orbital frontal area 13 projects to medial regions of DM and PM. Area 32 on the medial surface of the frontal lobe sends projections primarily to dorsomedial DM, with a slight caudal extension into the rostralmost portion of PM. In contrast to the preceding cases, area 24 shows no evidence of projections to PM, but does project heavily to medial DM. Projections to the medial pulvinar are again evident from rostral area 23, occupying a dorsomedial to central region of that nucleus. In addition, rostral area 23 sends projections to central DM, and also to dorsal LP. Caudal area 23 differs from rostral area 23 in projecting to LD but not to DM. Caudal area 23 also projects strongly to dorsal LP and to dorsal and central PM. Among the limbic cortices of the temporal lobe, area TF projects to dorsal PM and to ventromedial LD, while area TH projects to dorsal PM and to lateral and medial regions of LD. Area 35 projects only to dorsal PM, with evidence of terminal label extending to the caudal pole of the thalamus. Finally, area TG of the temporal pole projects heavily to medial PM and to dorsomedial DM.

In summary, each of the areas investigated, with the exception of area 24, projects to PM--frontal areas medially and more caudal areas predominantly dorsally. Areas 13, 32, and 24, rostral area 23, and area TG project to DM, while caudal area 23, and areas TF, TH, and 35 do not show evidence of such connectivity. Projections to LD are from areas 23, TF, and TH, while those to LP are from area 23 only. Although each limbic cortical area has a distinctive overall pattern of projections to thalamic association nuclei, each area projects to at least one such nucleus. Thus, projections to thalamic association nuclei appear to be a general feature of limbic cortices in the rhesus monkey.

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106.10 Thalamocortical mechanisms underlying non-barbiturate spindles in the cat association system. M. Avoli, R. McLachlan*, D. Giaretta* and P.Gloor. Montreal Neurological Institute and Dept. of Neurology and Neurosurgery, McGill University, Montréal, P.Q., H3A 2B4, Canada.

Extracellular single unit recordings were performed simultaneously from pairs of neurons, one located in the cortex (middle suprasylvian gyrus, MSS), the other in the thalamus (n. lateralis posterior and pulvinar) of painlessly immobilized, awake cats. The unitary activity was studied during spontaneous non-barbiturate EEG spindles recorded with the intracortical and thalamic microelectrodes and with epicortical electrodes. Data were statistically analyzed by a computer which generated EEG averages and histograms of single unit activity (EA-HSU) which were triggered by: (a) peaks of EEG transients (i.e. spindle waves) or (b) action potentials (aps) occurring during selected sections of EEG (i.e. spindle bursts).

As previously reported for barbiturate spindles those recorded in the present experiments displayed mixed features of type I (augmenting) and type II (recruiting spindles) (J.Neurophysiol. 24, 50-65, 1961). When EA-HSUs were triggered by cortical spindle waves, (a) 14 of 31 cortical neurons showed rhythmic oscillation between maximum and minimum firing probability at the same frequency as the cortical and thalamic EEG spindle bursts, while in the remaining neurons no relationship with spindle waves was observed; (b) 11 of 31 thalamic neurons displayed a rhythmic modulation of firing probability at the frequency of the thalamic and cortical spindles, while the others did not show any clear change of firing probability throughout spindle bursts. A similar pattern of neuronal behavior during non-barbiturate spindle was also observed when EA-HSUs were triggered by thalamic spindle waves or by cortical or thalamic aps. In those cases in which both cortical and thalamic cell firing was modulated during non-barbiturate spindles, the thalamic peak of firing probability preceded that of the cortex by 5-40ms in 7 cases and followed that in the cortex by 30-40ms in 2 cases.

These data show that at least in nearly 30% of pairs of neurons studied in thalamus and cortex a mechanism similar to that described for barbiturate spindles (J.Physiol. 192: 282-307, 1967) is seen to operate in the absence of barbiturates. The lack of any relationship between EEG and unit firing probability at both the thalamic and cortical levels in a large number of neuronal pairs in the absence of barbiturates might in part be due to a lesser degree of effective synaptic coupling of neurons involved in spindle genesis. The action of barbiturates in enhancing neuronal synchronization may be due to an increased effectiveness of gating effect exerted by thalamic inhibitory interneurons.

106.11 CORTICAL-SUBCORTICAL MECHANISMS FOR "INTERICTAL AFTER-DISCHARGE" IN FOCAL PENICILLIN EPILEPSY. Edgar L. Gasteiger and Birgit Albowitz(1)*, Department of Physiology, New York State College of Veterinary Medicine and Section of Physiology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

Local application of sodium penicillin to the presigmoid gyrus (projection area of the ventral lateral nucleus of the thalamus) in urethane anesthetized cats produced regular interictal spikes, each followed by cortical after-discharge (AD) that required 10-30 min to develop. The AD occurred 170-200 msec after the spike and consisted of 16-20/sec oscillations usually starting with positive polarity and lasting up to three cycles. Cooling selective subcortical sites revealed that AD could be readily and reversibly blocked without altering the spikes when the cooling thermode was placed in VL as compared to other sites. This observation plus current views of the thalamic pacemaker of cortical rhythms suggest two models in which the cortical spike triggers VL to fire synchronously. Then, after a delay of 170-200 msec caused by a thalamic ipsp, a rebound discharge activates either an independent oscillator in the cortex or a thalamic oscillator that in turn drives a phase-locked oscillator in the cortex. Extracellular recordings were made from over 60 VL units to test these two models. Consistent with the expected trigger action of the cortex on VL, sixty percent of the VL units fired during the penicillin spike. When AD was present all units recorded in VL, in contrast to other sites, showed a discharge pattern strongly related to AD. All units were inhibited for 170-250 msec following a spike indicating the presence of the expected long lasting ipsp. The units fired in bursts on either the positive or negative phases of AD but not immediately preceding or following it. Thus the units did not appear to initiate or show independence from the cortical AD. Although this is not conclusive evidence for differentiating between the two models, it does favor the independent cortical oscillator model. Additional support for this view is seen in increased delay of AD with cooling of VL without a change in AD frequency.

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106.12 **CORTICAL-STRIATAL DOPAMINE INVOLVEMENT IN AN ANIMAL MODEL FOR TARDIVE DYSKINESIA.** **A. Hitri, G. Pasinetti*, R.L. Borison and B.I. Diamond. Psychiatry Department, Medical College of Georgia, Augusta, Georgia 30912.**

Chronic neuroleptic treatment can produce tardive dyskinesia in man. This is a result of chronic blockade of extrapyramidal dopamine receptors which results in a paradoxical increase in the numbers of these striatal receptors. The tardive dyskinesia model in animals produces not only the increase in the numbers of striatal dopamine receptors, but also a behavioral sensitization to dopamine agonst type drugs. Since involuntary movements also have a cortical involvement, we have studied the role of the cortex in an animal model for tardive dyskinesia. Male Sprague-Dawley rats (200 - 250 g) were treated for 18 days with haloperidol (1 mg/kg, i.p.). Half of these animals had stereotactically placed 6-hydroxydopamine lesions of the cortex above the striatum. After a five day washout from haloperidol, animals received either apomorphine (0.5 mg/kg) or d-amphetamine (3.75 mg/kg) and stereotyped behaviors were rated. Two days later brains were removed and the cortex above the striatum and the caudate-putamen nucleus were dissected out. These areas were analyzed for the affinity and number of dopamine receptors using ^{3}H-spiroperidol as the ligand. Compared to control animals, haloperidol treated rats demonstrated an increase in stereotyped behavior to apomorphine and d-amphetamine and both cortical and striatal dopamine receptor numbers were increased over controls. In animals with cortical lesions, and haloperidol treatment for 18 days, there was no increase in the number of cortical or striatal dopamine receptors. Moreover, apomorphine produced a decrease in stereotypy whereas d-amphetamine increased stereotyped behavior in these animals. These results suggest that striatal dopamine hyperactivity is modulated by the cortical dopaminergic system.

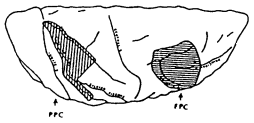
106.13 TECTAL METABOLIC DEFICITS IN MONKEYS WITH HEMISENSORY NEGLECT. R.K. Deuel, Departments of Pediatrics and Neurology, Washington University, St. Louis, MO.

The syndrome of hemisensory neglect includes conditional but severe hemifield visual and somesthetic, and motor performance deficits. These are reliably produced by frontal or parietal polysensory association cortical lesions (FPC or PPC) in monkeys. Complete recovery from symptoms occurs over 4 to 6 weeks after the lesion (Deuel and Collins, 1979). We used quantitative ^{14}C-2-deoxyglucose autoradiography (DG) to study local cerebral glucose utilization (LCGU) in the brains of animals demonstrating neglect after FPC and PPC lesions and in recovered animals. Behavioral responses were the same in the acute lesion groups. In contrast, LCGU deficits in di- and tel-encephalic structures differed completely between the two groups. With FPC lesions, basal ganglia and selected medial and ventral thalamic nuclei of the damaged hemisphere (DaH) were affected. With PPC lesions, DaH posterior and lateral thalamic, and cortical regions were affected. The only structure that exhibited LCGU deficits in both neglect groups was the superior colliculus (SC) of the tectum. The ratio of metabolic activity in the DaH, compared to the intact hemisphere (InH) differentiated the deeper layers of the colliculus in both FPC and PPC neglect brains from control brains. In brains of behaviorally recovered FPC and PPC animals, SC LGU deficits had improved.

The cardinal symptoms of hemisensory neglect, namely lack of orientation to and sustained attention for a spatial hemifield, may be related to decreased synaptic activity in the contralateral tectum in the monkey rather than dysfunction of di- or telencephalic structures.

Ratio of Damaged/Intact Hemisphere Metabolic Activity (group medians)

	Acute		Recovered		
	FPC	PPC	FPC	PPC	Control
Suprior colliculus superficial	.89	.91	.98	1.00	1.02
Superior colliculus deep	.84	.85	.94	.91	1.03



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106.14 DIFFERENTIAL PROJECTIONS OF THE BASAL FOREBRAIN AND NUCLEI OF THE SUBTHALAMUS TO VISUAL CORTEX IN THE RAT. Russell G. Carey and Richard W. Rieck (SPON: A.S. Schwartz). Div. of Neurobiol., Barrow Neurol. Inst., Phoenix, AZ 85013, and Dept. of Anatomy, Tulane Univ., New Orleans, LA 70112.

Previous reports (Mesulam & VanHoesen, '76; Rieck & Gould, '79; Wenk et al., '80; Jackson & Crossman, '81; Ribacak & Kramer, '82) have shown that large multipolar and fusiform AChE-positive neurons in the the basal telencephalon region of the substantia innominata (SI) and the thalamic region of the subthalamic nucleus are retrogradely labeled following HRP injections in cortex. Destruction of portions of these AChE regions by either electrical or chemical lesions has resulted in a conspicuous depletion of both AChE and choline acetyltransferase activity throughout the neocortex (Struble et al., '82; Johnston et al., '79). Thus, these subcortical regions are suggested to contribute substantially to the AChE plexus in cortex.

We have recently reported (Rieck & Carey, '83) that, counter to previous beliefs, the terminal locus of the AChE projecting neurons is maximally in the infragranular cortical layers and only minimally in layer I of visual cortex. The present report extends this finding and further illustrates the existence of a rough topography of the projection of these AChE neurons onto visual cortex.

After injections of WGA-HRP into infragranular layers of cortex immediately medial to area 17 (area 18M), numerous labeled cells are localized in the rostral portion of nucleus basalis of SI and continue dorsomedially into the diagonal band of Broca. Caudally a few labeled cells are found within the medullary laminae of the globus pallidus. No labeled cells are localized in either the subthalamus or within the ansa lenticularis. In contrast, after injections into cortical regions lateral to area 17 (area 18L) numerous labeled cells are found in the subthalamus as well as within the the ansa lenticularis and other regions of caudal SI. Further, in comparison with injections of 18M, fewer labeled cells are found in the rostral nucleus basalis. The pattern of labeled cells seen after injection in area 17 is intermediate between that seen with the more medial or lateral injections.

Thus, these data suggest that the proposed cholinergic afferent system arising from these nuclear regions not only terminate in the infragranular layers of visual cortex, but also in a very preferential manner.

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106.15 PHYSIOLOGY OF CORTICALLY PROJECTING NEURONS IN RAT BASAL FOREBRAIN. G. Aston-Jones, R. Shaver* and T. Dinan*. Center for Neurobehavioral Sciences, SUNY, Binghamton, NY 13901.

Recent studies have confirmed a direct cholinergic projection from basal forebrain neurons to cerebral cortex in many species. These cells are of interest because they represent a diffusely projecting, non-thalamic cortical afferent system in addition to monoamine tracts, and because degeneration in this cholinergic system has been linked to Alzheimer's Disease. Unit recordings in waking monkeys have revealed that neurons in this area discharge in relation to feeding behavior. However, there have been no physiological studies of basal forebrain neurons that are known to innervate cortex. We examined the physiology of antidromically (AD) identified cortically projecting basal forebrain neurons in the rat.

Extracellular recordings from single neurons were obtained in 46 chloral hydrate-anesthetized rats. Electrical stimulation of frontal cortex (FCx) revealed that 67 of 858 (7.8%) cells tested were AD activated, while 12 of 357 cells tested with parietal cortex (PCx) stimulation (3.4%) were AD driven. Antidromic activation was verified by constant latency driving, activation at frequencies greater than 100 Hz, and occlusion of driven spikes by collision with spontaneous impulses. All AD driven cells were histologically localized to the globus pallidus area, corresponding to anatomical maps of acetylcholinesterase-positive cells. Latencies from FCx ranged from 1.0 to 13.0 msec (mean = 4.8 $\pm$ 0.4 msec), reflecting conduction velocities of about 0.6 to 8.0 m/sec. Latencies for PCx activation ranged from 1.6 to 24.0 msec (mean = 7.4 $\pm$ 1.1 msec), yielding conduction velocities of approximately 0.5 to 7.5 m/sec. Additional experiments revealed that fibers with the slower conduction velocities had lowest thresholds in superficial cortex, while the faster fibers were most easily driven from deeper cortical placements. Single neurons often exhibited multiple antidromic latencies as a function of stimulation depth or amplitude. These results indicate that efferent fibers from these basal forebrain neurons are highly branched in cortex, and that they may become non-myelinnated as they reach terminal fields in cortical gray matter. Spontaneous discharge rates and spike waveforms differed among AD driven neurons, indicating a physiologically heterogenous cell population. This work was supported by NINCDS Grant NS19360 and BRSG Grant SO7RR7149-09 from NIH.

106.16 N-METHYLASPARTATE, A USEFUL EXCITOTOXIN FOR LESIONING SUBSTANTIA INNOMINATA NEURONS IN THE RAT. Gregory Stewart*, Madelon T. Price and John W. Olney, Washington Univ, Dept Psychiatry, St. Louis, MO.

A loosely clustered group of neurons in the substantia innominata (SI) region of the basal forebrain comprises the major source of extrinsic cholinergic inputs to the cerebral cortex. Evidence in recent years that these neurons are lost in Senile Dementia Alzheimer's Type (SDAT) has led to attempts to develop an animal model of SDAT by selectively lesioning SI neurons. Two excitotoxins, kainic acid (KA) and ibotenic acid (Ibo), have been used to delete SI neurons from rat and monkey brain respectively (Johnson et al.,PNAS 76, 5392, 1979; Struble et al., Neurosci Abst 8, 211,1982). Efficacy of the lesioning approach, as reflected by loss of cholineacetyltransferase (CAT) from the cerebral cortex was reported as a 45-50% loss induced by KA (rat) or "significant" loss induced by Ibo (monkey). Since both of these agents have disadvantages as lesioning agents, KA being a potent convulsant that causes seizure-linked distant lesions and Ibo being exhorbitantly expensive, difficult to synthesize and unstable, we undertook the present experiments to test the efficacy of N-methyl-DL-aspartate (NMA) a relatively potent excitotoxin that has none of these unfavorable properties.

Adult female rats (~300g) were stereotaxically injected with NMA unilaterally at two dorsoventral levels (-6.0 and -7.0 from dura) through a single needle insertion (skull raised to 4.0, AP at bregna, L=2.6). Each injection consisted of 100 nmol NMA in 1 µl of sterile distilled H_2O (pH adjusted to 7.0±0.2). Ultrastructural analysis of the SI lesion revealed sparing of most axons and acute degeneration of SI neuronal perikarya. CAT measurement in ipsilateral frontal and parietal cortices revealed a loss of 47% and 41% respectively (compared to homotopic contralateral cortex) and was accompanied by a striking loss of cholinesterase staining. Electron microscopic evaluation of the ipsilateral cortex at appropriate post-treatment intervals revealed numerous degenerating axon terminals, the majority of which were in synaptic contact with small caliber dendritic branchlets and were distributed diffusely through both superficial and deep cortical layers. The long-term fate of denervated cortical cholinergic receptors and ability of spared cholinergic neurons to reinnervate such receptors remain under investigation. We conclude that NMA is a useful agent for selectively lesioning SI cholinergic neurons and studying the synaptic organization and functional role(s) of their cortical projections. Supported in part by Research Career Scientist Award MH 38894 (JWO).

107.1 EFFECTS OF BRAINSTEM COOLING ON TREADMILL LOCOMOTION EVOKED FROM THE MESENCEPHALIC LOCOMOTOR REGION. L.M. Jordan, S.J. Shefchyk and R.M. Jell. Department of Physiology, University of Manitoba, Winnipeg, Canada R3E OW3.

Electrophysiological and anatomical studies of projections from the mesencephalic locomotor region (MLR) have revealed pathways to pontobulbar reticulospinal nuclei (n. reticularis gigantocellularis and magnocellularis and n. raphe magnus) and to lateral sites corresponding to the pontobulbar locomotor strip (PLS). We have examined the role of these sites in MLR-evoked locomotion by reversible synaptic blockage with local cooling. Locomotion was induced in high decerebrate, decerebellate cats by stimulation of the MLR at stereotaxic coordinates P2-4, L2-4, H4.5-6.5. Locomotor activity was monitored by EMG recordings from the biceps brachii, triceps brachii, tibialis anterior, and lateral gastrocnemius muscles bilaterally. Selected brainstem sites were cooled using ice water circulating at a rate of 15 ml/min through a coaxial 18 gauge stainless steel probe. The temperatures reached in the closely surrounding tissue were within the range required for blocking synaptic but not axonal transmission. When locomotion was initiated by stimulation of the most lateral sites in the MLR (L3.5-4.0), cooling in the midline region 5mm rostral to the obex at a depth of 2-3mm below the surface of the medulla always reversibly abolished or severely impaired MLR evoked locomotion. Cooling within the ipsilateral PLS at a site 5mm rostral to the obex, 4mm lateral to the midline and at a depth of 2-3mm abolished or impaired locomotion induced from the lateral MLR in some animals and was without effect in others. Cooling within the contralateral PLS was always without effect when lateral MLR sites were stimulated. When the stimulating electrode was positioned in the medial MLR (L2.0-2.5), the evoked locomotion was unaffected by midline cooling; but cooling within the ipsilateral PLS (L4.0) reversibly abolished the locomotor response. These results suggest that relays within the medulla are required for the "locomotor command" from the MLR to reach the spinal cord. Furthermore, they suggest that lateral MLR sites form functional relays in both the midline reticulospinal areas and in the ipsilateral PLS, while the functional relays of medial MLR sites are in the ipsilateral PLS.

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107.2 LOCOMOTOR STEPPING ELICITED BY ELECTRICAL STIMULATION OF THE LATERAL HYPOTHALAMUS IS MEDIATED BY AN IPSILATERAL DESCENDING PATHWAY, Sun Hi Lee,* Harry M. Sinnamon and David G. Adams. Department of Psychology, Wesleyan University, Middletown, CT 06457.

The effects of unilateral midbrain lesions on stepping produced by ipsilateral MFB stimulation was determined in acute experiments. Rats were anesthetized with nembutal (50 mg/kg), mounted in a stereotaxic apparatus and suspended over a 30-cm diameter wheel. Stepping by the rat caused the wheel to rotate. Supplemental anesthesia was provided by nembutal (7.5 mg/kg). Stimulation consisted of 10-sec trains of 50 Hz cathodal pulses (0.5 msec) at currents up to 200 uA delivered through monopolar #00 insect pins. Lesions were made by radio-frequency current (6 or 8 mA) continued for 40 sec. The stimulation sites were in the MFB mainly at the level of the subthalamic nucleus. Prelesion baseline locomotor scores were determined and one side of the brain was randomly selected to receive a midbrain lesion. Following the lesion, the MFB electrodes were tested periodically for approximately 1 hour.

Certain midbrain lesions (N=6) abolished stepping elicited from ipsilateral MFB stimulation but produced no significant effect on contralaterally elicited stepping. These lesions destroyed a region which included the medial two-thirds of the medial lemniscus, the ventral red nucleus, the medial third of the substantia nigra compacta (SNC) and the ventral tegmental area (VTA). Partial reductions were produced by lesions that were generally more lateral. Lesions without effect spared the dorsomedial aspect of the VTA but damaged extensively either the SNC and SNR, ventrolateral VTA, red nucleus, medial lemniscus, central gray and superior colliculus. To determine if an ascending system was responsible, the effects of MFB lesions were determined on stepping elicited from VTA stimulation. In none of five cases was stepping reduced. We conclude that stepping elicited from MFB stimulation in this preparation requires a descending system projecting through the VTA.

107.3 CATALEPSY CAUSED BY INJECTION OF CHOLINERGIC AGENTS INTO THE MESENCEPHALIC RETICULAR FORMATION. S.L. Hartgraves* and P.H. Kelly. Dept. of Physiology and Biophysics, USC School of Medicine, Los Angeles, CA 90033.

Catalepsy in rats is an experimentally-induced state which shares a common characteristic with Parkinson's disease, akinesia. Catalepsy can be caused by systemic injection of neuroleptics, cholinergics or narcotics, or by injection of these agents into specific brain regions. Whereas neuroleptic-induced catalepsy seems to depend upon an intact caudate nucleus, cholinergic-induced catalepsy is produced at another location(s) (Costall and Olley, Neuropharmacology, 10:297-307, 1971). There is a relationship between neuroleptic- and cholinergic-induced catalepsy, however, since systemic atropine can antagonize the catalepsy caused by systemic neuroleptic. This relationship may ultimately occur in the mesencephalic reticular formation (MRF), due not only to its connection with the substantia nigra (Beckstead, et al., Brain Research, 175:191-217, 1979), but also because of its involvement in extra-pyramidal movement (Imperato, et al., Brain Research, 216:437-443, 1981). To examine this hypothesis male Sprague-Dawley rats were stereotaxically implanted with cannulae into the ventral MRF at the level of the red nucleus. Bilateral injection of a mixture of 50 µg acetylcholine and 2.5 µg eserine in 0.5 µl saline caused pronounced catalepsy as measured by the bar test (front paws placed on a bar 10 cm above the table surface, average of two tests with the maximum score being sixty seconds). To examine the effects of atropine in the MRF on catalepsy elicited by haloperidol, rats were injected systemically with 1.5 mg/kg haloperidol two hours prior to bilateral MRF injection of atropine sulfate (5 µg in 0.5 µl saline) or saline. Atropine, but not saline, significantly reversed the cataleptic effects of haloperidol. This effect was apparent immediately following the completion of the injection.

This study suggests that the MRF may be a site involved in the mediation of catalepsy by cholinergic agents, and in the reversal of neuroleptic-induced catalepsy by anticholinergic agents.

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107.4 FORELIMB AND HINDLIMB STEPPING BY THE ANESTHETIZED RAT ELICITED BY ELECTRICAL STIMULATION IN THE DIENCEPHALON AND MESENCEPHALON, Harry M. Sinnamon, Laboratory of Neuropsychology, Wesleyan Univ., Middletown, CT 06457.

Thirty rats anesthetized with a combination of chloralose, urethane and nembutal or nembutal alone were fixed in a stereotaxic apparatus and suspended over a moving treadmill belt. Electrical stimulation (100µA, 10-sec trains, 0.5-msec cathodal pulses, 50-Hz pulse frequency) was applied every 200-µm through 109 movable electrodes. The patterns of stepping elicited ranged from well-coordinated stepping of all four limbs to spastic stepping of only one limb. In the hypothalamus, stepping-positive sites were found in and dorsal to the medial forebrain bundle (FMP), the paraventricular, dorsomedial and posterior nuclei, and the supramammillary area. Dorsally, effective regions extended to the medial zona incerta and the fields of Forel. In the thalamus only the parafasicular nucleus and the anterior parts of the rhomboid and reuniens nuclei were positive. Positive sites were particularly dense in the ventral tegmental area but were infrequent in basal regions caudal to the interpeduncular nucleus. In the dorsal midbrain positive sites were most common at caudal levels dorsal and dorsolateral to the central gray. At sites in the area of the medial raphe, stepping was elicited only at the offset of the train. The distribution of positive sites resembled that found in unanesthetized rats and indicates that locomotor initiation pathways are separated into dorsal and ventral systems at the level of the midbrain.

107.5 BRAINSTEM AXONS MEDIATING CIRCLING: BEHAVIORAL MEASUREMENT OF CONDUCTION VELOCITY DISTRIBUTIONS. J.S. Yeomans and L. Linney*. Dept. of Psychology, Univ. of Toronto, Ontario, M5S 1A1.

Electrical stimulation delivered near the crossed tectospinal tract elicits rapid ipsiversive circling at low currents. In this study the trajectory and conduction velocities of systems mediating this behavior were measured using the collision method of Shizgal et al. (J. Comp. Physiol. Psychol., 94, 227, 1980). C pulses were delivered through a midbrain electrode and T pulses through a pontine electrode, or vice versa. If axons passing near both sites mediate the circling, collision will occur between antidromic and orthodromic action potentials from the two electrodes at short C-T intervals, but not at long C-T intervals. Collision was measured by comparing T pulse effectiveness, i.e. the decline in frequency required to produce a constant amount of circling (Yeomans, Physiol. Behav., 15, 593, 1975; Miliaressis, Physiol. Behav., 26, 891, 1981.), at short and long C-T intervals. Clear evidence of collision was found for 3 electrode pairs, suggesting that a longitudinal bundle of axons mediates the circling behavior. In particular, 1) Double electrode (i.e. collision) measures of T pulse effectiveness rose at longer C-T intervals and rose more gradually than single electrode (i.e. refractory period) measures. This suggests a range of conduction velocities in the axons. 2) No local potential summation was observed in double electrode curves at C-T intervals of 0.2 msec. 3) Collision and spatial summation increased systematically as current increased.

Conduction velocity was measured in two ways, one yielding a low estimate and the other yielding a high estimate. These estimates were 10-30 m/sec for the initial rise, 2.6-9 m/sec for the 50% level, and 1.1-3.7 m/sec for the final rise. For comparison, physiological estimates of the conduction velocity of tectal units driven contralaterally from the same brainstem sites that produced circling were determined. These units had conduction velocities that ranged from 1-20 m/sec. These crossed tectospinal axons, then are likely candidates for the substrate that produces circling.

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107.6 A ROLE OF NIGRAL DOPAMINE RELEASE IN DRUG-INDUCED CIRCLING BEHAVIOR AND LOCOMOTOR ACTIVITY. E.A. Jackson* and P.H. Kelly (SPON: B.C. Abbott). Dept. of Physiology and Biophysics, USC School of Medicine, Los Angeles, CA 90033.

Evidence has accumulated recently to indicate that dopamine is released from dendrites in the substantia nigra (Cheramy et al., Nature, 289:537-542, 1981). In the present studies behavioral effects of intra-nigral dopamine receptor stimulation and blockade were investigated. In rats pretreated with systemic pargyline, contralateral circling was elicited by unilateral infusion of dopamine or d-amphetamine into the substantia nigra pars reticulata (SNR). The latter of effect was of short duration, and could be reinstated 2 hours after intracranial infusion by systemic d-amphetamine. Unilateral intranigral infusion of the putative D-1 receptor agonist SKF38393 also caused short lasting contralateral circling, which could be reinstated by systemic d-amphetamine. In rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway, amphetamine-induced circling was markedly reduced by prior infusion of haloperidol into the SNR bilaterally. Infusion of a GABA-transaminase inhibitor, γ-acetylenic GABA (GAG) into one SNR caused pronounced contralateral turning when followed immediately by systemic d-amphetamine. Prior infusion of haloperidol into the SNR bilaterally reduced the circling caused by amphetamine after unilateral intranigral GAG. In pargyline-pretreated rats bilateral intranigral dopamine elicited pronounced stimulation of locomotor activity, whereas the locomotor activity elicited by systemic amphetamine was markedly reduced by bilateral intranigral haloperidol.

The results support a role for dopamine released from dendrites in the substantia nigra in both circling behavior and locomotor activity, and suggest the substantia nigra is a site involved in the behavioral effects of psychostimulants and neuroleptics.

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107.7 CONTROL OF LOCOMOTION USING LOCALIZED INJECTIONS OF GABA AGONISTS AND ANTAGONISTS INTO THE MESENCEPHALIC LOCOMOTOR REGION (MLR). E. Garcia-Rill, R. D. Skinner and J. A. Fitzgerald*. Dept. of Anatomy, Univ. of Arkansas, Little Rock, AR 72205.

Following a precollicular-postmamillary brainstem transection in the cat, controlled locomotion on a treadmill can be induced by electrical stimulation of the MLR. This study was undertaken to determine whether or not locomotion could be induced in the same preparation by localized, chemical activation of the MLR. An ensemble of stimulating wires about a 35 g cannula was lowered into the posterior midbrain until the tip was located in the MLR. Location was verified by the induction of controlled locomotion on a treadmill using current pulses of 50 μA or less. Several neurotransmitters, their agonists and antagonists (concentrations 0.001-1M), were infused (rates 1-5 μl/min) in standard volumes of 1.5 μl. To date, only infusions of the GABA antagonist, picrotoxin (PIC), have resulted in locomotion on a treadmill in the precollicular-postmamillary cat. Amounts of 3-6 μg of PIC (0.005M) resulted in a 10-30% decrease in the threshold for electrically-induced locomotion. Amounts of 6-9 μg induced greater than 50% decreases in threshold, leading within minutes to spontaneous and constant (over 1 hr) locomotion without need of electrical stimulation. PIC-induced locomotion could be blocked by infusion of the GABA agonist, muscimol (MUS) (0.005M) within seconds. Additional injections of PIC reinduced locomotion which could again be blocked by MUS. Injection spread was verified using similar volumes of Fast Green, Evans Blue or Bisbenzimide dyes. Only injections which included the cuneiform and pedunculopontine nuclei resulted in locomotion. Injections of more than 10 μg of PIC which included the reticular formation were found to induce convulsions. Injection sites other than the MLR produced no locomotor movements. Sites in the inferior and superior colliculi had no effect.

Our findings demonstrate for the first time that localized chemical infusions into the MLR can induce and inhibit locomotion (Qualitatively, chemical-induced locomotion appears more "natural" than electrically induced locomotion). The first indication of the presence of gabaergic receptors in the MLR is provided by the observed blockage by MUS of PIC-induced locomotion. The source of the gabaergic input is unknown, but the substantia nigra has been reported by others to provide ascending gabaergic projections. We have reported the presence of a descending substantia nigra projection to the MLR, the only afferent to the MLR located posterior to the precollicular-postmamillary transection.

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107.8 ROLE OF SUBSTANTIA NIGRA IN LOCOMOTION. E. Eidelberg, J. Yu and M.H. Droge. U. of Texas Sch. of Med. and V.A. Hospital, San Antonio, TX, 78284

Thalamic cats walk on a treadmill when the subthalamic region (SLR) or the area of N. cuneiformis (MLR) are stimulated electrically. We tested the possibility that the cell bodies in the SLR and MLR are crucial to this by localized injection of glutamic and homocysteic acids. There was no locomotor response there, but electrical and chemical stimulation of the S. Nigra, pars reticulata, did evoke walking. Bilateral coagulation of this region, however, did not abolish walking in trained cats. Total cerebellar ablation did not prevent later elicitation of controlled locomotion. These data indicate that the crucial elements in the SLR and MLR are probably axons going to and/or from the substantia nigra. This seems to be important in view of the gait disorder in Parkinson's disease.

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107.9 ACTIVITY OF RETICULOSPINAL NEURONES DURING LOCOMOTION IN UNRESTRAINED CHRONIC CATS. T. Drew*, G. Blanchette* and S. Rossignol. Centre de recherche en sciences neurologiques, Université de Montréal, Montréal (Québec) CANADA.

Experiments in high decerebrate cats have suggested that during locomotion reticulospinal (RS) cells in the medullary reticular formation (MRF) are related principally to the electromyographic (EMG) activity of flexor muscles (Orlovsky, G.N., 1970, Biophysics. 15: 761). Experiments in chronically implanted, unrestrained animals suggest, however, a more complex interaction.

Under aseptic conditions a plate designed to hold a microdrive was stereotactically positioned to allow access to the MRF at coordinates in the range of AP-3 to AP-12 and L 0.5 to L 4.0. Teflon-insulated stainless steel wires were sewn into flexor and extensor muscles of all four limbs as well as into back and neck muscles; Tri-ML insulated 50μm wires were implanted at L_2 into the spinal cord for antidromic identification of RS cells. Recordings were made using conventional glass-coated tungsten micro-electrodes (1-5MΩ at 1KHz). Temporal relations, on a step by step basis, were studied between the unit discharge and each recorded EMG.

76 reticulospinal neurons from 3 cats have been recorded during locomotion. 27 of these neurones (36%) showed a clear frequency modulation during locomotion, displaying either one or two peaks of activity in each step cycle (20 and 7 neurones respectively). All 7 double burst neurones seemed best related to flexor muscle activity whereas the 20 neurones having only one burst in each step cycle were strictly related to the phase of onset, and/or the duration, of extensor (12 neurones) or flexor (8 neurones) muscle activity. Correlations were found with forelimb and hindlimb muscle activity both ipsilateral and contralateral to the recording site.

A further 26 neurones (34%) showed an intermittent but clear frequency modulation at the locomotor rhythm and could generally not be strictly correlated with any one of the recorded EMGs. It is noteworthy that the majority of these cells were excited by passive manipulation of both the neck and/or all four limbs so that they might be related to aspects of interlimb or head-limb coordination. The remaining 23 cells (30%), although projecting to the lumbar cord, either discharged in a completely unrelated manner to the rhythmical patterns of locomotion or were silent.

It is concluded that during walking RS neurones are related to the activity of flexor or extensor muscles, both ipsilateral and contralateral to the recording site. These results are consistent with those obtained by microstimulation of the MRF (Drew, Dubuc & Rossignol, Soc. Neurosci. Abstr., 8, p. 163, 1982).

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107.10 MODULATION OF DENTATE NEURONS DURING THE PERTURBED AND UNPERTURBED STEP CYCLE IN DECEREBRATE CATS. A. B. Schwartz, T.J. Ebner and J.R. Bloedel. Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Mpls., MN 55455.

The purpose of these experiments was to examine the modulation of dentate neurons in spontaneously walking cats with and without two types of perturbation applied during specific phases of the step cycle. Employing an electrocoagulator, cats were decerebrated by transecting the brainstem at an angle of 23° to the horizontal from A3.7 dorsally to A7.2 ventrally. These cats walked spontaneously on a treadmill driven by a speed controlled motor. Approximate limb displacement was recorded with potentiometers attached to the front legs. The biceps and triceps EMG activity was recorded bipolarly. Cells recorded with glass microelectrodes from the right dentate nucleus were antidromically identified using stimuli applied at the decussation of the brachium conjunctivum. Electrode tracks were verified histologically. The discriminated spike activity, the integrated rectified EMG activity, and front leg displacement were averaged (100-200 step cycles) using a specific phase of the right front leg displacement to trigger each sweep used to construct the histogram. Two types of perturbation were used. All four legs were perturbed simultaneously by a dynamic brake which rapidly stopped the belt, halting the limbs in a given position for about 400 msec. On reacceleration of the belt, the cat resumed normal locomotion. The second type of perturbation placed an obstacle in the path of a single moving limb. A pneumatic cylinder was triggered to thrust a rod, impeding the leg during a single step cycle only. Separate histograms were constructed from step cycles with and without perturbations. Some dentate neurons were modulated in relation to the unperturbed step cycle, usually exhibiting a peak in firing rate once per step cycle. The phase of this peak varied among the neurons with this characteristic. In cells that were well modulated in normal locomotion, perturbation of all four legs arrested the modulation during the perturbation. In some dentate cells that were poorly modulated, this type of perturbation elicited a response corresponding to the time course of the perturbation. Perturbation of a single leg did not produce a pronounced change in the response of well modulated cells. However, in poorly modulated cells a short decrease in rate was evoked. These data show that dentate neurons can be modulated during the step cycle and that discharge of these neurons can be modified by at least two types of perturbation. This work was supported by NIH Grants RO1-NS 09447 and RO1-NS 18338.

107.11 COORDINATION OF LOCOMOTION AND THE PAW SHAKE RESPONSE IN THE HINDLIMBS OF SPINALIZED CATS. M.C.Carter and J.L.Smith. Dept. Kinesiology, UCLA, Los Angeles, CA 90024

In the normal cat, generation of the hindlimb paw shake response (PSR) during treadmill locomotion is confined to the swing phase of the step cycle (Neurosci. Abst. #47.6, 1982). To accommodate both actions, the PSR is usually limited to 1 to 4 cycles and the time devoted to the swing phase of the shaking limb is increased, as is the stance phase of the contralateral hindlimb. Although both treadmill locomotion and the PSR can be generated independently in the hindlimbs of a spinalized cat, it is not known whether the lumbosacral cord alone can coordinate the simultaneous execution of the two behaviors. The purpose of this study was to examine the coordination of locomotion and the PSR in the spinalized cat.

Young adult cats were spinalized at T_{12} and surgically implanted with bipolar fine wire electrodes in the right and left soleus (RSO, LSO), right lateral gastrocnemius (RLG) and tibialis anterior (RTA) muscles. EMG records obtained during unperturbed treadmill locomotion were compared with walking and concurrent shaking at speeds of 0.4 and 0.6 m/s. Paw shaking was induced by wrapping tape around the right paw. Cycle duration (CD, measured from successive LSO onsets) and burst durations (BD) normalized to a percent of CD were obtained from the EMG records.

Data analyses were limited to periods when the hindlimbs maintained stable weight support and rhythmical stepping. Average CD decreased from 1007 $\pm$ 51 to 780 $\pm$ 75 ms when the treadmill speed increased from 0.4 to 0.6 m/s. Similar to normal cats, when tape was applied to the hindpaw, the PSR occurred intermittantly and was initiated in the swing phase of the step cycle. However, the average number of PSR cycles generated by the spinal cat was greater than that observed for the normal cat, 12 $\pm$ 2 and 4 $\pm$ 2, respectively. To accommodate the complete PSR elicited in the right hindlimb, the left hindlimb of the spinalized cat simultaneously produced multiple steps. At 0.4 m/s, two short steps were usually completed by the left leg with average CD of 677 $\pm$ 45 and 850 $\pm$ 83 ms. At 0.6 m/s, three steps were completed with the first two steps shorter than the third, 585 $\pm$ 81, 559 $\pm$ 121 and 884 $\pm$ 108 ms, respectively.

We conclude that the lumbosacral cord can coordinate the simultaneous generation of the PSR during locomotion. Rather than being limited to a single swing phase, the PSR in the spinalized cat continues its full course, 9 to 12 cycles, and the contralateral limb compensates by taking multiple steps. This behavioral strategy was not observed in the normal cat and suggests that supraspinal input constrains the completion of the PSR in the normal cat, whereas generation of the PSR in the spinalized cat is unmodified.

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107.12 ONSET AND PERTURBATION OF AIRSTEPPING IN THE CHRONIC SPINALIZED CAT. C. A. Giuliani and J. L. Smith. Neuromotor Control Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024.

Previous studies have identified the temporal characteristics of airstepping (ASTP), a commonly observed behavior in chronic spinalized animals, as similar to those of locomotion (Giuliani et al, Neurosci. Abst., #47.7, 1982). The purpose of the present study was to identify the onset of ASTP following cordotomy and to determine the effect of sensory perturbation on ASTP patterns.

Five adult cats were cordotomized at T-12 and bipolar electrode wires were chronically implanted in the soleus (SO) and the tibialis anterior (TA) of both hindlimbs. The animals were tested 2-3 times wkly for 3 mos following cordotomy. EMG parameters analyzed included: cycle duration (CD, defined as the interval between sequential SO burst onsets), burst duration, and integrated EMG (I-EMG). The effects of tonic sensory perturbation were studied druing immobilization of the ankle (110°) and knee (115°) in a plaster cast, with tape application to the hind paw, and after limb deafferentation.

The earliest onset of ASTP occured at 13 days following cordotomy and required tail pinching to elicit the behavior, while the latest onset occurred at 29 days. Spontaneous ASTP, without additional exteroceptive stimuli, occurred between 21 and 39 days following cordotomy. Prior to the onset of ASTP, all cats exhibited paw shaking responses, myotatic, flexion, and crossed extension reflexes, all of which were present within 4 days of cordotomy.

The effects of sensory perturbation were determined by comparing the EMG parameters of perturbed ASTP to those of spontaneous ASTP. Immobilization by casting had no effect on average CD (656 ms); however, burst durations of the ipsilateral SO and TA both increased 53%, while increases in I-EMG were 456% and 94% respectively. With tape application, ASTP was slower by 42% and exhibited a larger amplitude of movement in both limbs. Also, SO burst duration increased on average 60% and I-EMG increased 156%.

In one cat, the left hindlimb was partially deafferented (L4-S2) to determine the effects of decreased segmental input. Preliminary results indicate that the spinal cord was still capable of generating a characteristic ASTP pattern, although tonic input (tail pinching) was required to elicit ASTP. The pattern of intralimb coordination was essentially unchanged while the normal interlimb coordination pattern occurred infrequently.

Our results show that although some parameters of ASTP (CD and burst durations) may be modified substantially by sensory augmentation, others such as intra- and interlimb coordination remain invariant. Reduction of the segmental input by deafferentation alters interlimb coordination, but not intralimb control.

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107.13 EMG - FORCE RELATIONSHIPS IN CAT MG AND SOLEUS DURING TREADMILL LOCOMOTION. R.J. Gregor,* R.G. Lovely,* R.R. Roy and V.R. Edgerton, Brain Research Institute and Dept. of Kinesiology, UCLA, Los Angeles, CA 90024.

Neural control of locomotion has typically been described through the phasic electromyogram. While individual muscle forces have been reported (J. Neurophysiol., 41:1203-1216, 1978) little attention has been given the temporal relationship between EMG and subsequent force production of individual muscles in vivo. This study was designed to assess the phasic relationship between selected EMG and force parameters in vivo in the cat MG and SOL during unrestrained treadmill locomotion.

A total of 180 step cycles in three cats walking/running at three different speeds (0.8 m/s, 1.6 m/s, and 2.2 m/s) were chosen for analysis. Each cat was surgically implanted with a force transducer (J. Biomechanics, 14:489, 1981) of both MG and SOL tendons and with a pair of electrodes (50 μm) implanted into the belly of each muscle (Brain Res. 117:529-533, 1976). Records were stored on FM tape and subsequently digitized off-line by a digital MINC 23 minicomputer.

As treadmill speed increased, mean peak tension in the MG increased (40%) while mean SOL peak tension declined (20%). While impulse duration (Ft) was similar for both MG and SOL, at each speed EMG burst duration was always longer for the MG. The difference was 43 ms at 0.8 m/s and 23 ms at 2.2 m/s. Electrical activity in the MG began 12 ms before the SOL at the slow speed but 4 ms after the SOL at 2.2 m/s. The delay between onset of EMG and the onset of force was 10 ms longer (34 vs 44) for the MG than the SOL at 0.8 m/s but similar (∿55 ms) for both muscles at 2.2 m/s. Peak force in the SOL always occurred before the MG with differences of 14 ms at 0.8 m/s and 6 ms at 2.2 m/s. Additionally, the time between peak force and EMG cessation was always greater for the MG than the SOL. Although this time decreased as speed increased, a consistent difference of 15-20 ms remained.

These data suggest that in spite of the marked differences in contraction time, relaxation time and maximal velocity of shortening of the SOL and MG when tested in situ, the actual time to peak tension, time to complete relaxation and the duration of the force pulse are similar in the SOL and MG muscles when the cat walks at a slow speed or trots at a fast speed.

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107.14 ARCHITECTURAL DESIGN OF ADULT CAT HIP MUSCULATURE. R.R. Roy, M.D. DiGiuro* and V.R. Edgerton. Brain Research Institute and Dept. Kinesiology, UCLA, L.A., CA 90024.

The hip musculature has been implicated as a "trigger" to initiate and maintain locomotory patterns in the normal and spinal cat (Grillner and Rossignol, Brain Res. 146:269, 1978). Although some of the sensory factors involved in this apparent hip joint control have been studied, details of the architecture of the musculature in which many of the sensory receptors are located have not been described. The design of the muscle appears to be important in determining the responsiveness of the muscle to length changes (Walmsley and Proske, J. Neurophysiol. 46:250, 1981). Spindle density (#/g muscle) is often considered to be an important variable in this responsiveness. But receptor density can be misleading when comparing muscles differing in their architectural design. In addition, the force and velocity properties of a muscle are known to be dictated in large part by its architectural design (Bodine, et al., J. Neurophysiol. 48:192, 1982; Spector, et al., J. Neurophysiol. 44:951, 1980).

Thus, the purpose of this study was to detail the architectural features of the major extensor flexor, adductor and abductor muscles at the hip. Muscle wet weights (MW), muscle lengths (ML), fiber lengths (FL), approximate angles of pinnation (θ) and average sarcomere lengths (SL) were used to calculate the physiological cross-sectional area (CSA) of each muscle as described by Sacks and Roy (J. Morphol. 173:185, 1982).

Some data were provided by the latter study. MW's ranged from .2 (gemellus inferior) to 29.6 (adductor femoris) g. Average FL normalized to a SL of 2.2 μm ranged from 7.0 (obturator externus) to 105.5 (sartorius) mm. Generally, there was a tapering of FL from one surface of the muscle to the other. Average FL to ML ratios were consistent for a given muscle. Theta was less than 6° in all muscles except for the quadratus femoris (∿10°). The CSA of the extensors was ∿4 times that of the flexors and the CSA of the abductors was ∿3 times that of the adductors. In general, the uniarticular extensors and flexors seem to be more appropriately designed for force production while the biarticular extensors and flexors appear to optimize velocity and displacement. These data suggest that the appropriate control mechanisms of the hip could be accounted for by the marked variation in the basic architectural design of the involved musculature.

(Supported by NIH Grant 16333)

107.15 DISCHARGE PATTERNS OF DYNAMIC AND STATIC GAMMA MOTONEURONES DURING LOCOMOTION IN THE PREMAMMILLARY CAT AND THEIR IMPLICATIONS FOR CONTROL OF LOCOMOTION. R.B. Stein, J. Taylor* and P.R. Murphy. Dept. of Physiology, University of Alberta, Edmonton, Canada T6G 2H7

Although suggestions have been made for thirty years on the role of gamma motoneurones, few direct recordings are available in behaving animals to test these suggestions, and the function of gamma motoneurones remains unclear. To clarify their role, we recorded triceps surae gamma motoneurones from cut nerve filaments in a premammillary cat during locomotion. Even if one leg is largely denervated except for the muscles of interest and fixed in place for recording, the other three legs walk spontaneously on a treadmill, and the fixed leg generates appropriate motor outputs.

In this preparation, two distinct patterns of gamma activity were observed. One type of fibre (γ_{dm}, 11 units) had a high resting discharge (41 i.p.s.), which changed little during walking (mean = 34 i.p.s.), but was dynamically modulated (mod. = ±82%) with each step. Another type (γ_{sm}, 16 units) had a low resting discharge (11 i.p.s.), which increased at the onset of walking (static modulation) to a high mean level (58 i.p.s.) which varied less (±24%) during each step. During each step, peak γ_{dm} activity generally occurred after peak EMG (10 out of 11 units) and sometimes even after the time of peak tension (3 out of 11 units). In contrast, peak γ_{sm} activity generally preceded peak EMG (13 out of 15 units).

In other experiments with premammillary cats, we found that the sensitivity of soleus muscle spindle primary afferent endings to small sinusoidal stretches (<1 mm, 4-10 Hz) is reduced near the time of peak γ_{sm} activity and increased near the time of peak γ_{dm} activity. In contrast, the sensitivity of secondary muscle spindle afferents to stretch is decreased throughout the stretch cycle. Finally, in anaesthetized cats, various stimulus patterns were applied to dynamic and static gamma motoneurones during stretch of muscle spindle afferents. For our parameters of stretch, we generally confirmed that dynamic gamma motoneurones increase and static gamma motoneurones decrease the sensitivity of primary afferents.

We conclude that γ_{dm} and γ_{sm} units correspond to dynamic and static gamma motoneurones respectively. The observed patterns of activity permit the velocity sensitivity of triceps surae spindles to be maximized when the active muscles are transiently stretched by the weight of the body during stance and minimized when the muscles are passively stretched by antagonists during the swing phase of locomotion.

Supported by Canadian Medical Research Council (RBS) and Alberta Heritage Foundation for Medical Research (JT and PRM).

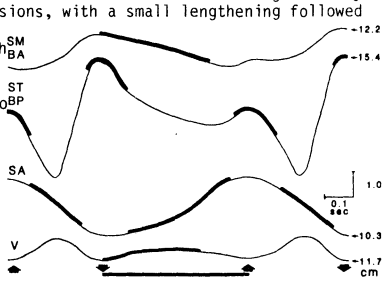
107.16 THE KINEMATICS AND TASK GROUP ORGANIZATION OF BIFUNCTIONAL MUSCLES DURING LOCOMOTION. G.E. Loeb, W.B. Marks, A.J. Rindos, M. O'Malley*, J.P. Chapelier*, and W.S. Levine*, Lab. of Neural Control, NINCDS, Betnesda, MD 20205, and Dept. of Electrical Engineering, U. of Maryland, College Park, MD 20747.

The motor units of the cat anterior sartorius muscle (SA) are divided into task groups which each contribute to either stance or swing phase movements but not both (Hoffer et al., J. Physiol. 308:20P, 1980). This division may be ascribed to one or more of the following characteristics peculiar to that muscle:

1) mixed polyarticular action - hip flexor and knee extensor
2) recruitment in both half cycles - stance and swing phases
3) kinematic dichotomy - active lengthening vs. shortening

The biarticular hamstrings muscles (semitendinosus - ST and posterior biceps - BP) provide a test case of the importance of these factors. They are complete anatomical antagonists of SA, causing hip extension and knee flexion, and they also generate two bursts of EMG per step cycle (early flexion phase and early stance phase). However, there is evidence from thalamic locomotor preparations that single motoneurons projecting to ST are depolarized and can be activated during both EMG bursts (Perret and Cabelguen, Brain Res. 187:333-352, 1980), in contrast to SA motoneurons.

The figure indicates the timing of EMG bursts (thick regions on muscle length curves) recorded by chronically implanted bipolar "patch" electrodes. The lengths were calculated from digitized video stick figures (60 fields/sec) and anatomical measurements of origin, insertion, lever arms, and pulleys as appropriate. The nearly-monarticular hip extensors semimembranosus (SM) and anterior biceps (BA) undergo steady active shortening during stance, while the monarticular vastus knee extensors (V) undergo slight active lengthening. The biarticular ST and BP muscles undergo considerable length change during the step cycle, but the two bursts of EMG activity both occur as the muscles undergo virally identical velocity excursions, with a small lengthening followed by active shortening.



This is consistent with kinematics being the determining factor in the division of a muscle into task groups, presumably to cope with the different motor control problems presented by active shortening vs. lengthening, regarding both the fusimotor control of spindles and the use of stretch reflexes.

107.17 RECRUITMENT OF MOTONEURONS IN BIFUNCTIONAL MUSCLES BY CUTANEOUS NERVE (FRA) STIMULI DURING WALKING IN INTACT CATS. W.B. Marks, G.E. Loeb, and J.A. Hoffer, Lab. of Neural Control, NINCDS, NIH, Betnesda, MD 20205

We have previously reported on the activity patterns of single, identified hindlimb motoneurons during normal walking in the cat (Neurosci. Abst. 272.5-7, 1982). In the anterior sartorius muscle, there appear to be two independent groups of motor units which are similar in anatomical location and action but are independently recruited during the two bursts of activity of this muscle in each step cycle. We have tentatively identified these "task groups" as a flexor group, recruited during swing phase to perform a rapidly shortening contraction related to hip flexion, and an extensor group, recruited during stance, which undergoes active lengthening to assist in antigravity knee extension. Within each group, there is an orderly and strict recruitment based on conduction velocity, but tne groups are never coactivated during normal walking.

Chronically implanted nerve cuffs were used to stimulate saphenous nerve with single shocks 4-10X threshold for the largest fibers. The stimuli were delivered at random phases of normal treadmill walking. The responses included an inhibition of pure extensor muscles (15-25 msec post-stimulus) and a double-peaked excitation of pure flexor muscles (10 msec and 25 msec latencies; Duysens and Loeb, J. Neurophysiol. 44:1024-1037, 1980). In this study, all single motoneurons projecting to the vasti muscles (pure knee extensors) demonstrated the same inhibition as their parent muscle EMGs, with occasional late excitation probably related to rebound and/or stretch reflex activation by yielding during the inhibitory reflex. Six motoneurons projecting to the bifunctional sartorius and rectus femoris were studied; four were normally recruited with the extensor task group (during stance) and two with the flexor task group (during swing). In response to the cutaneous nerve stimuli, all six participated in a typical double-peaked flexor reflex. Units were activated at both latencies, although rarely during a single trial.

The flexor reflex recruitment task group thus appears to include motoneurons from both the flexor and extensor locomotor task groups of bifunctional muscles (probably all motoneurons in tnese pools), providing an efficient way to achieve maximal mechanical synergy for ballistic movements which do not require orderly proprioceptive feedback. A similar analysis of cutaneous reflex EMG's from hamstrings and ankle muscles supports the notion that tne FRA pathway generates two basic task groups: a double-peaked excitatory response in all muscles with significant flexion action, and an inhibition in all pure extensor muscles. Further refinements of these patterns are consistent with the effects of pre- and post-synaptic gating by the step cycle generator and proprioceptive sequelae of short latency responses.

107.18 Locomotor Activity Patterns of Single Motor Units in Compartments of Cat Lateral Gastrocnemius Muscle. A. W. English Department of Anatomy, Emory University, Atlanta, GA 30322

Cat lateral gastrocnemius (LG) muscle is composed of four compartments, each of which contains a discrete population of motor units. To examine the extent to which they are used independently during locomotion, the behavior of motor units in the different compartments was determined using chronic unit recording techniques (eg. Hoffer et al, Science 214:343-345, 1981). Single LG and soleus (SOL) motor units were recorded from "floating" "hatpin" microelectrodes which had been placed in the lateral gastrocnemius-soleus nerve under general anesthesia. Each motor unit was identified using the spike-triggered averaging technique (STA), in conjunction with chronically-implanted EMG electrodes and tripolar nerve cuffs. A unit was identified as a motor unit and its axonal conduction velocity determined by averaging onto a cuff placed on the sciatic nerve just distal to the branching of the hamstring nerve. Motor units were further characterized by averaging onto EMG recordings from each of the different LG compartments and SOL. From such records, the compartment into which the motor unit projected could be determined. Timing patterns of single motor units were then analyzed as the cats stepped on a motor-driven treadmill belt at a number of different speeds ranging from 0.3 to 1.0 m/sec. All single units examined displayed one of two temporal activation patterns. They were active either early or late in the EMG burst of their compartment, but not throughout. A few relatively fast-conducting LG units in different compartments displayed clear recruitment thresholds and firing patterns: they displayed no activity during quiet standing; they began activity only above certain treadmill speeds; and they displayed a fairly consistent spike train pattern. For most units, recruitment thresholds were less clear and firing patterns changed appreciably at different stepping speeds. A few were active during quiet standing but most were not. Most fired at all stepping speeds examined, and increased their firing rate with increases in stepping speed. At any given speed motor units in different compartments displayed firing rates which were quite variable, ranging from silence or a few spikes per step cycle to fairly rapid discharge rates. These results suggest that rate coding of LG motor units may be an important factor in the neural control of stepping at slow speeds and that considerable flexibility in activation patterns exists between motor units in different compartments.
Supported by AM19916 from the USPHS.

107.19 MECHANICAL WORK IN CAT HINDLIMB MUSCLES DURING LOCOMOTION: IMPLICATIONS FOR MOTOR CONTROL. R. G. Lovely*, R. J. Gregor*, R. R. Roy, and V. R. Edgerton (SPON: J. J. Vidal). Department of Kinesiology and Brain Research Institute, UCLA, CA 90024.

In vivo measurements of length, tension and electrical activity, although important in studying muscular function during locomotion, are limited indicators of a muscle's role in movement generation. This study employed in vivo length-tension and mechanical work measures to further assess the function of cat soleus (SOL) and medial gastrocnemius (MG) muscles in generating locomotion at different speeds.

Three adult cats were trained to locomote on a treadmill. Muscle lengths were determined using 3-D filming techniques (Med. Sci. Sports & Exercise 14:144-5, 1982), and forces were recorded using surgically implanted tendon buckle transducers (J. Biomech. 14:489, 1982). Length-tension curves were derived from digitized data, and mechanical work during the stance phase of a step cycle was calculated as the integral of tension with respect to length. Slow walking, fast walking and trotting corresponded to speeds of 0.8, 1.4, and 2.2 m/s.

No significant differences were observed between the two walking speeds in negative, positive or net mechanical work for either muscle. Each muscle did however, exhibit significant differences in work measurements between walking and trotting ($P<.05$). Values for negative, positive, and net mechanical work (average±SE) in the SOL of an exemplar cat during fast walking were -13.93±1.45, 17.22±1.38, and 3.29±1.41 mJ, while during trotting these values were -7.38±1.32, 24.87±3.06, 17.50±4.04 mJ, respectively. Negative, positive and net work measurements in the MG during fast walking were -14.08±1.2, 5.78±0.76, and -8.30±1.34 mJ, while those during trotting were -9.38±1.62, 32.39±5.09, and 23.01±4.52 mJ, respectively.

It is concluded that during walking, the SOL is active and producing tension to help control yielding, and to generate ankle extension for propulsion. In contrast, the MG at walking speeds, is active and producing tension to assist in the control of yielding, but provides little energy for propulsion. During trotting, both muscles retain their roles in the control of yielding, but also become major sources of energy for ankle extension and propulsion.
Supported by NIH grant NS 16333-03.

108.1 EVIDENCE FOR THE PRESENCE OF A HIGH FREQUENCY OSCILLATOR IN THE CPG FOR RHYTHMIC JAW MOVEMENTS IN THE GUINEA PIG. L.J. Goldberg and S.H. Chandler. Depts. of Oral Biology, Kinesiology, Anatomy and the Brain Research Institute, UCLA, L.A., CA 90024.

We have previously demonstrated the occurrence of spontaneous rhythmical jaw movements (SRJMs) in the ketamine anesthetized guinea pig. The frequency of the SRJMs are ≃3-4 Hz. The burst phase of each cycle (≃90 ms) is composed of simultaneously occurring depolarizations in jaw opener motoneurons and hyperpolarizations in jaw closer motoneurons. The interburst phase of the cycle is ≃200 ms in duration.

In the present experiments intracellular recordings were obtained from either digastric (jaw opener) or masseter (jaw closer) motoneurons along with EMG activity of the digastric muscle during SRJMs in the ketamine HCl (100 mg/kg i.v.) anesthetized guinea pig. In many cases the digastric EMG response in the burst phase of the cycle was seen to be composed of several distinct segments (sub-units) of EMG activity. The present experiments were designed to determine the frequency of these sub-unit bursts and their relationship to membrane potential fluctuations in jaw opener and closer motoneurons. Cross correlation analyses were performed between the rectified digastric EMG activity and opener and closer motoneuron membrane potential activity during SRJMs. Results of this analysis showed that the digastric EMG bursts were composed of sub-units (≃25 ms in duration) which occurred at a frequency of ≃30 Hz. Synchronized with these EMG sub-units were membrane potential oscillations; depolarizing sub-units in the case of opener motoneurons and hyperpolarizing sub-units in the case of closer motoneurons.

These results demonstrate that within the digastric burst phase of the ≃3-4 Hz SRJM cycle a separate high frequency oscillation (≃30 Hz) can be observed at the level of the membrane potentials of both opener and closer motoneurons. This is similar to what we have previously observed when RJMs are evoked by repetitive (20-60 Hz) stimulation of the masticatory area of the cortex in which the depolarizing and hyperpolarizing sub-units are time locked to each cortical stimulus. This suggests that the mechanism for SRJM generation is similar to that for cortically evoked RJMs. Furthermore, the sub-unit activity in both cases demonstrates that there is a centrally generated tightly coupled depolarization in opener motoneurons and reciprocal hyperpolarization in closer motoneurons.

This research was supported by NIH grant DE4166.

108.2 THE EFFECT OF THE SEROTONIN AGONIST (QUIPAZINE) ON CORTICALLY INDUCED RHYTHMIC JAW MOVEMENTS IN THE GUINEA PIG. S.H. Chandler, L.J. Goldberg, and B. Alba*. Depts. of Kinesiology, Oral Biology, Anatomy and Brain Research Institute, UCLA, L.A., CA, 90024.

Little information is available on neurotransmitters which may be involved in the control of the central pattern generator (CPG) for mastication, although significant advances have been made in this area with respect to the CPG for locomotion. In a previous study we showed that the CPG responsible for rhythmic jaw movements (RJMs) evoked by cortical stimulation is not critically dependent on glycine. In the present study we investigated the role of serotonin on cortically induced RJMs.

5 albino guinea pigs (400-600g) were anesthetized with ketamine HCl (100mg/kg). Prior to the administration of quipazine (3mg/kg i.v.), RJMs were produced by repetitive stimulation of cortex at 40Hz and an intensity sufficient to consistently evoke RJMs. Stimulating electrodes were also implanted in the tongue. Cycle time and EMG burst durations of the RJM's were measured. In addition, control amplitudes of the digastric EMG reflex response to single shock tongue stimulation were obtained.

8 to 12 minutes after the injection of quipazine the threshold intensity for evoking RJMs was markedly elevated. In some instances RJMs could not be evoked at any stimulus intensity. Accompanying the increase in threshold was a significant alteration in the RJM pattern. The pattern became arrhythmic with an increase in the variability of cycle times and burst durations. The occurrence of spontaneous RJMs was completely blocked by quipazine but the digastric EMG reflex response to tongue stimulation was not remarkably affected by the drug. Pretreatment with the serotonin antagonist methysergide (5mg/kg i.v.) blocked the above mentioned effects of quipazine.

The finding that RJM cycle times and burst durations became irregular while the short latency cortico-brainstem-trigeminal pathway remained intact suggests that serotonin has a direct effect on the CPG network responsible for RJM production. The inability of quipazine to modify the jaw opening reflex as evoked by tongue stimulation further indicates that the quipazine effect on RJMs is not the result of significant depression of digastric motoneurons. These results support the model we have previously presented indicating that the CPG for RJMs acts by modulating a cortico-brainstem-trigeminal short latency pathway and not by directly affecting trigeminal motoneurons. It further implicates the role of serotonin in the operation of the CPG for RJMs.

This research was supported by NIH grant DE4166 and Academic Senate Funds at UCLA.

108.3 EFFECT OF SYNCHRONOUS ACTIVATION OF MEDULLARY INSPIRATORY PREMOTOR NEURONS ON PHRENIC NERVE DISCHARGE IN THE CAT. D.F. SPECK, D.R. McCRIMMON* and J.L. FELDMAN. Departments of Physiology and Anesthesia, Northwestern University, Chicago, IL. 60611.

These experiments examined the effects on respiratory neural outflow elicited by synchronous activation of bulbospinal respiratory neurons. Experiments were conducted in chloralose-urethane anesthetized, paralyzed, vagotomized and artificially ventilated cats. Phrenic nerve activities were recorded bilaterally. Descending respiratory axons were activated in the ventrolateral spinal cord at the C2 level using either monopolar or bipolar stimulation (25-100 uA, 100 us, 1-300 Hz). Stimulation at intensities of 100uA antidromically activated approximately half of the inspiratory premotor neurons tested in the ventrolateral nucleus tractus solitarius (vlNTS) or the nucleus retroambigualis (NRA). Activation of descending inspiratory pathways was determined by recording both orthodromic phrenic excitation and antidromic invasion of single, inspiratory modulated units which was confirmed by the criteria of high frequency following and collision tests. Stimulation pulses delivered to the spinal cord elicited a 2-6 msec orthodromic excitation of the ipsilateral phrenic nerve during inspiration. The onset latency of excitation was 2-4 msec and decreased as inspiration progressed. Following the initial excitation there was a 4-30 msec period of inhibition of phrenic nerve discharge. Continuous trains of stimuli or phrenic gated trains delivered during every fourth inspiratory or expiratory cycle had little effect on the duration of inspiration or expiration. Similarly, brief (400 msec) trains of bilateral spinal cord stimulation delivered at various delays from the onset of inspiration had only a transient effect on the amplitude and pattern of phrenic nerve discharge, with no noticeable effect 60 msec after the termination of stimulation. Since synchronous activation of a portion of a central pattern generator would be expected to phase shift or reset the rhythm, we conclude that the bulbospinal respiratory neurons do not themselves generate respiratory rhythmicity and have limited, or no, collateral projections to the neurons that do. (Supported in part by NIH grants NS-17489, HL-23820, HL-00554, and HL-06331 and the Parker B. Francis Foundation).

108.4 MORPHOLOGY OF EARLY VS. LATE RECRUITED PHRENIC MOTONEURONS AS REVEALED BY INTRACELLULAR INJECTION OF HRP. W.E. Cameron, D.B. Averill* and A.J. Berger. Dept. Physiol. & Biophysics, Univ. of Washington, Seattle, WA 98195.

Respiration represents a unique motor system for study because the descending central respiratory drive onto respiratory motoneurons persists during anesthesia. Rather than implying the order of a motoneuron's recruitment based on the mechanical properties of the muscle fibers it innervates, the present study of phrenic motoneurons (PMs) classified units based upon their recruitment relative to the onset of inspiratory activity in the phrenic nerve. The purpose of this study was to determine whether the morphology of PMs is related to their recruitment order.

PMs of the C5-C6 spinal cord were studied in anesthetized, paralyzed and ventilated cats. Antidromically identified PMs were impaled with beveled glass microelectrodes and HRP was iontophoresed into the cell. The cervical cord was fixed, frozen, sectioned and reacted with DAB. Neuronal reconstructions were made.

PMs were classified based on their extra- and intracellularly recorded discharge and the amplitude and trajectory of the central respiratory drive potential. Early recruited units initiated their discharge within the first quarter of the inspiratory cycle, while late recruited units fired within the second half of the cycle. A third group of cells was found that had no spontaneous discharge; these were classified as high threshold units. The mean ± SD of several measured variables are summarized below.

	Early (N=6)	Late (N=11)	High Thres. (N=7)
Axonal CV (m/sec)	45±6	57±5*	69±14**
Axon Diam. (μm)	3.4±0.8	4.9±1.5*	5.4±1.5**
Somal XS area (μm^2)	1741±588	1755±624	2488±892*
# of stem dendrites	8.8±1.6	9.0±1.1	10.1±1.7*

*significantly different (p<.05) than preceding mean
**significantly different (p<.05) than both early & late means

The dendrites of two PMs (one early and one high threshold) were studied in detail. This analysis revealed no significant difference in either the number of end branches per stem dendrite, average or combined dendritic length or total dendritic surface area of these two cells. The two unit types did differ in the regional distribution of their dendrites. The high threshold cell had long dendrites coursing rostrocaudally within the motor column. In contrast, the early cell had many dendrites directed laterally and generated twice as many end branches that terminated outside the column. When compared to the early PM, the high threshold PM had a greater dendritic surface area within the confines of the phrenic motor column. Supported by USPHS grant NS 14857 and NRSAs HL 06 06474 & 06233.

108.5 EFFERENT CONNECTIONS OF THE VENTRAL RESPIRATORY GROUP IN THE MEDULLA OF CATS: AN AUTORADIOGRAPHIC STUDY. J.L. FELDMAN AND D.F. SPECK. Depts. of Physiology and Anesthesia, Northwestern University, Chicago, Ill. 60611.

A large population of medullary respiratory premotor neurons is concentrated in the ventral respiratory group (VRG), which corresponds anatomically with the nucleus ambiguus-retroambigualis from the spino-medullary junction rostral to the retrofacial nucleus. Anterograde transport of tritiated amino acids was used to examine the spinal projections of VRG neurons. Cats were anesthetized with chloralose-urethane and placed in a rigid headholder. An occipital craniotomy was performed to expose the brainstem. At a given level relative to the obex, the VRG was mapped with a tungsten microelectrode. Subsequently, a glass micropipette containing tritiated leucine, lysine and proline (equal amounts totalling 90-120 uC/ul) in saline was inserted into the VRG. The micropipette was mounted in a holder which permitted simultaneous recording and pressure injection. Pressure injection of amino acids (20-50 nl) was controlled using visual examination of the miniscus level in the micropipette. After a two week survival period, cats were anesthetized and perfused. The entire neuraxis was removed and processed using standard autoradiographic procedures*. Examination of the processed sections revealed a marked bilateral projection to the phrenic motor nucleus at the C4-C6 level and a primarily contralateral projection to upper thoracic motoneurons, presumably intercostal. The ipsilateral phrenic projection seems to include inputs both from axons descending in the ipsilateral spinal cord and from axons that descend contralaterally and recross at a cervical level. There seems to be some somatotopic distribution of projections since injection sites at different rostral-caudal sites labelled different regions of the phrenic nucleus. Propriobulbar projections from the VRG included a bilateral ascending projection to the nucleus parabrachialis medialis, the Kölliker-Fuse nuclei and the preolivary nuclei. There was also a projection to the contralateral nucleus retroambigualis-ambiguus. (Supported by NIH grants NS-17489, HL-23820, HL-00554 and HL-06331.) *Tissue was processed by A.D. Loewy.

108.6 PROJECTIONS OF MEDULLARY INSPIRATORY NEURONS TO PHRENIC AND INTERCOSTAL MOTOR NERVES ASSESSED BY CROSS-CORRELATION ANALYSIS D.R. McCRIMMON*, D.F. SPECK AND J.L. FELDMAN, (Spon:P.M. LALLEY) Departments of Physiology and Anesthesia, Northwestern University, Chicago, IL. 60611.

Most of the bulbo-spinal neurons transmitting respiratory neural discharges to phrenic and intercostal motoneurons have cell bodies located within the medulla in the ventrolateral nucleus of the tractus solitarius (vlNTS) or nucleus retroambigualis (NRA). We attempted to determine whether axons of single neurons within these populations projected to populations of inspiratory motoneurons in one or a few neighboring cervical or thoracic segments. Experiments were conducted on chloralose-urethane anesthetized cats which were bilaterally vagotomized, paralyzed with gallamine triethiodide and artificially ventilated. End-tidal PCO2 was continuously monitored with an infrared CO2 analyzer and maintained constant at a level sufficient to maintain inspiratory neural discharges on phrenic and intercostal motor nerves. Discharges of single neurons within the vlNTS or NRA were recorded with extracellular tungsten microelectrodes. Simultaneous recordings were also made of the contralateral phrenic nerve, a rostral (T2-T4), an intermediate (T6-T7) and a caudal (T10-T12) external intercostal nerve. Nerve activities were digitized and the cross-correlations between medullary neurons and whole nerve recordings were computed for each nerve. Preliminary results indicate that single medullary inspiratory neurons may project differentially to these nerves. We observed neurons with short latency correlation peaks (4 to 8 msec) with one or more of the intercostal nerves but no noticeable peak with the phrenic nerve. (Supported by NIH grants NS-17489, HL-23820, HL-00554, and HL-006331. DRM is recipient of a Parker B. Francis Fellowship in Pulmonary Research).

108.7 SYNAPTIC INPUT TO INSPIRATORY AND EXPIRATORY INTERCOSTAL MOTONEURONS IS HIGHLY COHERENT WITH THE INSPIRATORY ACTIVITY OF THE PHRENIC AND RECURRENT LARYNGEAL NERVES OF THE CAT. C.A. Richardson, D.A. Herbert*, R.A. Mitchell*, Depts of Physiology and Anesthesia, and the CVRI, Univ of Calif, San Francisco CA 94143.

It has been proposed that the ~100Hz synchrony observed in the inspiratory motor outputs of the cat is a signature of the central respiratory pattern generator. We recorded the membrane potentials of 6 inspiratory and 3 expiratory intercostal motoneurons (ICMNs) to determine if they too received the 100Hz inspiratory drive. Cats were anesthetized with halothane, decerebrated at the midcollicular level, paralyzed, and ventilated with a volume respirator. The thoracic spinal cord was exposed (T4-6) and dorsal roots severed. ICMNs were localized by stimulating the internal or external intercostal nerves and searching for the antidromically evoked fields in the spinal cord with low impedance electrodes. Intracellular recordings were made with high impedance (20-40Megohm) glass microelectrodes. We recorded the ICMN membrane potentials and the whole nerve activities of the phrenic (PN) and recurrent laryngeal (RLN) nerves for 25 to 50 respiratory cycles. We computed the autopower, crosspower, and coherence spectra for the three neural signals and applied a statistical test to detect significant peaks in the coherence (see below). The coherence at the high frequency peak (96Hz [80,110]) for the three pairs of signals were (PN x ICMN) = .37 [.22,.84], (RLN x ICMN) = .27 [.15,.82], (PN x RLN) = .45 [.18,.90] (mean [range]). There were no significant peaks between 25 and 80 Hz in the coherence of either nerve paired with the ICMN. We conclude that intercostal motoneurons receive synchronous synaptic input (at about 100Hz) during inspiration, but do not receive any detectable input at the 55Hz found in the RLN. Furthermore, the significant coherence for inspiratory nerve activity and expiratory ICMNs is evidence that the expiratory ICMNs are actively inhibited during inspiration.

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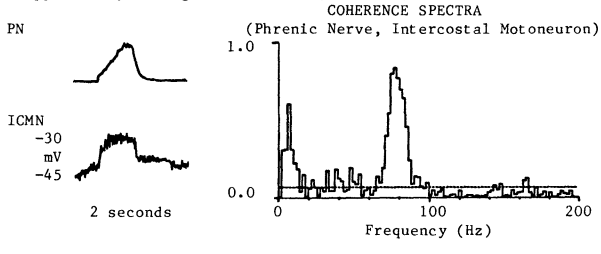


108.8 MORPHOLOGY OF PHYSIOLOGICALLY DISTINCT INSPIRATORY NEURONS IN THE VENTROLATERAL NUCLEUS OF THE TRACTUS SOLITARIUS (vl-NTS) STUDIED BY UTILIZING INTRACELLULAR INJECTION OF HRP. A.J. Berger, W.E. Cameron and D.B. Averill*. Department of Physiology & Biophysics, University of Washington, Seattle WA 98195.

In cats, two types of physiologically distinct inspiratory (I) cells have been described in the dorsal medulla associated with the vl-NTS. One type, Iα, exhibits reduced activity when the lungs are inflated simultaneously with central inspiratory activity compared with activity observed when the lungs are not inflated. A second type, Iβ, exhibits increased activity during lung inflation compared with activity observed when not inflating the lungs. Different functional roles have been postulated for these neurons. The purpose of the present study was to determine if these different cell types are morphologically distinct. HRP was injected into physiologically classified I-neurons of the vl-NTS in anesthetized, paralyzed and artificially ventilated cats. The brain stems were sectioned at 100 μm in either the transverse (6 cases) or horizontal (4 cases) plane. These sections were preincubated in $CoCl_2$, reacted with DAB and counterstained with cresyl violet. To date, 10 well-stained I-cells (4 Iα and 6 Iβ) have been reconstructed. All cells were located within the vl-NTS. We compared the somal, axonal and dendritic morphology of the Iα and Iβ cells at the light microscopic level. Mean somal diameters for the two cell types were similar in the two planes of section: 32 μm (2 Iα) versus 30 μm (4 Iβ) in transverse plane and 38 μm (2 Iα) versus 37 μm (2 Iβ) in horizontal plane. Axons of both cell types coursed ventrally from the vl-NTS then turned medially to cross the midline of the medulla. Axonal diameters were 2.8±0.5 μm ($\bar{x}$±SD) versus 3.4±0.8 μm for Iα (N=4) and Iβ (N=3), respectively. These mean values were not statistically different ($p>0.2$). Both cell types produced a similar mean number of stem dendrites (7 for Iα and 6 for Iβ). When viewed in horizontal plane, the main dendritic projection from these inspiratory cells was observed to travel parallel and ventrolateral to the tractus solitarius. The extent of these main dendritic arbors were similar between cell types. Dendrites of both types possessed spines and appendages. Based upon the lack of observable differences between Iα and Iβ cells, we conclude that they are not morphologically distinct. Therefore, the different responses of these two classes of neurons to lung inflation must arise from the differences in afferent connectivity. This work was supported by USPHS Grant NS 14857 and NRSAs HL 06233 and 06474.

108.9 ULTRASTRUCTURAL EVIDENCE FOR PRIMARY AFFERENT SYNAPSES ON PREGANGLIONIC SACRAL AUTONOMIC NEURONS. G.M. Mawe, J.C. Bresnahan, and M.S. Beattie. Dept. of Anatomy, Div. of Neurosurg., Neurosci. Res. Lab. The Ohio State University, Columbus, Ohio 43210

Primary afferent input to the cat sacral parasympathetic nucleus (SPN) has been examined by injury filling of sacral dorsal root axons with HRPin 24 cats. The relationship of primary afferent terminals to preganglionic neurons was studied by applying HRP to ventral root axons in 17 of those cats. Twenty hours after HRP application, the cats were perfused intracardially, and the next day appropriate spinal segments were sectioned, processed with DAB, and flat-embedded for sequential light and electron microscopic analysis.

At the light microscopic level, the lateral collateral pathway described by Morgan et al. (1981) was clearly visible in our material. This fascicle of fibers passes ventrally along the lateral edge of the dorsal horn to the region of the SPN then sweeps medially across the intermediate zone. Swellings, which may represent synaptic boutons, are seen throughout the course of the axons, but are particularly abundant in the SPN. Injury filling of the ventral root axons resulted in Golgi-like labelling of preganglionic neurons and their dendritic arbors. Neurons located ventrally and laterally in the SPN were oriented dorsoventrally; those located more dorsally in the nucleus were primarily arranged mediolaterally, but some were dorsoventrally oriented within the fascicle of primary afferents as they enter the nucleus. Primary afferent varicosities were often found in close apposition to labelled preganglionic neurons and their dendrites.

Upon ultrastructural examination of this tissue we found that labelled afferent terminals contained clear spherical vesicles; some of these terminals also contained dense core vesicles. Of particular interest was the presence of labelled terminals synapsing on labelled preganglionic neurons. Careful documentation of tissue at the light microscopic level prior to thin sectioning indicates that at least some of these label-label contacts represent monosynaptic input of sensory fibers onto preganglionic neurons. However other possibilities for the labelled presynaptic profiles include recurrent collaterals and motoneuron dendrites which extend into the SPN. Any of these connections provide interesting and as yet unexplored possibilities for autonomic reflex integration. Supported by NIH Grant NS10165 to J.B. and M.B., and a Neuroscience Research Laboratory Fellowship to G.M.

108.10 AFFERENT PROJECTIONS TO THE FACIAL NUCLEUS IN THE RAT AS MAPPED BY RETROGRADE TRANSPORT OF HORSERADISH PEROXIDASE. Masako Isokawa and Barry R. Komisaruk. Institute of Animal Behavior - Rutgers Univ., Newark, NJ. 07102.

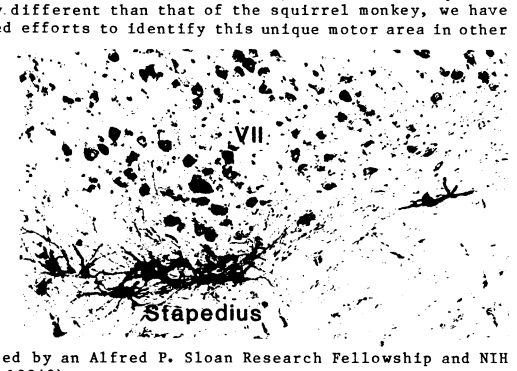
In order to study pathways involved in rhythmical vibrissae movements (Komisaruk, JCPP, 70:482, 1970; Semba et al., Br. Res., 195:281, 1980), we injected HRP iontophoretically into the lateral (L), dorsolateral (DL), medial (M), intermediate (I) and ventral (V) subdivisions of the facial nucleus (NVII), using positive DC current delivered in 0.5Hz square wave pulses of 1-2 μA for total on-time of 5-15 min. Current was applied between a Ag-AgCl lead inserted into a micropipette containing a 4% HRP solution and a Ag-AgCl ground wire attached to exposed hind limb muscle tissue. After survival time of 44-48 hr the brains were processed for HRP-visualization using tetramethyl benzidine (Mesulam, J. Histochem. Cytochem., 26:106, 1978). The results are summarized in the following table (I: ipsilateral, C: contralateral, -: no projection, blank: inconclusive).

Nucleus containing dense reaction product	Div. of Facial Nucleus injected with HRP L	DL	M	I	V
red n.	C	C			C
retrorubal n.	-	-	C	-	
substantia nigra (retic.)		-	I	-	-
Lateral lemniscus*	I,C	I	I		I
ventral n. lat. lemn.*	I	-			
ventral parabrach. n.**	I	I		-	I
Kölliker-Fuse n.**	I	I		-	I
superior colliculus	C	C	I,C	C	C
interstitial n. Cajal		I,C	I,C		I,C
n. Darkschewitsch			I,C	-	
central gray	C	-	I	-	I
oculomotor n.	C	-	I	-	I
lat. vestibular n.		I>C		-	
med. vestibular n.		I<C		-	-
spinal trigem. n.	I	I		I	-
parvocell. retic. n.	I>C	I>C	I>C	I>C	
ambiguus n.***	I>C	I>C	I,C	I>C	I>C

Note: *paralemniscal region has a major ipsilateral projection to lateral NVII, which differs from cat (Henkel et al., J. Comp. Neurol., 182:763, 1978) and opossum (Panneton et al., Br. Res., 168:493, 1979); **parabrachial zone projects densely to lateral NVII ipsilaterally as in cat, but different from opossum; ***Ambiguus n. sends fibers bilaterally to NVII with a predominantly ipsilateral distribution, which also differs from cat and opossum. No direct cortico-facial connection was observed. We thank Drs. Thomas R. Akesson and Mei-Fang Cheng for help with the HRP reaction technique. Supported by NSF grant 78-24505.

108.11 STAPEDIAL COMPONENT OF THE FACIAL NUCLEAR COMPLEX. G. C. Thompson, A.M. Cortez* and M. Igarashi. Dept. of Otorhinolaryngology & Communicative Sciences and Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030.

While studying the motor innervation of the stapedial muscle in squirrel monkeys, we identified a previously-undescribed separate origin for those motoneurons. Based upon their cellular morphology and location, these motoneurons are not contained within the classical facial motor nucleus. But, because of their proximity to the facial nucleus and because of their projection via a component of the facial nerve, they should be considered as yet another distinct nucleus comprising the facial nuclear complex. Since the origin of the stapedial motoneurons has been studied in cats without describing the discrete pool of motoneurons we saw in squirrel monkeys, we began comparative studies to determine if these motoneurons are unique to primates or characterize mammals in general. Therefore, we replicated the experiment in bush babies, a primitive primate. Horseradish peroxidase was injected into the stapedial muscle of four animals. After 24 hours, the animals were perfused with 1.25% gluteraldehyde and 1% paraformaldehyde. Frozen sections were cut at 40 micra and reacted with tetramethylbenzidine. Alternate sections were counterstained with neutral red. When HRP was wholly contained to the stapedial muscle, a group of motoneurons was heavily labelled just outside the ventromedial edge of the facial motor nucleus (see photo). Since the location of the bush baby stapedial motoneuron pool is slightly different than that of the squirrel monkey, we have initiated efforts to identify this unique motor area in other mammals.



(Supported by an Alfred P. Sloan Research Fellowship and NIH Grant NS-10940).

108.12 THE ORGANIZATION OF DIVERGENT AXONAL PROJECTIONS FROM THE MIDBRAIN RAPHE NUCLEI IN THE RAT. D. A. Steindler*, H. Imai*, S. Afsharpour, and S. T. Kitai, (SPON: R. A. Pax), Dept of Anatomy, Michigan State University, East Lansing, MI 48824

There is a paucity of information on the discrete intranuclear organization of divergently projecting midbrain raphe neurons. We have examined the order of axonal projections arising from the midbrain raphe nuclei in the rat using a recently-developed double retrograde axonal tracing technique. Patterns of divergence in the axonal projections of the midbrain raphe were found after paired pressure or iontophoretic injections of the axonal tracers horseradish peroxidase (HRP) and wheat germ agglutinin (WGA),N-[acetyl-^{3}H] were placed within known projection targets of the midbrain raphe nuclei (caudate-putamen, amygdala, hippocampus, substantia nigra, and locus coeruleus) in 67 Long-Evans hooded rats. The positions of single and double labeled raphe projection neurons in combined histochemical/autoradiographic sections were reconstructed through the light microscope. Retrograde labeled projection neurons were found within the dorsal raphe, caudal B_6 group, and linearis and centralis superior divisions of the median raphe complex after forebrain or brainstem injections of the tracers. Each structure receiving midbrain raphe projections has its own unique representation within a topographically distinct portion of one or more of the raphe subgroups, and an overall rostrocaudal topography in the intranuclear distribution of raphe projection neurons results in the formation of complex overlap zones where collateralized neurons reside. Raphe neurons projecting to the neostriatum occupy a rather rostral position within the dorsal raphe as well as linearis nuclei of the median raphe and neurons projecting to the substantia nigra also reside within rostral portions of the dorsal raphe. Neurons projecting to the hippocampus and/or locus coeruleus are situated more caudally in the dorsal raphe, B_6 and centralis superior nuclei. Neurons with collateralized axonal projections were found within a distinct portion of the dorsal and median raphe after injections of the two tracers in the amygdala and hippocampus, but no overlap zone and thus no double labeled raphe neurons were ever seen after paired injections in the neostriatum and hippocampus. Injections of the two tracers in the neostriatum and locus coeruleus, two structures not directly interconnected with each other, also resulted in no double retrograde neuronal labeling in the raphe. It is possible that raphe axonal projections to the forebrain and brainstem are thus organized according to a plan of target structure interrelation i.e., raphe-innervated structures possessing interconnections with each other are interrelated by a topographically distinct group of collateralized raphe projection neurons. (Supported by grants NS 15931 to D.A.S. and NS 14866 to S.T.K.from NIH/NINCDS).

108.13 ORGANIZATION OF THE DESCENDING NORADRENERGIC PATHWAYS TO THE RAT SPINAL CORD. R. Grzanna and E.W. Akeyson* (SPON: J.C. Hedreen). Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

To determine the organization of the noradrenergic (NA) spinal projections from the pontine cell groups subcoeruleus (SC), the A5 and A7 groups, we have combined the retrograde transport of fluorescent dyes (True Blue, TB and Diamidino Yellow, DY) with immunocytochemical (ICC) staining using antibodies to dopamine-β-hydroxylase (DBH). Injections of dye solution were made in a series of 5 penetrations of the spinal cord within a three segment area, delivering 0.1 µl per penetration for a total of 0.5 µl. Seven days after injections rats were perfused and the brains processed for DBH-ICC staining. DBH neurons and retrogradely labeled cells were identified in the same tissue section in the fluorescence microscope using different filter combinations and the percentage of DBH cells which were retrogradely labeled in each of the SC, A5 and A7 groups was determined. Retrogradely labeled DBH-cells were found within each of these groups. In addition, we observed numerous retrogradely labeled cells which were not DBH-positive, indicating the existence of non-NA projections from these regions to the injection sites.

Injections of TB into a single level of the spinal cord, either cervical, thoracic or lumbo-sacral segments, revealed that A5 and A7 cells project primarily to plexus levels whereas subcoeruleus cells project uniformly throughout the spinal cord. Injections of TB into two levels of the spinal cord, either cervical/thoracic or cervical/lumbo-sacral segments, indicated that the majority of the cells of the A5 and A7 groups project to a single level whereas subcoeruleus cells project to multiple levels of the cord. These organizational features were confirmed with injections of TB into cervical levels followed by injections of DY into either thoracic or lumbo-sacral segments.

The data indicate the existence of two distinct NA projections to the rat spinal cord. The A5 and A7 cells form a focused projection which is organized such that the majority of these cells project primarily to restricted portions of the spinal cord and are thus in a position to selectively influence the activity of neurons in a fraction of the spinal cord segments. In contrast, the subcoeruleus cells form a highly collateralized projection which is organized such that cells in this group project to all levels of the spinal cord and are thus in a position to influence the activity of neurons throughout the entire spinal cord.
(Supported by USPHS grants NS15199 and NS16654)

108.14 STATE-DEPENDENT ALTERATIONS OF MEMBRANE POTENTIAL AND EXCITABILITY OF MEDIAL PONTINE RETICULAR FORMATION NEURONS IN DESYNCHRONIZED SLEEP AND WAKING IN NATURALLY BEHAVING CATS. R.W. McCarley, K. Ito and M. Rodrigo. Harvard Medical School, Boston, Mass. 02115

Characteristics of medial pontine reticular formation (mPRF) neurons during behavioral state changes are of interest because of evidence for the presence of the neuronal generating machinery for PGO waves and rapid eye movements of desynchronized sleep (D) in this area, and we have hypothesized that a tonic depolarization of mPRF neurons during D might lead to the occurrence of these D events. However, extracellular recordings showing mPRF activity present during waking (W) as well as in D have raised questions about whether mPRF neurons have distinctive membrane polarization and excitability characteristics during D.

To help resolve this question we have performed chronic intracellular recordings in unanesthetized, undrugged cats during naturally occurring sleep-wake cycles. Cats were prepared with electrodes for recording behavioral state variables and for microstimulation (0.2 mS, <80 uA) in contralateral mPRF and ipsilateral mesencephalic and bulbar reticular formation. All recording and stimulation sites were histologically verified by marker lesions, and in some instances by intracellular injection of HRP; all neurons had membrane polarization levels more negative than -45 mV. We have thus far obtained continuous intracellular recordings of seven mPRF neurons during D and the succeeding portion of W.

During D, as we have previously reported, there was a tonic depolarization of membrane potential (mp) upon which was superimposed phasic runs of further depolarization associated with spontaneous discharge activity. With state change from D to W all neurons showed a tonic increase in mp (repolarization, p=.008, sign test) with the membrane potential becoming more negative by an average of 7.6 mV (SD=2.3 mV). In contrast to the slow time course of mp change we observed on transition from synchronized sleep to D the passage from D to W as electrographically more abrupt and mp repolarization became complete within 30 sec. During W the background level of the mp remained more polarized than in D, although in association with some eye and somatic movements in W some neurons showed EPSPs, sometimes with spikes. All seven neurons studied in W and D showed state-dependent excitability increases in D, as measured by the probability of antidromic and orthodromic responses to constant current microstimulation during D and W.

These data provide clear evidence for state-dependent alterations in mPRF mp and excitability in D and W and are compatible with a generating role for mPRF neurons in D sleep phenomena.

Supported by grant BNS 81-15786 and an RSDA to RWM.

EVOKED POTENTIALS

109.1 Developmental Analysis of Cortical Topography in Normal Children. Johnson, J., Buchsbaum, M.*, Clark, C.*, King, A.*, Coppola, R. NIMH, Lab. of Psychology and Psychopathology, 9000 Rockville Pike, 10/4N317, Bethesda, MD 20205.

Despite the proliferation of studies examining electrophysiological development, it has generally been agreed that the normal development of this phenomena has not been clearly defined. Now, multilead studies of cortical topography, made possible with improved hardware and software development, cohesively present the complex data of event related potentials (ERPs) and make it possible to examine the relationships between age and topographical distributions of ERPs.

Utilizing a 16 lead montage, this study examined the ERPs of 85 normal subjects ranging in age from 5 to 18 years (40 females, 45 males). Sixteen electrodes were attached to the left hemisphere (12 according to the 10-20 system plus 4 additional interpolated leads: FTC, CP, TCP, PO). ERP stimuli consisted of a) 4 intensities of light flashes (2,30,80 and 240 ft La) presented in a random sequence and, b) alternating lights and tones consisting of 4 intensities of light flashes (as above) and 4 intensities of tones (40,50,60,70 db) each presented 64 times in a randomized sequence at 1-second intervals. Instructions to the alternating lights and tones were to attend to the brightest light, and ignore the tones. Only the N120 component (116-152 msec area data) will be reported.

For the lights only condition, a significant multivariate F was found for age (Hotellings TSQ=86.32; F=4.41; df=16, 67; p=.000). Univariate tests indicated that the following leads were significantly correlated with age: Fz, Cz, Pz, Oz, F3, P3, and PO. Significant main effects were found for intensities (F=23.33; df= 48, 697; p=.0000) with significant intensity x lead interactions for Fz, Cz, Pz, Oz, F3, C3, P3, O1, F7, T3, T5, and FTC. No significant sex differences were revealed. For the light attention condition, a significant multivariate F was also found for age (TSQ=74.72; F=3.79; df=16, 65; p=.0001). Univariate tests indicated that the following leads were significantly correlated with age: Fz, Cz, Pz, Oz, F3, C3, P3, T3, and PO. Significant main effects for intensities were also revealed (F=21.78; df=48, 679; p=.0000) while significant intensity x lead interactions were found at leads Fz, Cz, Pz, Oz, F3, C3, P3, O1, F7, T3, T5, FTC. Again, no significant differences for sex were found. Overall, younger children revealed larger amplitudes at each of the 16 leads than did the older children.

These results show that: a) differences in age are highlighted in midline leads while 1 frontal and 2 posterior sites reflect similar changes, b) in addition to these sites, light attention is reflected by ERP activity in the temporal midline area.

109.2 EFFECT OF α-CHLORALOSE ON REFRACTORY PERIOD OF ASSOCIATION CORTEX RESPONSE TO SENSORY STIMULATION. Norbert J. Pontzer*, Douglas M. Wilkison* and Michael J. Hosko (SPON:Zeljko Bosnjak). Dept. of Pharmacology and Toxicology, Med. College of Wisconsin, Milwaukee, WI 53226.

α-Chloralose has long been a standard anesthetic for neurophysiological and neuropharmacological experiments since it preserves autonomic, respiratory, reflex, and cortical association function. Small sub-anesthetic doses increase the amplitude and decrease the variability of the sensory-evoked long latency field potential measured at association cortex. This is in contrast to most other commonly used anesthetics such as barbituates which abolish association cortex response at sub-anesthetic doses. Thus much of what is known about association cortex response has come from α-chloralose anesthetized animals; 35-100 mg/kg commonly being used.

One frequently used paradigm for studying the refractory period after a response is entrainment of paired stimuli. The relative response amplitude to the second (test) stimulus of two stimuli is determined as a function of time after the first (conditioning) stimulus. It was noticed in our lab that the refractory period for association cortex response to sensory stimuli appeared to decrease with time in α-chloralose anesthetized cats (70 mg/kg i.v.). We studied this phenomena by giving an initial dose of 50 mg/kg i.v. α-chloralose and measuring within 6 hours the refractory period of the test response to either the same or a different modality conditioning stimulus. Responses were measured at the four feline association areas: anterior sigmoid; anterior marginal; anterior suprasylvian; and posterior suprasylvian. Two additional doses of α-chloralose (50 mg/kg i.v.) were infused and the refractory period measured 45 minutes after each dose, as drug effects developed quite slowly. We found that the refractory period dramatically increased after the first additional dose with only a small additional increase after the second. Because of the length of action of α-chloralose, cumulative dosing can be assumed. These changes in refractory period occurred with minimal changes in the amplitude of the responses. One explanation for this effect would be an α-chloralose mediated increase of recurrent inhibition at a cortical or sub-cortical level. (Supported by USPHS Grant DA 01754).

109.3 EVENT RELATED POTENTIAL INDICES OF CROSSMODAL INFORMATION PROCESSING. R. Erwin* and P. Tanguay* (SPON: D. Molfese). Electrophysiology Lab, Neuropsychiatric Institute, UCLA, Los Angeles, CA 90024.

Previous research (Thatcher, R., Behav. Biol., 19:1, 1977) has indicated that a late positive component LPC of the event related potential (ERP) is enhanced for stimuli judged to be matches (i.e., related on some dimension) as compared to stimuli judged to be mismatches. The present study now reports similar findings using a crossmodal match-mismatch procedure in which subjects were trained to associate visually presented geometric figures with auditorily presented tones of different frequencies. Event related potentials were recorded over the temporal (T5, T6), parietal (P3, P4) and occipital (01, 02) regions of 12 adult volunteers. Stimuli were presented in two trial blocks. Each trial consisted of presentation of a stimulus in one modality (auditory or visual) followed by a stimulus in the alternate modality after a brief interstimulus interval (2 to 4 sec.) Subjects were required to press a button to match stimuli (as determined by previous training) on one trial block and to mismatch stimuli on the other block. Amplified ERPs for each condition were averaged independently at 10 ms intervals over a 750 ms period following stimulus onset (for 75 points). The averages for each modality were then formed into a covariance matrix and submitted to a principle components analyses (PCA). A factor which occurred in the latency range of the LPC was obtained for both the visual PCA and the auditory PCA. The peak latency of the auditory factor was 320 ms, while the peak latency of the visual factor was 350 ms. Factor scores were computed for each of these factors and submitted to independent analyses of variance for Block (2) x Sites (6) x Stimulus (2) x Condition (2) to determine if either factor varied systematically as a function of experimental conditions. The visual factor indicated that the LPC to the match stimulus was enhanced in comparison to the LPC for the mismatch stimulus, $F(1,11)=11.07$, $p=.01$, over all recording sites and trial blocks. For the auditory factor, the match LPCs were enhanced only over right-hemisphere sites (T6, P4) during the trial block in which match responses were required, and over the left hemisphere (T5, P3) during the block in which mismatch responses were required. Overall these findings suggest that crossmodal processing can be assessed using a match-mismatch procedure with the ERP as a dependent measure. In addition, the findings indicate that endogenous components, such as the LPC, may be more sensitive to task demands when obtained in one modality as compared to other modalities. (Supported by grants HD04612 and HD05958).

109.4 CORTICAL EVOKED POTENTIALS AND WORD AND PICTURE RECOGNITION. R.J. Chabot*, D.H. York, and W. A. Waugh*. Departments of Physiology and Neurosurgery, U. of Missouri Sch. of Med., Columbia, MO 65212.

Whether or not separate substages of the word and picture recognition process can be identified in the cerebral activity following stimulus presentation was investigated using averaged evoked potentials. Eight right handed volunteers (x age = 29.6 ± 4.7 yrs) served as subjects. Cerebral electrical activity was recorded from Cz, C3, C4, and Pz (standard international 10-20 system) with amplifier gain set at 10^4 and bandpass filters set at .1 to 300 Hz. Eighty trials were averaged for each experimental condition and the averaged waveforms were analyzed using the IBM Statistical Analysis System.

Subjects made perceptual and cognitive decisions about either pairs of visually presented words or pictures. For the word pairs subjects made decisions associated with three substages of the word recognition process. These substages corresponded to feature detection and synthesis, lexical access and semantic memory access. Analogous decisions were made for pictures with decision levels corresponding to feature level decisions, basic category decisions, and superordinate category decisions. Principal components factor analysis with varimax rotation described the evoked waveforms well with 7 factors which accounted for 93% of the total variance. Subsequent analyses of variance on the factor scores associated with each component showed that the type of cognitive decision made effected the maximum amplitude and the duration of a late positive component which extended from 418-754 msec after stimulus presentation. Variability associated with the latency and duration of this component was highly correlated with the response latencies of the various experimental conditions. It appears that this late positive component provides an internal index of the neural processes which accompany cognitive decisions. As such, this component serves as a source of converging evidence, along with response latency measures, that the pattern recognition processes for both words and pictures can be tapped at several different levels. The averaged waveforms were also examined for differences associated with accessing different superordinate categories in memory.

The procedures outlined above should be applicable for studying disruptions to the pattern recognition process which accompany stroke, head trauma, and neurological deficits associated with learning disorders. These techniques should be particularly sensitive for defining neurological disorders which influence central processing mechanisms while leaving peripheral processing mechanisms relatively intact.

109.5 THE HABITUATION OF HUMAN EVENT-RELATED BRAIN POTENTIALS ELICITED BY SPEECH SOUNDS AND TONES D. L. Woods and R. Elmasian*, Clinical Neurophysiology Laboratory, Dept. of Neurology, U.C. Davis, V.A. Medical Center, Martinez, CA 94553 and Dept. of Neurosciences, UCSD, La Jolla, CA

When auditory stimuli are repetitively presented to alert listeners, the event-related brain potentials (ERPs) which they elicit are reduced in amplitude. This habituation has been divided into two phases 1) short-term habituation which occurs when stimuli are repeated at interstimulus intervals (ISIs) of 20 sec or less; and 2) long-term habituation which occurs minute-by-minute over the course of an experiment. In this experiment we examined the short- and long-term habituation of exogenous (N100) and endogenous (P300) brain potentials elicited by speech sounds and various control stimuli.

METHODS. Pure tones, synthetic vowels, and digitized speech sounds were presented monaurally to normal listeners (ages 18-34 yrs). ERPs were recorded from midline sites and from electrodes overlying auditory cortical regions bilaterally. The stimuli were presented in trains, with ISIs fixed within a train (either 0.5 or 1.0 sec), but varying randomly between trains. Inter-train intervals were also randomized (2.0 or 6.0 sec). Half of the trains included either a single "target" stimulus which served to cue a motor response, or a "deviant" stimulus (a word or tone which was presented frequently in other conditions) which required no response.

RESULTS. 1). The ERPs elicited by "frequent" stimuli habituated rapidly within trains, and the habituation was partially stimulus specific. 2). Habituation processes were similar for speech and non-speech stimuli. 3). The degree of short-term habituation of the N100 to the "deviant" stimulus was related to its acoustic similarity to the standard stimuli. The long-term habituation of the N100 component was partially specific for the stimulus which was repeated. 4). P300s elicited by "deviant" stimuli habituated rapidly at all scalp sites, while P300s elicited by target stimuli habituated gradually over frontal sites only.

DISCUSSION. ERPs recorded from the human scalp show habituation patterns which reflect mnemonic persistence in several neural systems. A similar persistence is evident for linguistic and non-linguistic stimuli.

109.6 GENERATOR SUBSTRATES OF THE VERTEX SHORT LATENCY AUDITORY EVOKED RESPONSE WAVE 6. C. Shipley*, G. Strecker* J. Buchwald Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiology, UCLA Med. Center, Los Angeles, CA 90024.

In the cat, the "auditory brainstem response" recorded from the vertex consists of 5 potentials generated by auditory relays from the level of the eighth nerve to the inferior colliculus which occur within 6-7 msec of a click stimulus. Subsequently, a less well defined wave 6 is generally seen at a latency of 7-8 msec. This potential is of interest because it develops at a later age than waves 1-5 and may provide a sensitive index of maturation within the auditory pathway. In the present study, a combination of depth recording of local field potentials and surgical lesions was used to identify possible generators of wave 6. These experiments were carried out on a total of 10 cats with each procedure being performed on at least two animals.

Previous research has reported that local field potentials within pars principalis of the medial geniculate body occur in the latency range of wave 6 suggesting this structure as a likely generator substrate. This result was confirmed in the present study. However, precollicular decerebration which isolated the medial geniculate body from the brainstem changed the form of wave 6 slightly but did not result in its disappearance, suggest-that the medial geniculate body is not the major source of activity reflected in vertex wave 6. Depth recording of auditory evoked activity in the superior colliculus revealed an evoked potential that was highly correlated with the surface wave 6 in form, latency, response to parametric manipulation of stimulus rate and response to monaural and binaural stimulation. Aspiration confined to the medial portion of the superior colliculus, i.e. which did not extend to the brachium of the inferior colliculus, reduced the amplitude of wave 6 substantially although the wave was not completely eliminated.

These results suggest that activity present in the superior colliculus is reflected in the vertex short latency auditory evoked response wave 6. Although a number of studies have shown that neurons within the superior colliculus are responsive to sound, this is the first report of involvement of superior colliculus in the generation of vertex recorded short latency auditory evoked potentials. (This work was supported in part by HD 04612 and HD 05958).

109.7 A STUDY OF THE LONG LATENCY COMPONENTS OF THE AUDITORY EVOKED POTENTIALS IN THE CAT. K. T. Tam*, J. C. Hwang, and P. W. F. Poon. Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

When tone bursts of short duration were presented at low rates (less than 0.1 Hz) to choralose anaesthetized (35 mg/kg) cats prominent and stereotyped potential changes could be recorded over the scalp of the animals. This response was not affected by the intravenous injection of gallamine triethiodide (3 mg/kg). With vertex-to-nuchal differential recordings, the averaged response typically consisted of two large surface positive deflections. The deflections were identified as an early wave with a peak latency measured at 45 ± 3 msec, and a late wave delayed at 115 ± 15 msec to the stimulus. This response appeared to be reminisent to the auditory evoked potentials measured in unanaesthetized preparations (G. M. Gerken and W. D. Neff, Electroenceph. Clin. Neurophysiol., 1963, 15: 947-957). Systematic mapping of this evoked potential over the scalp of the animal showed that the early wave appeared with the greatest amplitude (140 uV) over the frontal region (peak latencies 42 to 48 msec), while the late wave was recorded with the largest amplitude (280 uV) at the vertex (peak latencies 100 to 130 msec). Intracranial recordings showed that strong positive deflections, with delays comparable to those of the early and late waves of the vertex potentials respectively could be differentially detected at the frontal cortex and at the corpus callosum. This suggested that the activities originated from both the frontal lobes and the corpus callosum might contribute to the early wave as well as the late wave of the auditory evoked responses measured over the scalp of the animal.

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109.8 IS A PLACE MECHANISM RESPONSIBLE FOR WAVE V LATENCY DECREASE WITH INCREASING CLICK INTENSITY? Robert Burkard* and Kurt Hecox* (SPON: G. Moushegian). Waisman Center, University of Wisconsin, Madison WI 53706.

The base-to-apex, frequency-to-place representation along the cochlear partition results in decreasing distance to traveling-wave maxima with increasing stimulus frequency. Electrophysiologically, this is manifested as decreased peak latencies of the whole-nerve action potential and brainstem auditory evoked response (BAER) with increasing stimulus frequency. Increasing stimulus intensity also causes a decrease in peak latencies. This has led to the hypothesis that increased stimulus intensity produces a basalward shift in the response-generating region of the cochlear partition, thereby reducing its time delay. This thesis may be tested by restricting the site of response-generating activity along the cochlear partition through the use of high-pass masking. If the site of response generation is displaced basally at higher stimulus intensities, then there should be a smaller intensity-dependent latency shift (shallower latency/intensity function) with decreasing masker cutoff, as the high-pass masker progressively restricts the response to more apical locations. Eggermont and Don (1980; J. Acoust. Soc. Am. 68 : 1671-1675) used a similar technique, but varied both stimulus and masker intensity. However, if the region of response emanation is dependent on stimulus intensity, then the cochlear distribution of high-pass masking might also be level dependent.

The thesis that a place mechanism explains the intensity-dependence of wave V latency is reexamined by holding noise level constant while varying click intensity and high-pass masker cutoff. Click intensities ranged from 15 to 65 dB nHL. Broadband noise was presented at an intensity adequate to perceptually mask 70 dB nHL clicks. Keeping spectrum level constant, the noise was high-passed at 20 (unmasked), 6, 5, 4, and 3 kHz. There was an increase in BAER threshold with decreasing high-pass masker cutoff, possibly a result of remote masking. With decreasing high-pass masker cutoff there was a steepening of the wave V latency/intensity function. Thus, restricting the cochlear region responsible for generating the response to progressively more apical regions did not result in a shallower latency/intensity function. This finding is difficult to reconcile with the theory that place shifts are responsible for intensity-dependent latency shifts of wave V of the BAER. (Supported by NIH grant #16436).

109.9 BRAINSTEM AUDITORY EVOKED POTENTIALS (BAEPs) IN MICE RECEIVING CORONAVIRUS SD: D.L. Asher*, L.M. Nelson*, S. Staller*, J.S. Burks*, (SPON: J. Nolte). Depts of Otolaryngology and Neurology, Rocky Mountain Multiple Sclerosis Center, University of Colorado School of Medicine, Denver, CO 80262.

Coronavirus SD was previously isolated while working with fresh multiple sclerosis autopsy brain material (Burks et al {1980}, Science 209:933). Following intracerebral inoculation of 3-4 month old mice with coronavirus SD, hyperexcitability and hind limb paresis were observed from days 4-9 followed by a lack of clinical signs until day 45. At that time, hind limb anesthesia and paresis was again observed (Mendelman et al {1983}, Arch. Neurol., in press). Viral antigen was identified in brain and spinal cord sections with autoradiography (J. Immun. Methods {1982} 54:191). Histopathology revealed demyelination in temporal cortex and spinal cord at times when infectious virus was not recoverable. This study reports preliminary BAEP findings observed in an additional group of coronavirus SD infected mice.

After normative BAEP data were collected from 4 and 6 week old C57 B16/J control mice, we measured the BAEPs of two groups of mice inoculated intracerebrally with either a sham inoculation (N=15) or with coronavirus SD (N=15). Baseline BAEP measurements were made for each mouse prior to inoculation and a minimum of two BAEP measurements were obtained weekly from each ear of each mouse for 6 consecutive weeks beginning with day 0 (6 wks. old). Click stimuli were presented at 60 dB SPL through TDH 39 earphones. Two hundred fifty clicks of 0.1 msec. duration were delivered unilaterally at 13.1 presentations per second. Recordings were obtained from needle electrodes placed subcutaneously at the vertex and referenced to ipsilateral ear lobe.

Five vertex-positive peaks were identified in the BAEPs of the normative control mice. Mean latencies for BAEP controls were Wave I = 1.03, Wave II = 1.76, Wave III = 2.73, Wave IV = 3.45 and Wave V = 4.24 msec. Mean interpeak latencies were 1.70 for I-III, 1.52 for III-V and 3.22 for I-V. There were no significant morphology or latency changes in the sham inoculated mice over the eight-week test period. However, BAEP morphology and latency changes were observed in the coronavirus SD injected group. These changes included diminished Waves II and III and prolonged latency of the Wave IV-V complex; abnormalities presented both unilaterally and bilaterally.

These observations indicate that the BAEP may be useful as a non-invasive probe for assessing the effects of viral induced central demyelination in an animal model.

(Supported by the Rocky Mountain Multiple Sclerosis Center)

109.10 EFFECTS OF WHOLE BODY HYPERTHERMIA ON AUDITORY BRAINSTEM, SOMATOSENSORY AND VISUAL EVOKED POTENTIALS. B.E. Lyons, R.H. Britt, T.P. Ryan*, E.L. Saxer*, W.G. Obana*, and G.T. Rossi. Div. Neurosurgery, Stanford Univ. Sch. of Med., Stanford, CA 94305.

A model of whole body hyperthermia has been developed in the cat using a cardiopulmonary bypass circuit with a heat exchanger (Britt, R.H. and Rossi, G.T., J. Neurosci. Meth. 6:231-244, 1982). The effect of systematically raising core and brain temperature from 36° to 45°C was studied in 18 pentobarbital anesthetized cats using multimodality evoked potentials (brainstem auditory, somatosensory and visual). Core temperature was monitored by esophageal and rectal probes. Brain temperature was monitored by copper/constantan thermocouple junctions housed in 26 awg stainless needles in the lower medulla and in the parietal cortex at depths of 0.5 and 1.0 cm. The effect of hyperthermia on the 5 component waves (I-V) of the brainstem auditory evoked response was studied in 6 cats. In general, both amplitude and latency of each peak decreased as temperature was increased to a "critical" value at which the latencies increased and the amplitudes continued to diminish. In 3 experiments this "critical" temperature was 42.2°C and in 3 other experiments it ranged from 40.2 to 41.7°C. An Arrhenius equation has been adapted for analyzing temperature dependent effects of evoked potential waveforms (Rossi, G.T. and Britt, R.H., Electroenceph. clin. Neurophysiol. In Press.). Arrhenius plots of 1/latency or k (rate) versus temperature (1000/°K) for the temperature range from 36°C to the "critical" temperature showed similar slopes for all five component waves of the brainstem auditory evoked response. Above the "critical" temperature, there was an inflection in the slope of each component wave reflecting the increase in latencies with further temperature elevations. This "critical" temperature was lower than the temperature at which there was a significant reduction in waveform amplitude. The Arrhenius analysis suggested that the abrupt change in slope at the "critical" temperature was probably due to a deleterious change in the rate-limiting biochemical processes involved in neural transmission. Similar effects on latency and amplitude were observed with visual and somatosensory cortical evoked potentials in experiments on 12 animals. Studies in our laboratory using ultrasound-induced (Britt, R.H., Lyons, B.E., Pounds, D.W., and Prionas, S.D., Med. Instrument. 17:172-177, 1983) and microwave-induced (Lyons, B.E., Britt, R.H., and Strohbehn, J.W., IEEE Trans. Biomed. Engin. In press.) hyperthermia of normal brain tissue have shown that neurons begin to show cytological evidence of damage after heating at 42.2 to 42.5°C for 50 min. This study demonstrates that neurons begin to show deleterious physiological changes in or near this "critical" temperature range.

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109.11 CHANGES IN SOMATOSENSORY EVOKED POTENTIALS WITH HYPOTHERMIA IN HUMANS. M.J. Taylor*, J.G. Coles*, D.S. Borrett*, N.J. Lowry*, J.M. Pearce*, G.A. Trusler*, W.G. Williams* (SPON: W.J. Logan). Departments of Neurology and Cardiovascular Surgery, The Hospital for Sick Children, University of Toronto, Toronto, Ont., Canada M5G 1X8

Somatosensory evoked potentials (SEPs) were recorded over the cervical spine and contralateral sensory cortex (C3' or C4') in 15 children during cardiopulmonary bypass surgery with hypothermia alone (n=11) or with profound hypothermia and complete circulatory arrest (n=4). SEPs in response to median nerve stimulation at the wrist were recorded throughout the surgical procedure in all patients. The lowest temperatures reached in the patients ranged from 9 - 28°C (oesophageal).

The latencies of the spinal and cortical SEPs increased systematically in all patients with hypothermia, showing marked slowing of conduction with decreasing oesophageal temperatures. The latency changes occurred as a nonlinear function of decreasing temperature.

The cervical SEP divided into a double response with hypothermia in all cases; this was in effect a widening of the notched N13 found in the patients and normal control children at normothermia. The slower of the two responses (N13s) was more sensitive to hypothermia - its latency increased more rapidly with cooling and it disappeared 2 - 6 degrees before the faster response (N13f).

In patients in whom SEPs were recorded at Erb's point, lower and upper cervical spine, the double response was seen at all three sites. Over the cervical spine the absolute latencies increased with the more rostral recording site as did the N13f-N13s interpeak latency. These latency differences were enhanced with hypothermia. These data suggest that the N13f and N13s represent travelling waves in two populations of primary afferent neruons, which have small but consistent differences in conduction speed. The existence of these two distinct populations of neurons contributing to the SEP is clearly shown by these studies with hypothermic patients.

109.12 SOMATOSENSORY EVOKED POTENTIALS (SEPs) AND SINGLE UNIT ACTIVITY EVOKED BY AIRPUFFS IN ALERT MONKEYS. H. Hamalainen*, S. Warren*, W. Young*, J. Davis* and E.P. Gardner (SPON: E.S. Flamm). Dept. of Physiology/Biophysics, NYU Sch. of Medicine, New York, NY 10016

In order to develop more physiological techniques for eliciting SEPs, we have applied airpuffs lasting 10 ms to the skin of the monkey's hand and arm. Unlike electrical shocks to whole nerves, airpuffs are highly selective for specific mechanoreceptors, and permit stimulation of a restricted area of skin in a variety of spatial and temporal patterns. Short latency SEPs recorded epidurally from the postcentral gyrus of 3 alert monkeys consist of an early positive potential (onset latency, 8-10 ms; duration, 25 ms) containing several peaks (P10, P16, P25, P30), a negative wave (N40) of 30 ms, and a late slow positivity (P70). Exact times of occurence and amplitude of the individual peaks varied somewhat from monkey to monkey, but each animal showed a consistent and reproducible SEP that fit the general pattern. The early positive complex appears restricted to cortical regions whose receptive fields encompass the stimulated skin areas. N40 is more widespread on the cortical surface, but is maximum in amplitude at regions of greatest early positivity. P70 is recorded over even wider regions of the pre- and postcentral cortices, and also displays less variability in amplitude. Short latency SEPs were seen only over the contralateral hemisphere, and could be shifted from right to left cortex depending upon which arm was stimulated. Bilateral stimulation evoked activity in both SI cortices, although the N40 wave appeared somewhat attenuated. These results indicate that the SEPs we recorded are specific to the tactile stimulus. P16, P25, and N40 amplitudes depend upon both the position of the airpuffs on the forearm, and the number of points stimulated. SEPs were largest when the stimulus position coincided with the receptive field centers of the neurons recorded in the underlying cortex.

Single unit responses to airpuffs in monkey SI cortex typically consist of a short, high-frequency burst of impulses (12-15 ms latency, 25 ms duration). Thus the earliest positivity in the SEP occurs before cortical spike activity begins, and may be due to the thalamo-cortical volley. The remainder of the early positive complex coincides with airpuff initiated impulse activity in SI, while the onset of N40 is mirrored in the decay of unit responses. The latency, duration and spatial distribution of N40 correspond to those of in-field inhibition. No single unit activity in SI cortex could be attributed to P70, nor did we observe poststimulus facilitation in this time period. We suggest that P70 originates elsewhere in the cortex. Thus SEPs elicited by mechanical stimuli provide opportunities for assessing the spatial and temporal integrative capabilities of the somatosensory pathways, and for evaluating the neural mechanisms underlying pattern recognition. (Supported by NIH Grant NS11862).

109.13 OPERANT CONDITIONING OF SEP AMPLITUDE: SPECIFICITY OF THE CONDI- CHANGES IN AMPLITUDE AND ITS ASSOCIATED CHANGE IN FACIAL NOCICEPTIVE SENSITIVITY. R. Dowman and J.P. Rosenfeld. Dept. of Psychology, Northwestern Univ., Evanston Il., 60201.

Our present work focuses on extending our earlier finding that operantly conditioning somatosensory evoked potential (SEP) amplitude is correlated with changes in facial nociceptive sensitivity (FNS). In the present study SEPs were recorded monopolarly via a screw placed over the SI cortex contralateral to the evoking stimulus (non-aversive stimulation to the trigeminal tract). Animals (rats) were rewarded for increasing (uptrain) or decreasing (downtrain) the amplitude of a surface positive component of this SEP (latency= 30msec; P30). Ipsilateral SEPs and non-aversive reflex movements evoked by the stimulus were also recorded. FNS tests were taken immediately following each conditioning session. Four animals have successfully completed training. Uptraining the P30 component was correlated with decreased FNS and downtraining with increased FNS on the side of the face contralateral to the trained cortex ($p<.05$) but not on the ipsilateral side ($p>.10$). Changes in the conditioned SEP were restricted to components P30 and N14 (uptrain>downtrain, $p<.05$). There were trends seen in other components (uptrain>downtrain) but these were not significant ($p>.05$). Likewise, there were trends seen in the ipsilateral SEP components and the non-aversive reflex movements (uptrain>downtrain) but these were not significant ($p>.10$). These data demonstrate that the conditioned changes in SEP amplitude are relatively specific to the trained component and that this specificity is reflected in the correlated changes in FNS.

Supported by NIH grant DE05204

109.14 SOMATOSENSORY EVOKED POTENTIALS (SEPs) TO MUSCLE STRETCH STIMULATION PRODUCED BY TENDON TAPS IN HUMANS. L G Cohen*and A Starr. Department of Neurology, Univ. of California, Irvine, Ca 92717. SEPs were recorded after gastrocnemius-soleus, anterior tibial, and quadriceps tendon displacement producing muscle stretch in normal humans. The techniques of recording involved the placement of surface electrodes at several levels of the somatosensory system and using a computer to define the small amplitude time-locked potentials to muscle stretch stimulation. We measured amplitude and latencies of the evoked potentials and defined the effects of age and stimulus variables on these parameters. Results were correlated with the SEPs to electrical stimulation of posterior tibial nerve at the ankle, anterior tibial nerve at the ankle and posterior tibial nerve at popliteal level. Control experiments defined the role of cutaneous afferent contributions to the evoked potentials. The latency of the initial scalp positivity to gastrocnemius-soleus tendon taps occurred earlier than that obtained after posterior tibial nerve at the ankle. The effects of anesthesia, muscle contraction and tendon vibration suggest that tendon tap stimulates predominantly muscle spindles and that the afferent volley travels along fast central pathways to the cerebrum.

109.15 EVALUATION OF SELECTED SITES FOR RECORDING TRIGEMINAL EVOKED POTENTIALS IN MAN. C.G. Widmer* and W.D. McCall, Jr., Sch. Dentistry, S.U.N.Y. Buffalo NY 14214

Important features of trigeminal evoked potentials may depend on the location of the recording electrodes. Our purpose was to select sites for reference and active electrodes to record these potentials. We focused on the reliable waveform at 19 ms.

Each of 13 subjects gave informed consent. An acrylic splint held a pair of stimulating electrodes over the left greater palatine nerve. Each of 6 trials at each of 2 sessions included 128 pulses, 0.2 ms width, 1.4/sec, and twice sensory threshold. Four sites were recorded simultaneously. Each channel was sampled at 2 kHz and averaged by a PDP 11/03 computer. Analysis time was 64 ms prior to and 256 ms after the stimulus.

Our criterion for a reference electrode site was minimal electrical activity in the interval 15 to 25 ms in trials where a 19 ms potential was observed with an amplitude of 3 uV or more in the C4-A2 trace. Electrical activity was quantified by dividing the variance of the averaged signal in the cited post-stimulus interval by the variance of a like interval prior to the stimulus. These variance ratios were obtained from signals recorded from FZ, A2, and mastoid with reference to the back of the neck.

Results from 6 subjects who had 19 ms potentials over 3 uV gave mean variance ratios of 1.6 for FZ, 3.9 for mastoid, and 10.0 for A2. All differed significantly ($p<.01$). Thus FZ had the minimum electrical activity and was selected as the reference site.

Our criterion for selecting the active electrode site was maximal electrical activity in the interval from 15 to 25 ms from those trials in which the A2-FZ recording contained a 19 ms potential over 3 uV. The activity was again quantified by a ratio of post-stimulus to pre-stimulus variances in the designated time intervals. The sites C4, C6, T4, and A2 were evaluated with reference to FZ.

Variance ratios from 7 subjects with 19 ms potentials over 3 uV were 6.1 for C4, 6.7 for C6, 11.2 for T4, and 28.3 for A2. All active sites differed ($p<.05$) except C4-C6. Thus the signal was largest at A2 and increased in the superior to inferior direction when FZ was the reference site.

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EVOKED POTENTIALS: VISUAL

110.1 ASSESSMENT OF RETINAL FUNCTION IN HUMAN AMBLYOPIA. Robert F. Hess* and Curtis L. Baker, Jr., Physiological Laboratory, Cambridge University, Cambridge, U.K.

In an attempt to assess whether amblyopia in humans has a retinal basis, we compared the psychophysical and electroretinographic responses between normal and severely amblyopic eyes using the same stimuli.

Our stimuli were either sinusoidally modulated uniform fields or sinusoidally contrast-reversing gratings. Electroretinographic responses which were measured using gold-foil lid electrodes, were averaged and Fourier analysed.

We found no electrophysiological analogue of the psychophysical deficit in correctly refracted, but severely amblyopic eyes. We assessed responses for stimuli of various spatial frequencies and contrasts. In one amblyope reduced electroretinographic responses were recorded but only at low spatial frequencies and high contrasts where the psychophysical deficit was minimised. Similar results were obtained for uniform field stimulation. The linear and non linear response were of similar amplitude in normal and amblyopic eyes over a wide range of modulation depths except in the one amblyope who exhibit a pattern-evoked deficit. In this case, only the non-linear component was anomalous and only for stimuli of large modulation depths. We conclude that some severe amblyopes do not exhibit an electroretinographic anomaly that parallels their psychophysical deficit.

(Supported by MRC and Wellcome Trust grants to RFH and NIH Postdoctoral Fellowship to CLB.)

110.2 THE CONTRIBUTION OF THE ON CHANNEL TO THE ELECTRORETINOGRAM OF MONKEYS AND RABBITS. A. G. Knapp* and P. H. Schiller* (SPON: R. Held). Dept. of Psychology, MIT, Cambridge, MA 02139.

The electroretinogram (ERG) is a complex signal reflecting the light-evoked electrical activity of several different retinal elements. For this non-invasive measure to be of use in assessing retinal function, the cellular origins of the various ERG components must be understood. In this study, we used 2-amino-4-phosphonobutyric acid (APB) to selectively inactivate the retinal ON channel (Slaughter and Miller, Science, 211:182, 1981) and examined the resulting changes in ERGs obtained from anesthetized monkeys (Macaca mulatta) and rabbits. The action of APB was monitored at the cellular level by obtaining microelectrode recordings from the lateral geniculate nucleus (LGN) in monkeys or the superior colliculus (SC) in rabbits. We have shown previously that ON responses in the central visual pathways of both species accurately reflect the functional state of the retinal ON channel (Schiller, Nature, 297:580, 1982; Knapp and Mistler, Neurosci. Abstr., 8:262, 1982).

ERGs evoked by a diffuse light stimulus (square-wave modulated at 1 Hz) were recorded with corneal ring electrodes, amplified, filtered (0.1 Hz-1 KHz or 3.0 Hz-1 KHz), digitized, and averaged. The responses of LGN or SC units to spots of light flashed in their receptive fields were recorded with conventional techniques.

APB injections yielding intravitreal concentrations of 50-500 μM diminished or abolished the ERG b-wave. The drug effects developed over 15-60 minutes and were maximal for several hours. The reduction in b-wave amplitude was accompanied by a diminution of ON responses observed centrally. When the b-wave was entirely absent, no ON responses could be elicited from the LGN or SC, but OFF responses appeared unchanged in both amplitude and receptive-field properties.

The results indicate that the ERG b-wave can serve as a non-invasive measure of activity in the retinal ON channel. ERGs obtained under our experimental conditions arise primarily from photoreceptors, bipolar cells, and non-neural elements. Of these, APB affects only the depolarizing bipolar cells, suggesting that these are the main neural elements underlying the b-wave.

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110.3 PATTERN ELECTRORETINOGRAMS IN THE HOODED RAT. C. Harnois, A. Kahan*, I. Bodis-Wollner, M. Marx*. Departments of Neurology and Ophthalmology, Mt. Sinai School of Medicine, New York, N.Y. 10029.

Recent studies in cats and humans suggest that pattern reversal electroretinograms (ERG) may be dominated by retinal ganglion cell activity, while the flash ERG has a preganglionic origin. We have simultaneously recorded ERGs and VEPs to pattern stimuli in slightly anesthetized hooded rats. ERGs were recorded with corneal silver chloride electrodes referenced to a subcutaneous electrode at the lower orbital margin. VEPs were recorded with chronically implanted screw electrodes placed over area 17 of each hemisphere referenced to the bregmatic suture. The stimulus was a vertical sinusoidal grating of 0.1 cpd and 50% contrast displayed on a CRT screen which subtended 46° of visual angle at the eye. This spatial frequency is optimal for the rat VEP as determined by our previous studies.

With square-wave counterphase modulation of the pattern at a rate of 1 Hz, the ERG of the rat shows three constant components: a small negative wave at 43 ms; a major positive at 66 ms; and a large negative at 111 ms. The ERG to on-off presentation differs in shape for the onset and offset of the pattern. The latency of the positive component at the onset is around 60 ms, which is 20 ms earlier than that for the offset of the pattern.

We studied the effect of the frequency of sinusoidal temporal modulation in the range of 2 to 30 Hz. The responses were analyzed by Fourier Transform, yielding both the amplitude and phase of the different harmonic components. The ERG to flicker stimulation is dominated by the first harmonic while the response to pattern stimulation is dominated by the second harmonic. The temporal transfer function is also different for the two types of stimuli suggesting differences in the origin for flicker and pattern ERG of the hooded rat.

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110.4 VEPs AS PREDICTORS OF DEVELOPMENT IN HIGH RISK INFANTS. I.P. Weiss, A.B. Barnet, and S.A. Reutter. Evoked Response Lab, Children's Hosp. Natnl. Med. Ctr., Washington, D.C. 20010

Visual evoked potentials (VEPs) to flash and Bayley tests of infant development were periodically administered as part of a longitudinal study of sick premature infants who were high risk for developmental delays. We report here the predictive value of the VEPs obtained at 6 months of age (192 ± 32 days) on development assessed at over 1 year of age (463 ± 120 days). The Bayley Psychomotor Development Index (PDI) and Mental Development Index (MDI) were each classified as high if 100 or above. The VEPs were scored according to the presence or absence of 14 peaks named N_1 through P_7 (Barnet et al. Electroenceph. Clin. Neurophysiol. 49, 476-489, 1980). Since several VEPs were recorded for each child, a total of 109 VEPs were analyzed on 18 subjects, nine of whom proved to have high Bayley test scores and nine low.

Component N_6, the most prominent wave on most VEPs, was present in 90% of high PDIs but only 56% of lows (χ^2 = 14.89, df = 1, p = .0001). Six VEP peaks were found to be present in at least half of the 109 VEPs, and all six were more frequent in the high PDIs than in the lows. The results were similar if the MDI was used as the index of development. Neither birth weight, gestational age, Apgar scores, number of days of neonatal hospitalization, nor incidence of respiratory distress was nearly as good a predictor of outcome in this population as was the 6 month VEP. The mean number of VEP components present at 6 months in children who were later shown to have high PDIs was 7.1 and 5.6 for children with low PDIs. VEPs to flash during infancy may be a useful tool to identify children most likely to experience developmental delay from a population already at considerable statistical risk.

110.5 DIFFERENTIAL EFFECTS OF HYPOTHERMIA AND ANESTHESIA ON FLASH-EVOKED POTENTIALS. Robert S. Dyer and William K. Boyes*. Neurophysiology Branch, Neurotox. Div., U.S.E.P.A., Research Triangle Park, NC 27711.

Chloropent, a commonly used anesthetic for rat surgery, contains chloral hydrate and pentobarbital as active ingredients. At normal room temperatures (22C), a rat injected with an anesthetic dosage of Chloropent undergoes a decrease in rectal temperature from about 37.5C to about 33.5C within a two hour period. Anesthetics and body temperature alterations are both known to alter parameters of sensory-evoked responses. However few studies have quantitatively assessed the contributions of hypothermia to anesthetic-induced changes. Two experiments were performed. In the first, chronically implanted rats were injected on different days and in counterbalanced order, with either 0, 0.05, 0.10 or 0.20 ml Chloropent/100 gm b.w., while body temperature was maintained within the normothermic range (37C-38C). Flash-evoked potentials recorded 30 min later showed increased latencies but only minor (not statistically significant) changes in amplitude. The latency increases of peaks P1, N1 and P2 reached asymptote at the 0.10, 0.10 and 0.05 dosages, respectively, while latencies of later peaks increased progressively at all dosages. In the second experiment the same rats were anesthetized with 0.35 ml Chloropent/100 gm b.w. and rectal temperature was systematically varied between 31C and 37C. Latencies increased extensively (>30%) with this level of hypothermia. P1N1 amplitude doubled when temperature was lowered to 31C, but P2N2 and N2P3 amplitudes declined over the same temperature range. These studies show that hypothermia is a more potent modifier of flash evoked potentials than is Chloropent anesthesia. The findings also suggest that previously reported alterations in evoked potentials following anesthesia may have been confounded with hypothermia. (* - Supported by NRC Research Associateship) This is an abstract of a proposed presentation and does not reflect EPA policy.

110.6 CHLORDIMEFORM INSECTICIDE PRODUCES SPATIAL FREQUENCY- AND INTENSITY-DEPENDENT CHANGES IN VISUAL EVOKED POTENTIALS. W.K. Boyes[1]*, D.E. Jenkins* and R.S. Dyer. Neurotoxicology Division, U.S.E.P.A., Research Triangle Park, NC 27711. (Spon. L. Reiter).

We previously reported that in hooded rats the formamidine insecticide chlordimeform (CDM) increased pattern reversal evoked potential (PREP) amplitude without changing flash evoked potential (FEP) amplitude, although the latency of both responses increased (Dyer and Boyes, The Toxicologist, 3:13, 1983). The present three studies further explore the effect of CDM on visual evoked potentials. First, PREPs were recorded from rats receiving either 0 (n=12), 5 (n=12) or 40 (n=13) mg/kg CDM to different spatial frequency patterns (horizontal square wave gratings generated on a TV screen, counterphase modulated at 0.25 Hz, 99% contrast, 30 cd/m^2). The 5 mg/kg group was not different from controls. Peak-to-peak amplitudes of the 40 mg/kg group were larger than those of the other two groups at spatial frequencies of 0.05, 0.10 and 0.44 cycles/degree (cpd), but were not different from the other groups at 0.8 cpd. To determine if the PREP changes were stimulus orientation-dependent, the second study examined PREPs to 0.20 cpd gratings oriented at 0 (horizontal), 45, 90 and 135 degrees in rats exposed to 0 (n=13) or 40 (n=14) mg/kg CDM. CDM increased amplitudes and latencies equally at all stimulus orientations. The third study investigated whether the failure of CDM to change FEP amplitudes resulted from the high intensity (4.5 x 10**7 Lux) or the absence of pattern in the flash stimuli. Evoked potentials were recorded from two groups of rats, 0 (n=18) and 40 (n=18) mg/kg CDM, under three stimulus conditions, a lower intensity strobe flash (1.6 x 10**5 Lux), a pattern reversl of 0.20 cpd horizontal bars, and when the TV went from all black to all white (2 to 33 cd/m^2). Preliminary data analysis revealed CDM increased amplitudes and latencies of certain components in all three responses. These experiments indicate that the CDM effect on visual evoked potential amplitudes is both stimulus intensity- and spatial frequency- but not orientation-dependent. ([1]-Supported by NRC Research Associateship) This is an abstract of a proposed presentation and does not reflect EPA policy.

110.7 CHOLINERGIC EFFECTS ON THE VISUAL EVOKED RESPONSE OF THE CAT. A.W. Kirby, R.W. Wiley* and T.H. Harding*. U.S. Army Aeromedical Research Laboratory, Ft. Rucker, Alabama 36362.

Anesthetized and paralyzed adult cats were held in stereotaxic headholder and fitted with appropriate corneal and auxiliary lenses to focus one eye onto a cathode ray tube (CRT). The other eye was occluded. Square wave luminance gratings were generated on the face of the CRT and phase alternated at 2 Hz. Visual evoked responses (VERs) were recorded with stainless steel bone screws over the visual and parietal cortex. Stimulus presentation and collection of response histograms were controlled by computer. Arterial blood samples were used to measure blood gases as well as acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) levels. Following determination of the baseline response and initial enzyme levels, diisopropyl fluorophosphate (DFP) (0.5 to 5.0 mg/kg) was administered IV over one minute. VERs and enzyme levels were then periodically measured.

Following DFP administration, reduction in VER amplitudes ranged from essentially no change after 0.5 mg/kg to 100% at higher doses. The maximum reduction was generally seen about 2 hours after DFP. Administration of atropine sulfate provided at least partial recovery. Coincident with VER reduction, blood AChE and BuChE reduction ranged from 40% to 97%. The VER showed slow recovery, returning to baseline about twenty hours after DFP, even though there was no recovery in enzyme activity. Three cats received increasing doses of DFP separated from each other by 2 hours. The VER reduction was greater and AChE activity lower as the DFP dose was increased. BuChE activity was reduced by the initial dose but remained unchanged with increasing doses.

Since atropine sulfate provided partial recovery of the VER, an initial accumulation of acetylcholine (ACh) is likely involved in the initial visual loss. Recovery of the VER without recovery of AChE or BuChE activity suggests that mechanisms in addition to ACh accumulation are involved. Finally, a graded reduction in VER and AChE activity but not BuChE activity following multiple doses of DFP suggests that only activity related to AChE is involved in this effect although both enzymes are found in the visual system.

110.8 VISUALLY EVOKED POTENTIALS TO CONTRAST STIMULI RECORDED AT THE SUBCORTICAL, EPICORTICAL AND SCALP LEVEL. G. Dagnelie*, J.Maier* and H. Spekreijse*(SPON: European Neuroscience Association). Dept. of Visual System Analysis, Neth.Ophthalmic Res.Inst,P.O.Box 6411, 1005 EK Amsterdam, The Netherlands.

Visually Evoked Potentials (VEPs) find an increasing number of applications in clinical diagnosis and monitoring of CNS and sensory lesions. Models of the electrical transfer of VEPs from cortex to scalp still lack precision to allow accurate analysis of cortical functioning by means of surface recordings. In order to improve such models developed in our department and in order to gain a better insight in the physiology underlying the VEP we have recorded VEPs in alert rhesus monkeys at the subcortical, epicortical and scalp level. The monkeys were trained to fixate an attention grating in the center of a high frequency monitor connected to a digital display system, on which a variety of stimuli could be presented.

Six electrode bundles, each consisting of 7 stainless steel wires, insulated except for .8 mm recording sites at relative distances of 5 mm, were implanted in the cerebrospinal fluid over the visual cortex and in deep brain layers under the visual cortex. Scalp electrodes were standard EEG cup electrodes. Simultaneous recordings were made from these 3 levels.

The data show that topology in the cortical VEP corresponds closely to known visual projections in primary visual cortex. In combination with the electrical transfer model a reasonable agreement between these VEPs and surface recordings can be obtained. Manipulation of stimulus parameters such as contrast, edge sharpness and temporal modulation of stimulus elements allow a comparison to known electrophysiological properties of cells in visual cortical areas. The use of sophisticated stimulus equipment and real-time computer facilities has been of great advantage in this analysis.

110.9 SPATIAL FREQUENCY TUNING IN THE VISUAL EVOKED POTENTIAL ELICITED BY SINE-WAVE GRATINGS. J.G. May*, J.L. Reed*, (SPON: R.W. Beuerman). Dept. of Psychology, University of New Orleans; LSU Eye Center, LSU Medical Center School of Medicine, New Orleans, LA 70112.

Recent reports indicate that the amplitudes of steady state visual evoked potentials (VEPs) exhibit a spatio-temporal interaction. Responses to low spatial frequencies are greatest at high temporal frequencies, while responses to high spatial frequencies are greatest at low temporal frequencies. It is not clear with such techniques which components of the transient VEP contribute to this effect. In the present study, transient VEPs were recorded monopolarly from the occiput of two subjects using constant-luminance, pattern-appearance techniques. These VEPs were obtained with sine-wave gratings that ranged in spatial frequency from 0.5 to 8.0 c/d in octave steps. Across this spatial frequency range, each grating was adjusted so that it was presented at equal apparent contrast. The amplitudes of two reliable components, N100-P140 and N180-P230, were found to be differentially tuned to spatial frequency. The early component was of greatest amplitude at high (> 4.0 c/d) spatial frequencies, while the later component was greatest in amplitude at low (< 4.0 c/d) spatial frequencies. These tuning functions were invariant over broad ranges of suprathreshold contrast (5-40%) and duration (25-200 msec). These results suggest that spatial tuning observed with steady-state methods may involve the differential predominance of these two components at different rates of stimulation. The possible role of transient and sustained mechanisms is discussed.

(Supported in part by USPHS grant EY03483, National Eye Institute).

110.11 PERIPHERAL AND CENTRAL CONTRIBUTIONS TO THE CAT VISUALLY EVOKED CORTICAL POTENTIAL: ANALYSIS IN A VISUAL MASKING PARADIGM. John S. Tootle* and Mark A. Berkley, Dept. of Psychology, Florida State Univ., Tallahassee, FL 32306

Human psychophysical studies have shown that two brief visual stimuli, presented close together in time, interact in ways which may make either stimulus less visible. This phenomenon, called masking, has been investigated in anesthetized cats in order to elucidate the neural mechanisms involved. Thus, the amplitude of a visually evoked cortical potential (VECP) to a test stimulus (S2) presented at various intervals after a prior stimulus (S1), was measured in anesthetized, paralyzed cats under stimulus conditions shown to produce masking in human psychophysical experiments. The stimuli were either pairs of sinusoidal gratings with a spatial frequency of .5 cycles/degree, a contrast of .5 and average luminance of 2.0 ft-L, or 3 ft-L spatially homogeneous flashes. S1 and S2 always had a duration of 50 msecs and were presented by instantaneously exchanging them for a 2.0 ft-L, 10° diameter circular adapting field. The amplitude of the VECP was found to be reduced when the test stimulus (S2) was presented after S1 at intervals shown to produce masking in the psychophysical experiments (onset asynchronies of 55 to 150 msecs).

Reduced VECP amplitude was observed with both monoptically and dichoptically presented gratings and flashes. Response reduction to S2 with dichoptic stimulation was assumed to be cortical in origin. However, the response to the test stimulus (S2) was reduced significantly less for dichoptically as opposed to monoptically presented stimuli, suggesting that the effect observed monoptically has both peripheral and central components.

To isolate these components, two procedures were used:

1) varying the orientation and phase between S1 and S2, and 2) simultaneous recording from optic tract and visual cortex.

The response reduction produced by monoptically presented gratings was greatest when the contours of S1 and S2 had the same spatial phase and orientation, and recovered by half when the orientation of the contours differed 6° to 15°. When S1 had a high contrast or low spatial frequency, VECP amplitude to the test stimulus showed some reduction at all orientations, indicating an additional non-orientation-selective effect. The simultaneous recordings of evoked potentials in the optic tract and visual cortex showed the response reduction measured in the optic tract was independent of test stimulus orientation.

The findings demonstrate that peripheral and central factors contribute to the VECP and probably to the masking phenomenon as well.

110.12 FAR FIELD VISUAL EVOKED POTENTIALS? T. J. Hoeppner and Fred Turner.* Dept. of Neurol. Sci., Rush Medical College, Chicago, Ill. 60618.

Theoretically, any electrical potential whose occurrence is synchronized to a stimulus should be detectable at a distance using signal averaging techniques. This has been applied usefully in recording brainstem auditory evoked potentials from the human scalp. The present study explores the application of this principle to the deep structures of the visual pathway of the cat.

In cats anesthetized with pentobarbital, an electrode array was constructed to determine the distribution of the electric field over the skull produced by visual stimulation (diffuse flash). We identified two relatively well separated electrical fields over the anterior and posterior skull, respectively. The prominent anterior field appears to be generated by the retina. The prominent posterior field is presumably generated by the visual cortex, possibly with a contribution from the lateral geniculate nucleus (LGN). To determine the extent and strength of the electric field generated by the LGN, electrodes were lowered through cortex and white matter to the LGN and the averaged visual evoked potential was measured at increments of 0.5 mm. At the dorsal surface of the LGN, there was a large (100 - 300 uV) positive potential occurring 20 - 40 msec after the stimulus onset. There was a phase reversal across the LGN with a negative potential ventral to the LGN. Dorsal to the LGN, the positive potential decremented monotonically with distance from the LGN until reaching the Clare-Bishop area where a local positive potential obscured the smaller potential from the LGN. If an oblique penetration to the LGN was utilized, thus avoiding the Clare-Bishop area, a monotonically decreasing potential was obtained from the LGN to the cortex. This suggests that appropriately placed electrodes on the skull or scalp may also detect the activation of the LGN. Studies combining ablation of visual cortex and radiations with recording of visual evoked potentials will be necessary to confirm this.

110.13 **RAPID VISUAL EVOKED POTENTIAL INDEX OF CORTICAL ADAPTATION.** J.I. Nelson, W.H. Seiple*, M.J. Kupersmith* and R.E. Carr*. Dept. Ophthalmology; NYU Sch. of Med.; New York, NY 10016

Contrast thresholds and acuity limits were measured in four observers with the swept visual evoked response (**VER**) technique. In this technique, sine wave grating contrast or grating spatial frequency is electronically varied while the subject's evoked response is retrieved in real time using lock-in amplifiers (without averaging). For example, contrast is made to slowly decrease in time, causing VER amplitude to fall. The threshold value of the stimulus may be inferred from the point at which VER response has fallen to zero. This is termed a downward contrast sweep.

Large contrast threshold elevations occur when downward rather than upward contrast sweeps are used. From previous analysis of the instrumentation we knew these shifts could not have a technical origin (Nelson, Seiple, Kupersmith & Carr, submitted). We hypothesized that downward sweeps are adapted. Thresholds averaged 4.25 times higher for adapted sweeps (contrast swept from 20% to 0.1% over 20 seconds). Differences in spatial frequency sensitivity were considerably less between up and down sweeps.

Rotating stimulus orientation during a sweep lessens adaptation, but typically not as much as using the unadapted sweep direction. 1 minute of prior exposure to a high-contrast stimulus elevates threshold, but typically not as much as using the adapted sweep direction. We conclude that up/down threshold differences are due to spatial frequency- and orientation- selective cortical adaptation.

The swept VER technique can reliably measure adaptation to contrasts as low as 1.25% which fade within seconds to threshold. Our findings are consistent with the view that cortical adaptation commences at threshold levels of excitation. The rapidity of adaptation's onset is consistent with the recent suggestion that adaptation provides a gain control for contrast sensitivity.

The novel ability to obtain a quantitative measure of cortical adaptability which is both psychophysically and neurophysiologically interpretable provides a useful tool for both basic and clinical research.

SPECIFICITY OF SYNAPTIC CONNECTIONS

111.1 THE SPECIFICITY OF NEW ELECTRICAL SYNAPSES IN THE ADULT HELISOMA NERVOUS SYSTEM DEPENDS UPON THE INTEGRITY OF EXISTING SYNAPSES. A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

Adult neurons of the mollusc Helisoma exhibit neuritic sprouting during organ culture which results in the development of new electrical synapses (Bulloch and Kater, J. Neurophysiol. 48, 569-583). Most of these new connections are transient, however, and the present study is part of an ongoing investigation into the signals responsible for elimination of these new synapses. Specifically, this study tested the possibility that the dissolution of new, normally transient synapses requires the integrity of existing synaptic connections.

The symmetrical, paired buccal ganglia of Helisoma were cultured in vitro using a modified Liebowitz-15 medium. This study focused on the normally transient, new electrical synapse between the paired neurons 19 and neurons 5. The development of this connection was induced by proximal crush of the axons of both neurons 19 and neurons 5, a condition which ensures overlapping central growth and subsequent electrical coupling of these neurons. In Control preparations the extant 19-19 connection was intact, whereas in Experimental preparations this connection was broken by crush of the commissure between the pair of buccal ganglia. Preparations were assayed after short term (2 days) or long term culture (7-14 days) to determine the strength and stability of 5-19 electrical coupling.

Transient 5-19 coupling was observed in Control preparations as expected. In Experimental ganglia, i.e., those in which the extant 19-19 connection was disrupted before 5-19 coupling could develop, 5-19 coupling was stabilized. Thus, in the absence of an existing synapse between the two neurons 19, a normally transient 5-19 connection is stable.

It is concluded that the signals to break normally transient connections between adult Helisoma neurons depend upon the integrity of existing connections. A previous study indicated that these signals require the presence of the soma rather than the axon of neurons with extant connections. Together these observations demonstrate that the persistence of the extant synapse itself in addition to the neuronal somata is needed to regulate new connections in this adult nervous system.

Technical assistance by R.M. Deverill, R.T.; supported by MRC (Canada) and the Alberta Heritage Foundation for Medical Research.

111.2 FORMATION OF ELECTRICAL COUPLING BETWEEN SPECIFIC REGENERATING NEURONS IN CELL CULTURE IS PREVENTED BY THE PRESENCE OF A RESIDUAL AXON. P. G. Haydon and S. B. Kater. Department of Zoology, University of Iowa, Iowa City, IA 52242.

Identified Helisoma neurons with different neurotransmitters can be isolated from their normal ganglionic environment and plated in cell culture where they grow neurites and form chemical and electrical synaptic connections. As has been previously demonstrated in situ (Murphy and Kater, Brain Res. 186:251, 1980) the presence of an axon in cell culture determines the locus of new neurite outgrowth and, furthermore, the presence of an axon affects the temporal competence of neurons to respond to a growth-promoting factor(s) (see Bodnar and Kater, these abstracts). Of primary importance to the present study is that the presence of an axon stump determines whether electrical synaptic connections can form between a pair of identified dopaminergic and serotonergic neurons. Electrical synaptic connections form where overlapping neuritic outgrowth occurs between cells initiating outgrowth as simple spheres. In contrast, in every case where either or both of the potential partners had even a small axon stump, overlapping neuritic outgrowth always failed to produce electrical synapses. Given the obvious importance of an axon stump in culture it is important to determine the conditions regulating whether or not axons are maintained or lost. We have found that we can reliably control whether these cultured neurons maintain their projecting axon or become essentially spherical. In cell culture axons remain only if they contact and adhere to the substrate, whereas neurons plated with their axon floating resorb their axon within hours.

These results on the effects of the axon stump on both the ensuing neurite regeneration and the potential for whether or not neurons interconnect indicates a significant stabilizing and regulatory influence for the normal morphologic features of these adult neurons.

Supported by PHS grant grant NS 18819.

111.3 SPECIFICATION OF THE STRENGTH OF ELECTRICAL CONNECTIONS BETWEEN ISOLATED IDENTIFIED NEURONS IN VITRO. C. S. Cohan and S. B. Kater. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242.

Connectivity in the central nervous system can be highly precise in terms of which neurons interconnect. Although less directly studied, the magnitude of particular connections also appears to be specified. This report asks whether the strength of specific connections is an intrinsic property of particular neurons that is expressed in isolation from the normal CNS environment.

The identified neurons of the buccal ganglia of the snail, Helisoma, allow high resolution analysis of synaptic connections. Electrical connections are common among buccal neurons and their strength can be readily quantified. In the ganglion the magnitude of the coupling between bilaterally symmetric pairs of identified neurons occurs within a narrow, neuron-specific range. For example, the coupling coefficient measured in situ, for salivary effector neurons 4R and 4L is 0.54±.11 which is about 4 times that of protractor motoneurons 19R and 19L. Such specificity is apparent not only in normal ganglia but also in new connections that are induced to form during regeneration in adult animals (e.g. 0.17±.03 for 5R and 5L). Thus, the strength of electrical connections between pairs of identified neurons in situ appears closely constrained.

The relative roles of intrinsic and extrinsic factors in determining the strength of electrical connections between buccal neurons was explored by removing neurons from their normal environment and plating them in cell culture. Identified neurons 4, 5, and 19 readily grew when cultured in a brain-conditioned medium and coupled to one another. All 3 neuron types were able to interconnect with one another. When like-neurons were plated together the strength of electrical connections was neuron-specific. The coupling coefficients for pairs of neurons 4 (0.27±.1), neurons 5 (0.50±.29), and neurons 19 (0.15±.09) were significantly different from one another. When different identified neurons were plated together, the strength of coupling appeared to be determined by the neuron with the lowest coupling in like-pairs (e.g. coupling coefficient between neurons 5 and 19 was 0.13±.07). These data suggest that the strength of electrical connections is determined, in part, by intrinsic neuronal properties. However, since the strength of coupling in situ differed from that of isolated neurons in cell culture, synaptic strength may be biased by extrinsic factors as well.

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111.4 TRANSITORY EXPRESSION OF BOTH MOSSY AND CLIMBING FIBER PHENOTYPE ON SINGLE DEVELOPING CEREBELLAR AXONS. C. A. Mason and E. Gregory*. Dept. of Pharm., N.Y.U. Med. Ctr., New York, NY 10016.

Many afferent axons wait in target zones before the onset of synaptogenesis. In the cerebellum, climbing and mossy fibers interact with different targets, forming axons with characteristic morphologies. In cerebellar slices, we have examined the initial steps in the expression of axon phenotype and specific interactions with their targets. Bundles of axons were filled with HRP and studied in the light and electron microscope (Mason, 1982, Soc. Neurosci. Abstr. 8:301). At birth, before formation of cortical layers, axons labeled via the peduncular tracts extend well into the cerebellar anlage, and few growing tips are found within tracts. All axons have a similar structure: they branch infrequently and have either bud-like endings or small growth cones (< 5 µm) with several filopodia. Immunocytochemical staining of cerebellum with antisera to neurofilament protein (Bovolenta et al., this meeting) reveals that axons arrive in the cerebellar anlage as early as 6 days prenatal and remain in the core of the anlage until birth, indicating a long waiting period.

By postnatal day 4-5, axon branches bear either large en passant or terminal expansions with many filopodia (mossy-fiber-like (MF)). These expansions establish glomerular contacts with granule cell dendrites. Others have finely branched endings with small foliate growing tips (climbing fiber-like (CF)), that contact Purkinje cell somatic spines. In the second postnatal week, each type is identifiable as CF- or MF-like, especially as CF extend over two or more adjacent Purkinje cells. However, during this period many individual axons display both morphologies and synaptic connections. That is, they bear some branches that extend into the granule cell layer and others that enter the Purkinje cell layer. The shape and synaptic connections of terminals on those branches are dictated by the respective layer, including the overbranching of CF-type branches in the Purkinje cell layer. Such fibers are rarely seen in late postnatal or mature cerebellum.

Thus, while afferent axons wait in the cerebellar anlage, they do not express their characteristic phenotype. Once target regions mature, some single axons then make supernumerary contacts not only with correct target cells, but also with incorrect cell populations. Whether these combination fibers evolve from expansion of axon terminals that have contacted both cell populations while in the waiting period or from secondary growth of filopodia must be determined. (Supported by NIH grant NS-16951. C.A.M. is recipient of a RCDA and an Irma T. Hirschl Career Scientist Award.)

111.5 SURVIVAL AND MATURATION OF CEREBELLAR GRAFTS IN SITU IN HOST CEREBELLUM. P. Bickford, H. Bjorklund+*, L. Olson+*, B. Hoffer*, and R. Freedman*. Dept. Pharmacology, Univ. Colorado Health Sci. Ctr., Denver CO 80302, and + Dept. Histology, Karolinska Inst., Stockholm, Sweden.

Cerebellar grafts in situ in host cerebellum were analyzed for survival, maturation, and organization of the grafted tissue. The cerebellar grafts survived transplantation well. Histological examination revealed typical trilaminar organization with clearcut foliation.

Two strategies were used to determine electrophysiological maturation of the cerebellar grafts: spontaneous Purkinje (P) cell discharge and responses to surface (local) stimulation. Adult P cells usually manifest a uniform and rapid discharge rate. P neurons recorded from intracranial cerebellar grafts also possess sustained regular activity. A total of 24 neurons from these grafts had a mean discharge rate of 19.3 ± 1.7 spikes per second. This compares with a discharge rate of 26.8 ± 1.0 for adult P cells in situ (N=30).

Patterns of activity can be readily analyzed using interspike interval histograms. P cells from adult animals usually have a small initial peak at 2-5 msec representing climbing fiber bursts and a much larger peak at 20-75 msec representing the most probable intervals for single spike discharge. Histograms from graft P cells show a prominent single spike modal peak. No initial climbing fiber peak is seen, nor are any complex spikes apparent on the oscilloscope tracings.

In the intact cerebellar cortex, surface (local) stimulation can excite and/or inhibit P cells. Excitation is mediated by parallel fiber synapses onto P cell dendritic spines. Inhibition is modulated by local GABAergic interneurons, the basket and stellate cells. As evaluated with post stimulus time histograms (PSTH's) both local excitation and inhibition can be obtained from intracranial cerebellar grafts in a graded fashion.

Taken together these data show that cerebellar grafts in situ in host cerebellum demonstrate organotypic development.

This research was supported by USPHS grant DA 02429.

111.6 CHOLINERGIC TRANSPLANTS EXHIBIT SPECIFICITY IN REINNERVATING THE ADULT RODENT HIPPOCAMPUS. Lawrence F. Kromer, Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, Vermont.

The dorsal hippocampus (HPC) of adult mammals can be completely denervated of its normal cholinergic input (originating in the medial septum and diagonal band) by transecting the dorsal fornix and fimbria. Moreover, this cholinergic septal projection can be reinstituted by transplanting a piece of embryonic septum to the lesioned surface of the host HPC (Björklund & Stenevi, 1977, Cell Tiss. Res. 185:289). The pattern of this reinnervation appears to mimic the normal cholinergic septal input to the HPC (Björklund et al., 1979, Brain Res. 173:57), even when there is a long delay between the cholinergic denervation and the septal transplantation (Kromer, 1982, Brain Res. Bull. 9: 539). These observations raise the question whether this cholinergic reinnervation can arise only from septal cholinergic neurons or whether other embryonic CNS cholinergic cells also can innervate the denervated adult HPC.

In the present study embryonic cholinergic neurons from the region of the nucleus cuneiformis in the brainstem of fetal rats (ED 13-14) were transplanted to the lesioned surface of the anterior HPC in adult rats which received a fornix/fimbria transection immediately prior to the transplantation. The presence of cholinergic cells and axons within the transplant and host HPC was monitored by staining for acetylcholinesterase (AChE). At all survival periods studied (1-6 months), the brainstem implants exhibited excellent survival and contained large numbers of AChE neurons and possessed a dense AChE plexus. However, in contrast to control specimens which received an embryonic septal transplant and exhibited a dense reinnervation of the host HPC by one month postoperation, specimens with cholinergic brainstem transplants demonstrated extremely sparse ingrowth of AChE fibers into the host HPC even after an in situ survival of six months. Many brainstem specimens exhibited a dramatic and abrupt termination of AChE staining at the interface between the implant and the host HPC pyramidal cells. Some specimens possessed AChE fibers within the host HPC but they usually were restricted to the dentate hilus immediately bordering the transplant. In no brainstem specimen was the density or pattern of AChE fibers in the HPC similar to that observed in normal animals or in specimens with embryonic septal transplants.

These results suggest that there is specificity in the cholinergic reinnervation of the HPC in an adult rodent when it is denervated of its cholinergic septal input. Thus, although embryonic neurons from different regions of the CNS may contain the same transmitter substance, only cholinergic cells from the appropriate presynaptic structure appear competent to replace this lesioned projection. (Supported by NIH Grant NS-18126).

111.7 SPECIFIC REINNERVATION OF DEVELOPING HINDLIMB FOLLOWING SPINAL MOTONEURON AXOTOMY. P.B. Farel and S.E. Bemelmans*. Dept. of Physiology, Univ. N. Carolina Sch. Med., Chapel Hill, NC 27514.

Regenerative specificity of an axotomized spinal motoneuron requires that the injured motoneuron first elongate its axon to the hindlimb and then form functional contacts with, and only with, its appropriate target. We are interested in the possibility that the developmental mechanisms responsible for the initial formation of specific patterns of neuromuscular connectivity can act to produce greater specificity of regeneration in immature animals than is found in adults. Experiments were performed on bullfrog tadpoles (Rana catesbeiana) because their large size permits surgical procedures to be performed with facility even at early stages of development.

The hindlimbs in frog are innervated by motoneurons of the lumbar lateral motor column (LMC). Muscles derived from the ventral premuscle mass are innervated by motoneurons located dorsally in the LMC, and motoneurons innervating more proximal limb regions are located in the rostral half of the LMC. Specificity of hindlimb reinnervation following transection of the three lumbar ventral roots was assessed by placing HRP in circumscribed hindlimb regions and comparing the locations of retrogradely labeled hindlimb motoneurons with those found in normal, unoperated tadpoles.

Previously, we have shown that normal specificity of innervation is lost following ventral root transection after stage XIV (Soc. Neurosci. Abstr., 8, 436, 1982). In the present study, specificity of reinnervation was assessed during limb bud and early foot paddle stages of development (stages IV-VII). At these stages, the major nerve trunks of the hindlimb are evident, and the muscle masses of the thigh are forming; however, individual muscle groups are not yet distinguishable. HRP was applied to the ventral thigh premuscle mass in normal tadpoles and in experimental tadpoles 6-8 weeks after lumbar ventral root transection. In normal tadpoles (n=8), 89% of the retrogradely labeled motoneurons are located in the dorsal half of the LMC. Similarly, in experimental tadpoles (n=6), 87% of the retrogradely labeled motoneurons were located in the dorsal half of the LMC. Further, a comparable tendency for clustering of labeled motoneurons in the rostral half of the LMC was seen in experimental and normal tadpoles.

Axotomy of immature motoneurons is thus followed by specific reinnervation of the hindlimb. The mechanisms underlying this specific reinnervation are presently under investigation.

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111.8 AFFERENTS AND EFFERENTS OF CORTICAL TRANSPLANTS IN HOST RAT CORTEX. F.-L.F. Chang, J.G. Steedman* and R.D. Lund. Dept. of Anatomy, Med. Univ. of S.C., Charleston, SC 29425. & Univ. of Pittsburgh, Pittsburgh, PA 15261.

An approximately 2 mm^2 superficial lesion of the left occipital cortex was made by suction on a series of newborn Long-Evans pigmented rats. In its place was laid a sheet of posterior cortex from E15 rat embryos. The objective of the experiment was to see whether the transplanted tissue would make connections with the host brain and whether these connections would reflect the origin and location of the transplants.

One to 3 days after transplantation, the host pups were injected intraperitoneally with tritiated thymidine (5 μCi/g body wt.) to help the later identification of the transplant. After 1-2 months, the transplants were located and labeled with a dry pellet of HRP. Brains were processed using TMB and DAB as chromogens. Seven animals with HRP clearly localized in the transplant were used for this study. Transplants were typically located on top of some remaining host cortical tissue. Labeled cells were seen in the host brain in the opposite cortex (6 animals), and ipsilateral thalamic nuclei (6 animals). Labeled cells in opposite cortices were frequently in areas more laterally located than the homotopic positions of the transplants. The thalamic nuclei labeled (following the terminology of Paxinos and Watson, '82) were lateral posterior (3 animals), centrolateral/paracentral (2 animals), ventrolateral (2 animals) and ventroposterior lateral (1 animal). The lateral geniculate nucleus ipsilateral to the transplant was largely degenerated as a result of the original lesion.

HRP filled fibers were also found in the dorso-medial part of the ipsilateral striatum after transplant labeling suggesting a transplant projection to the striatum. After HRP labeling the ipsilateral thalamus, filled cell bodies were found in transplants, suggesting that there is a reciprocal connection between transplant and thalamus. More detailed mapping of transplant efferents is now underway.

These results showed that rat cortical transplants located in cortex do connect with host brains. However, they do not substitute for the missing cortex but rather seem to make connections with regions which would be expected to project to cortex around the edge of the original lesion. This raises the possibility that the connections which define individual cortical areas are determined by their context. Supported by EY 03326, MUSC Fellowship (to F.-L.F.C.).

111.9 EMBRYONIC NEURAL RETINA TRANSPLANTED TO SPINAL CORD. L. K. McLoon, M. A. Sharkey and R. D. Lund. Dept. of Anatomy, Medical Univ. of South Carolina, Charleston, SC 29425.

Embryonic neural retina of rats, when transplanted over the tectum of newborns, develops connections with only those nuclei which normally receive retinal projections (McLoon and Lund, '80, Exp. Brain Res.). The possibility existed that the retina might also form projections when confronted with a developing non-visual sensory system. To test this, E14 retinae were transplanted over the dorsal columns in the spinal cord at the level of C1 or C2. At this time, the sensory fibers are still growing through this region. Two experimental paradigms were used. In one, the cord was left intact with only the meninges disrupted. In the second, a lesion was placed in the cord at the time of transplantation, which interrupted the dorsal columns.

One month after transplantation, the cords of the host animals were exposed. If a transplant was visible, it was injected with HRP. If no transplant was evident, the dorsal columns were injected with HRP. Both sets of animals were perfused with 2% glutaraldehyde and sectioned frozen. Sections were processed for HRP histochemistry by reaction with DAB or TMB.

Transplants were found in 75% of the experimental animals. As in previous studies, the retina differentiated and the normal cell and plexiform layers were clearly discernable. Injections of HRP both into dorsal columns or into the transplants failed to back-fill any ganglion cells in the transplants, even in cases where the transplants were embedded in the host spinal cord. After injections of HRP into the transplants, no fibers were seen coursing between the transplant and the host spinal cord. Neurofibrillar stains failed to demonstrate an optic fiber layer in these transplants. Electron microscopic examination of the transplants also demonstrated the lack of large cells in the ganglion cell layer. This suggests the cells in the ganglion cell layer may be displaced amacrine cells. Although retina survives and differentiates when placed into spinal cord, it is unable to form connections with this non-visual sensory system. The possibility of initial non-specific outgrowth which is retracted during development is currently under investigation.

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111.10 ALTERATIONS OF WING MOTILITY AND MOTONEURON NUMBER FOLLOWING HETEROTOPIC NEURAL TUBE TRANSPLANTATION IN CHICK EMBRYOS. P. Cauwenbergs*, E. Cosmos and J. Butler*. (Spon. J. Mazliah). Dept. of Neurosciences, McMaster Univ. Health Sciences Centre, Hamilton, Ontario. L8S 3Z5.

Thoracic neural tube segments transplanted to the brachial region of chick embryos are viable, form a brachial plexus and make contact with the brachial musculature during the first 10 days in ovo. However, as development proceeds, the nerve-muscle contacts uncouple and the muscles deteriorate (Straznicky, K., Acta.Biol.Hung.18:437,1967;Butler,J. et al.,Can.J.Neurol.Sci. 9:360,1982).

As a primary effort to understand the contributing roles of thoracic nerves and brachial muscles to the uncoupling phenomenon, we selected to examine both wing motility to monitor functional interaction and the status (number and structure) of motoneurons in the lateral motor column (LMC) of experimental versus control embryos.

To prepare experimental embryos, the brachial segment of the neural tube was extirpated at 48-52 hr and replaced by the thoracic segment of a donor embryo (50-53 hr). Controls were either unoperated or had received a brachial neural tube transplant. Daily observations of wing movements during 10 minute periods (M/10) were recorded from embryonic day-6 onward. Through day-8, the frequency of wing movements of experimental and control embryos was similar. By day-8, all embryos exhibited approximately 90 M/10. However, during subsequent development a dramatic change was noted. Whereas the frequency of wing movements of control embryos continued to increase and reached a peak value of 340 M/10 at day-14, experimental embryos demonstrated a reverse trend, i.e., the frequency attained a peak value of 140 M/10 at day-10 and, then, rapidly decreased. By day-16 only slight movements were detected.

These motility observations provide evidence that initially foreign thoracic nerves do make functional contact with the brachial musculature. However, at a crucial period in development, marked by the onset of the formation of permanent nerve-muscle junctions, the foreign nerves are deemed inappropriate for brachial muscles. The steady decline in wing motility of experimental embryos beyond day-10 monitors the progressive uncoupling of thoracic nerves and brachial muscles. Correlative analyses of the LMC revealed that, prior to the decline in wing motility, prolonged motoneuron survival occurred in experimental embryos. However, coincident with the loss of functional innervation, neurothanasia proceeded rapidly in the transplanted thoracic neural tube.

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111.11 TARGETTING BY SURGICALLY MISROUTED OPTIC FIBERS IN GOLDFISH WITH AND WITHOUT IMPULSE BLOCKADE Ronald L. Meyer, Developmental Biology Center, University of California, Irvine, California 92717

Optic fibers which innervate lateral posterior optic tectum were dissected from surrounding tectal tissue and redirected into the anterior medial edge of the opposite host tectum. The normal pathway at this entry site leads to medial posterior tectum. The eye innervating host tectum was removed at the same time as the deflection or up to 18 months prior to deflection. The growth of deflected fibers was followed by autoradiography from 1 to 11 months after deflection.

Roughly half of the deflected fibers immediately grew laterally across the anterior end of tectum and then posteriorly within lateral tectum. The other half grew in a posterior-laterial direction from the insertion site. By 2 months most label was clearly in the lateral half of tectum and showed a weak tendency to be heavier posteriorly.

This contrasts to the medial labelling previously seen when medial posterior fibers were inserted into this same anterior medial position (BR 155:213). The laterality of the label did not noticeably change from 2 to 11 months after deflection and was not diminished in tecta which had been denervated for 18 months prior to deflection.

To test whether the capacity of deflected lateral posterior fibers to bypass denervated medial tectum required impulse activity. Activity was chronically eliminated by periodic intraocular injections of tetrodotoxin (TTX) during regeneration. At 2 months after deflection in fish with simultaneous enucleation or enucleation 1 month prior to deflection, the innervation pattern was essentially the same as in fish without TTX. TTX also had no obvious effect on the innervation formed following deflection of medial posterior fibers. Thus TTX does not effect the "active" formation of gross topography. This suggests that the formation of refined topography which is inhibited by TTX (Dev. B. R. 6:293) represents a different patterning mechanism. (Supported by PHS Grant NS15381.)

111.12 **EFFERENT FIBER PROJECTIONS FROM TRANSPLANTED EMBRYONIC SUPERIOR COLLICULUS IN RAT.**
J.G. Steedman*, A.R. Harvey* and R.D. Lund (SPON: S.M. Bunt). Dept. Anatomy, Medical University of South Carolina, Charleston, SC 29425 and Dept. Anatomy, University of Pittsburgh, Pittsburgh, PA 15261.

Presumptive superior colliculus of embryonic day 15 Long-Evans Hooded rats was transplanted to the superior colliculus of newborns. It has been shown that such transplants connect with the host and receive afferents from a wide variety of host brain centers (Harvey and Lund, J. comp. Neurol., 202:505(1981)). The projections of transplant neurons to the host have been studied using horseradish peroxidase (HRP) and ^{3}H-proline injected into the transplant 1-8 months after the operation. Alternate sections through host brainstem and transplant were stained for the presence of HRP, with diaminobenzidine or tetramethyl benzidine, and transported ^{3}H-proline was demonstrated by autoradiography. Holmes' silver and Nissl stains were used to show general fiber and cell architecture respectively.

Whereas afferents to such transplants have been shown to come from remote parts of the brain (e.g. retina, cortex), the efferent projections were all to local structures at comparatively short range. Projections were never found to spinal cord or pontine nuclei. The principal output was to the host's superior colliculus and was largely confined to the deeper part of stratum griseum superficiale and below. The next most frequently found projections were to the central gray surrounding the cerebral aqueduct and to the midbrain tegmental nuclei lateral to this. Connections to these deeper parts of the brain stem were seen in those cases where the transplant was embedded in the host tissue; in those cases where the transplant was connected to the host by a stalk of fibers, projections tended to be localised to the adjacent superior colliculus. Other areas in which efferents were seen were the pretectum and inferior colliculus. Terminations in the latter have only been observed when the final position of the transplant in the host brain resulted in a connection bridge straight from the transplant to inferior colliculus. Connections with inferior colliculus demonstrate that such transplants are capable of connections uncharacteristic of the superior colliculus of the normal animal when mechanical constraints dictate. However, the best developed efferent projections from these transplants are clearly those to the host's own colliculus. The short-range nature of these projections contrasts with those observed for both cortical and retinal transplants performed by identical techniques (Jaeger and Lund, J. comp. Neurol., 194: 571(1980); McLoon and Lund, Exp. Brain Res., 40:273(1980)).

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111.13 NORMAL AND ABNORMAL DEVELOPMENT OF CALLOSAL PROJECTIONS TO THE OCCIPITAL CORTEX OF RATS. R.D. Lund, P.W. Land and F.-L.F. Chang. Dept. of Anatomy, Med. Univ. of S.C., Charleston, SC 29425 and Univ. of Pittsburgh, Pittsburgh, PA 15261.

In previous studies we (Cusick & Lund, J. Comp. Neurol., 212:385 '82) found that neonatal eye removal in rats substantially modified the extent to which callosal fibers invaded area 17 when examined as adults. In this study, we have investigated how the callosal projection develops and when in development the abnormality resulting from enucleation can be distinguished from the normal pattern. We were especially interested to know whether the inception of visual function or some other event correlated with the normal and abnormal development.

The projection was studied by injecting HRP or nuclear yellow into one cortex of a series of normal, one eyed or bilaterally enucleated rats of varying ages from 1 day to 18 days postnatal. After 1 day survival the brains were processed and reacted for peroxidase using TMB and DAB as chromogens. Two series of animals were studied using degeneration techniques after lesions involving one cortex to show orthograde projection patterns.

At birth, the callosal pathway arises from the whole occipital cortex, but the terminals do not appear to ramify substantially in the cortex at this stage. By day 5, a substantial callosal projection becomes defined at the 17/18a border and there is a loss of callosally projecting cells along the medial border of area 17. This sequence of events progresses in normal animals to day 12 when the adult pattern is achieved. During this process of retraction, the callosally projecting cells are focussed in bands above and below layer V. In the intermediate stages, the extent of area 17 occupied by labelled cells is greater than that occupied by the focussed terminal field. After eye removal at birth or 4 days postnatal, the callosal projection originates from a larger part of area 17 than normal in the cortex contralateral to the eye removal. This effect already can be seen by postnatal day 9; and while there is loss of callosally projecting cells in the medial part of area 17, it is less pronounced than in normals.

The timing of normal loss of callosally projecting cells in area 17 corresponds closely to the timing of segregation of crossed and uncrossed optic input into lateral geniculate nucleus (Maxwell and Land, Anat. Rec., 199:165A, 1981). Because of this coincident timing and because optic axon segregation does not occur after eye removal, it is suggested that the ordering of primary optic input is an important event in determining distribution of callosal axons. The establishment of an adult pattern of callosal distribution by day 12 and the fact that early eye removal already has an effect by day 9, indicate that normal visual function has no influence on the gross shaping of the rat's occipital callosal projection, since these events precede eye opening. Supported by NIH grants EY03414, EY03447, EY04064 and MUSC Fellowship to F.-L.F. Chang.

111.14 FIBER ORDER IN THE HAMSTER PYRAMIDAL TRACT. K. Kalil and J. Keifer*. Dept. of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

Recent studies have shown that the arrangement of fibers within nerve tracts may contribute to an ordered pattern of connections. We undertook a study of fiber order within the hamster pyramidal tract to determine how axons are arranged within the tract and whether this reflects the pattern of connectivity in corticospinal terminations.

Injections of HRP were made into the sensorimotor cortex or into their spinal cord targets. Animals ranged in age from newborns to adults. The TMB method was used to trace axons labeled in the anterograde or retrograde direction.

Localized injections of sensorimotor cortex resulted in a series of contiguous labeled fascicles within the pyramidal tract. The fascicles maintained the same relative position with respect to one another along the corticospinal pathway. Axons adjacent to one another in the pyramidal decussation, for example, could be followed into the same fascicle within the dorsal column, and fascicles arising from a similar rostro-caudal region of the cortex tended to cluster together within the pyramidal tract. This highly fasciculated pattern was apparent at stages early in pyramidal tract outgrowth.

Although neurons contiguous in the cortex appeared to maintain contiguity of their axons, it is not clear what unit of cortical organization is represented by individual fascicles. Moreover, since axons retrogradely labeled by HRP injections into a single or several adjacent spinal segments were widely dispersed throughout the pyramidal tract, it is not likely that individual fascicles simply represent axons destined for neighboring spinal segments.

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111.15 MAINTAINED TARGETS OF OCCIPITAL CORTICAL NEURONS WHICH TRANSIENTLY EXTEND A PYRAMIDAL TRACT AXON DURING DEVELOPMENT. Brent B. Stanfield and Dennis D.M. O'Leary, The Salk Institute, P.O. Box 85800, San Diego, CA 92138.

During the first postnatal week a large number of pyramidal tract neurons are present in the occipital cortex of the rat, but over the next two weeks pyramidal tract neurons become restricted to the more rostral cortical fields. We have previously shown that this developmental restriction in the distribution of pyramidal tract neurons is due to collateral elimination rather than to cell death, since neurons retrogradely labeled through their transient pyramidal tract axon can be identified in the adult occipital cortex.

In order to determine the sites to which the transient pyramidal tract neurons in the occipital cortex send a collateral which is maintained in the adult, injections of either True Blue or Fast Blue (0.3 µl of 2% TB or FB) were made into the pyramidal decussation during the first postnatal week, and three weeks later (12-24 hours before being killed) injections of Nuclear Yellow (0.05-0.3 µl of 2% NY) were made into one of five second sites: (1) In some animals the second injection (NY) was placed in the pyramidal decussation. NY- and FB-labeled cells within layer V were found throughout the rostral cortex; most of these neurons were doubly-labeled. Although the population of FB neurons continued back into the occipital cortex, no NY cells were seen in the occipital cortex, confirming our previous findings. (2) In the brains of animals in which the second injection (NY) was into the occipital cortex on one side, most of the NY-labeled neurons seen in the contralateral occipital cortex were outside of layer V; none of the NY-labeled cells within layer V, was doubly-labeled with TB. (3) Similarly, no doubly-labeled neurons were found in those cases in which the second injection labeled ipsilateral associationally-projecting neurons. (4) When the second injection (NY) was into the superior colliculus, NY-labeled cells in layer V were broadly distributed in the neocortex, and many of the NY-labeled neurons, both in the occipital cortex and in the more rostral cortical fields, were doubly-labeled. (5) In cases in which the second injection site (NY) was in the pons, NY-labeled neurons were also found in layer V of the occipital cortex and a great many of these were doubly-labeled with TB.

In an additional series of experiments the two dye injections were made and the rat pups were killed during the first postnatal week. None of the pyramidal tract neurons in the occipital cortex was doubly-labeled when the second injection was into the contralateral cortex, but even at this early age, many of these cells were doubly-labeled when the second injection was into the superior colliculus.

These findings suggest that none of the neurons in the occipital cortex that transiently send an axon into the pyramidal tract extends a callosal collateral, but that many of these neurons do send a collateral to the pons or to the superior colliculus, or to both regions and that these latter projections persist in the adult. Supported by NIH Grant NS-18506.

DEVELOPMENT AND PLASTICITY: SENSORY SYSTEMS

112.1 FUNCTIONAL DEVELOPMENT OF THE VENTRAL CORTICAL VISUAL PATHWAY MEASURED BY THE 2-DEOXYGLUCOSE METHOD. K.A. Macko, J.Bachevalier*, C.Kennedy, S.Suda*, L.Sokoloff, and M.Mishkin Lab. Neuropsych. and Lab. Cer. Metabol., NIMH, Bethesda, Md.

In primates, striate (area OC), prestriate (areas OB and OA), and inferior temporal cortex (areas TEO and TE) as far forward as the temporal pole constitute the ventral cortical visual pathway, which is now known to be critical for object recognition. The functional development of this pathway was traced metabolically in infant monkeys that were prepared with a unilateral optic-tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 3, and 5 months of age. This procedure allowed comparisons between "blinded" and functional cortical pathways. The 2-deoxyglucose method for quantitative measurement of local cerebral glucose utilization (LCGU) was applied an average of two days postoperatively in the two younger animals and one month postoperatively in the four older animals while each was awake and visually stimulated.

From the autoradiographs, representative sections were chosen at 1mm intervals throughout the ventral cortical visual pathway. Weighted averages of LCGU for each visual area were calculated by means of a computerized image-processing system. The results revealed systematic age-related changes in 1) absolute LCGU within the normal hemisphere and 2) differences in LCGU between the normal and deprived hemispheres.

In all cortical visual areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. As in adults, the intact hemisphere of infants shows a progressive decline in LCGU along the ventral cortical visual pathway from a high in area OC (ranging from 26.1 µmoles/100g/min at 9 days to 88.1 at 4 months) to a low in anterior TE (ranging from 17.6 at 2 days to 59.7 at 4 months). This gradient was present in all subjects, but was most shallow in the two youngest.

The deprived hemisphere showed reduced LCGU relative to the normal hemisphere in all areas of the cortical visual pathway at all ages. Also at all ages, hemispheric differences were greatest in area OC and smallest in anterior TE. These differences, however, varied systematically with the age of the animal. Thus, for each cortical area, the relative difference between the normal and deprived hemispheres was smallest in the youngest subjects and approached the differences seen in adults only at about four months of age, the time at which LCGU appeared to peak.

This finding that adult levels of metabolic activity are not reached until about 4 months of age is consistent with behavioral data indicating that the neural capacity for visual object recognition is probably not developed until about this time (Bachevalier and Mishkin, Int. J. Psychophysiol., 1983).

112.2 A COMPARISON OF THREE TASKS EVALUATING VISUAL FUNCTION FOLLOWING TRANSPLANTATION OF EMBRYONIC NEOCORTICAL TISSUE INTO THE LGN IN THE HOODED RAT. R.B.Wallace, G.D.Das*, P. Jasin*, K. Kaufman*, M. Lazar*, R. Smeyne*, and J. Thompson*. Developmental Psychobiology Laboratories. University of Hartford, West Hartford, CT 06117.

Embryonic forebrain tissue obtained from 17 day embryos was transplanted into the caudal diencephalon at the coordinates of the lateral geniculate nuclei in 4 month old Long-Evans hooded rats. Following growth and differentiation of the transplanted tissue, the animals were tested behaviorally on three tasks designed to assess visual function - a light avoidance task, an overhead orientation task, and a radial arm maze task. The light avoidance task employed an apparatus and procedure similar to that used by Altman in an earlier investigation (Amer. J. of Physiol., 202:1208, 1962). The specifics of the overhead orientation task are in a thesis submitted to the Department of Psychology, MIT (G.E. Schneider, 1966). Finally, the details of apparatus and procedure for the radial arm maze task are outlined in a paper by Barnes, Smith and Latto (Q.J. Exp. Psychol., 22: 239, 1970). Results from these animals on the set of three tasks were compared with data from animals that received surgical lesions at the coordinates of the LGN, with data from animals that received surgical lesions at the coordinates of the LGN followed by transplantation of embryonic neocortical tissue and finally with data from normal control animals. Two enucleated animals were also tested as an additional control condition. Following behavioral testing, all animals were sacrificed, perfused transcardially with 10% buffered formalin, the brains blocked in the coronal plane and stained with CV for microscopic evaluation. Histological results indicated that in the transplant and lesion/transplant animals, the neocortical tissue had replaced much of the ventral and lateral thalamic nuclear groups. Behavioral analysis for the light avoidance task revealed that all groups save for the enucleated condition spent significantly more time in the dark portion of the matrix; the enucleated animals distributed their time 50/50 between dark and lighted areas of the matrix. Results for the overhead orientation task (checkerboard pattern) indicated no overall significant differences across the treatment conditions although the enucleated animals had essentially random response frequencies and the control animals had the highest response frequencies. Finally, data from the radial area maze task showed all conditions to be significantly different except for the control-transplant comparison. Additional behavioral testing involving more complex visual tasks remains to be done in order to determine if more marked deficits become apparent under these conditions.

112.3 REFRACTIVE ERROR, GROSS MORPHOMETRY AND LIGHT MICROSCOPY OF EYES FROM CHICKENS FOLLOWING LID-SUTURE. Gail S. Tucker[1] and Uri Yinon*[2]. Bascom Palmer Eye Inst., University of Miami School of Medicine, Miami, FL 33136[1] and Goldschleger Eye Institute, Sackler School of Medicine, Tel-Aviv Univ., Tel-Hashomer, Israel[2].

Keratometry, retinoscopy, A-scan ultrasonography and gross measurements of the intact globe were used to study eyes in normal chickens and in chickens lid-sutured early in life. These analyses of the globe and dioptric apparatus demonstrated a consistent myopia in the treated eyes of the lid-sutured chickens. Survival times post-surgery ranged from 3-7 months. The observed changes favoring myopia were: development of a negative refractive error (9 normal eyes, $\bar{X}$ = +.75 D; 7 lid-sutured eyes, $\bar{X}$ = -14.03 D); increased axial length (normal/lid-sutured, $\bar{X}$ = 14.72 mm/17.5 mm); and increased equatorial diameter ($\bar{X}$ = 18.78 mm/21.16 mm); but these same eyes exhibited increased corneal radius ($\bar{X}$ = 4.99 mm/5.56 mm). The change in refractive error was variable in the lid-sutured eyes (Range, -7.0 D to -23.0 D) and unrelated to the age at lid-suture (3-29 days) or to whether one or both eyes were lid-sutured. Corneal diameter and anterior chamber depth were equally variable in normal paired eyes and in eyes from monocularly or binocularly lid-sutured chickens.

Light microscope analyses were done using glutaraldehyde fixed eyes embedded in paraffin, serially sectioned and stained with hematoxylin/eosin. Except for outer and inner segment length, all retinal layers were thinner in lid-sutured eyes, but the degree of retinal thinning also varied with retinal location. The planimetric density of cells in the nuclear layers was higher in retinas from lid-sutured eyes compared with normal retinas. Stratification in the inner plexiform layer was well-defined in the normal retina but was poorly defined in the lid-sutured retinas. Examination of the ciliary body demonstrated an augmentation of the surface folds in lid-sutured eyes, suggesting that an enlarged ciliary body might be associated with myopia in the chicken. Regional differences in the complexity of the surface convolutions may also be a factor.

The relationship of these changes to the dynamics of eye growth and the possible meaning of ciliary body augmentation following lid-suture in the chicken will be discussed.

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112.4 MITOSIS IN THE DIFFERENTIATED RETINA OF XENOPUS LAEVIS LARVAE: NISSL, GOLGI, AND COLCHICINE STUDIES. George J. Kokoris and Leslie J. Fisher. Neurosci. Prog., Univ. of Michigan, Ann Arbor, Mich. 48109, and Dept. Ophthal., Henry Ford Hospital, Detroit, Michigan 48202

The inner nuclear layer (INL) of the retina in Xenopus laevis larvae grows throughout larval life. In addition to the gradual addition of new cells at the retinal margins, we have demonstrated, with ^{3}H-thymidine, the appearance of DNA synthesis within the differentiated INL of the older, central retina at metamorphosis (Kokoris and Fisher, 1979). Subsequent ultrastructural analysis of cell types within the central retina revealed immature cells and variations in cellular stratification within the INL, consistent with our autoradiographic evidence of mitotic activity in this region (Kokoris and Fisher, 1982). To complete our analysis of anatomical plasticity in the central retina, and to verify the presence of mitosis in the INL, we have prepared retinae from Xenopus laevis undergoing metamorphosis for Nissl, Golgi, and colchicine analysis.

In Nissl-stained material, we observed cells in the central retina with dark, columnar nuclei, resembling germinal cells seen in the retinal periphery. These nuclei contain a varying distribution of aggregated heterochromatin, characteristic of dividing cells in various phases of the mitotic cycle. Moreover, we observed cells in the INL resembling immature, post-mitotic neurons, similar to those seen in newly-differentiated regions of the retina. These were scattered throughout the INL of the central retina.

Analysis of Golgi material revealed, in additon to recognizable neurons and glia, large columnar cells. These were identical to germinal cells seen at the retinal margins. Apical and basal processes extend from these cells to traverse the width of the INL to varying depths. These processes resemble cytoplasmic spindles of neuroepithelial cells dividing within the immature retina.

Retinae treated with colchicine, a mitotic inhibitor, contained many mitotic cells arrested in metaphase. Many of these cells were located at the retinal periphery; however, a number of metaphase figures were seen in the differentiated INL of the central retina.

Taken together, these data confirm that the INL of the central retina, although well-differentiated, displays a capacity for cellular addition, and extensive anatomical re-organization throughout metamorphosis.

112.5 TRANSPLANTATIONS BETWEEN NEONATAL VISUAL AND SOMATOSENSORY CORTEX IN PIGMENTED RATS. S. M. Lu,*D. E. Schmechel* and C.-S. Lin, Dept. of Anatomy, Duke University Medical Center, Durham, N. C. 27710

Previous studies have demonstrated that neonatal and embryonic brain tissue from the mammalian central nervous system may survive transplantation to an appropriate host. In our experiments, a piece of cortical tissue about 2 x 3 mm^2 wide and less than 0.5 mm deep was removed from the left somatosensory cortex and replaced with a similar size piece taken from the right visual cortex of the same animal. The purpose of these experiments was to determine the patterns of connections of the cortical grafts. In particular, we want to know whether the connections formed between the transplanted tissue and the host depend on the site of origin, or, on the new position of the transplant.

The transplantations have been carried out in several age groups, postnatal day 1, 2, 3, 4, and 5. This series also allowed us to investigate the effect of age on the formation of connections. All animals survived to 50 or more days of age and then were subjected to experiments designed to assess the connections of the transplants. Our present findings are summarized as follows:

1) From Nissl-stained sections, most of the grafted cortical neurons which survive in the transplant exhibit abnormal morphology.

2) In the acetylcholinesterase (Ach E) stained sections, the pattern of Ach E activity within the transplant is more random and less dense compared to the patterns seen in normal cortical areas.

3) In glutamic acid decarboxylase (GAD) antibody-stained sections, utilizing the immunoperoxidase technique, the density of the reactivity in the transplant is higher than in the surrounding normal cortical areas.

4) The physiological properties of neurons in the transplants also were studied. For example, visual cortical neurons transplanted to the somatosensory area are now responsive to somatic stimuli; however, the intensity of peripheral stimulation required to drive these neurons is much higher than for normal neurons.

5) In one case, a small amount of horseradish peroxidase was injected into transplanted visual cortex which responded to peripheral somatic stimulation. The retrogradely-labelled neurons were found in the ventrobasal nucleus of the thalamus. Further experiments are in progress.

In conclusion, our preliminary results suggest that thalamic afferents do invade and form synapses in the transplanted tissue, and that the new position of the grafts is the main factor in determining the connections between the graft and the host.

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112.6 BILATERAL TRANSITORY PROJECTIONS FROM AUDITORY TO VISUAL AREAS IN KITTENS. S. CLARKE*, G.M. INNOCENTI, Inst. of Anatomy, Univ. of Lausanne, rue du Bugnon 9, 1011 Lausanne, Switzerland.

Neurons connecting homologous and heterologous visual areas through the corpus callosum are more numerous and more widely distributed in newborn kittens than in adult cats; many callosal axons are eliminated during the first two or three postnatal months. Callosal connections between somatosensory areas and between auditory areas undergo a similar reshaping. We now report transitory projections from auditory areas to the contralateral and ipsilateral visual areas 17 and 18. The study was done by injecting - separately or simultanously - the retrograde fluorescent tracers Fast Blue (FB; 0.05 -0.1 µl at 1.5 % per injection) and Diamidino Yellow (DY; 0.1 -0.3 µl at 2 % per injection) into areas 17, 18 and into ectosylvian auditory cortex of 12 kittens on postnatal days 0 - 10. After injections in areas 17 and 18, labeled neurons were found ipsilaterally and contralaterally in all visual areas and in addition in the ectosylvian auditory region (A). The latter was identified by its position and also by the fact that when injected it yielded retrograde labeling of the medial geniculate body.

Axons from A reach most of the contralateral areas 17 and 18 since small injections into the gray and white matter at i) the medial bank of 17, ii) different positions along the 17/18 border, iii) lateral parts of area 18, all label a certain number of neurons in A. The projection seems to be roughly topographically organized: caudolateral parts of A project to caudal 17-18, rostro-medial parts of A to rostral 17-18. Simultaneous injections of FB and of DY show that neurons in A which project to the contralateral homologue are in layer III, deeper than those projecting to contralateral 17 and 18; very few neurons, if any, project both to A and 17-18.

The ipsilateral projection from A to 17-18 is heavier than the contralateral one. Its neurons of origin are in layers II, III and VI. Injections of the two tracers in 17-18 of the two hemispheres indicate that the ipsilateral and contralateral projections arise from different neurons (although less than 1 % of the callosal neurons do also project ipsilaterally).

Experiments with fluorescent tracers or horseradish peroxidase in adults indicate that the contralateral projection disappears completely during maturation and the ipsilateral one almost completely; the few remaining neurons are mostly in layer VI.

Thus, ipsilateral cortico-cortical projections, similarly to the callosal ones, develop through a phase of transitory exuberancy in which ephemeral "connections" are formed between areas belonging to different sensory systems. (NSF 3.628-0.80)

112.7 DEVELOPMENT OF SENSORY CELL ORIENTATION PATTERNS IN THE SACCULE OF THE TOADFISH, OPSANUS TAU. Bernd Sokolowski* (SPON. C. McCormick), Department of Anatomy, Georgetown University Medical School, Washington, D.C. 20007.

Sensory hair cell orientation, defined by the relationship of the kinocilium to the stereocilia, is a phenomenon observed in the inner ear of many vertebrates. In particular, different species of fishes have different hair cell orientation groups on the sensory epithelium of the saccule. For example, the adult toadfish has a four-quadrant saccule with two rostral groups that are oriented horizontally and two caudal groups that are oriented vertically. Hair cells lying in an area between the rostral and caudal patterns have neither of the four orientations but appear to be in transition toward a horizontal or vertical orientation. The purpose of the present study was to determine the inner ear development in the toadfish embryo, using SEM and TEM, in order to gain a better understanding of the factors involved in causing these orientation patterns.

The toadfish saccule is initially established at the caudal pole of the otocyst in the 14 somite embryo by the development of kinocilia with very short stub-like stereocilia. The initial cluster of approximately 20 hair cell bundles is grouped in a circular pattern, having neither an adult type horizontal nor vertical orientation. This cluster of cells is maintained in the 20-25 somite embryo at which time an otolith, without an otolithic membrane, begins to form on top of these cells. As the otocyst begins to divide into the pars superior and pars inferior, the initial cluster of hair cells begins to move and separate across the saccular macula, without the addition of any new hair cell bundles. The division of the pars inferior from the pars superior is completed by the invagination of epithelial cells at approximately 5 days prior to hatching. At this time the otolith and otolithic membrane have substantially increased in size. In addition, hair cell numbers have not significantly increased from the initial 20 cells, but bundles found in the rostral and caudal portions of the developing saccule have respective horizontal or vertical patterns. This observation suggests that orientation is established when movement occurs into these areas from the original central region. Once the embryo hatches, saccular hair cells proliferate over the entire macula with cells developing in the respective orientation of a region. Thus, the hair cells developing rostrally are horizontal, those developing caudally are vertical and cells developing in the central region are in transition. (Supported by grants from NSF, NIH and the Lerner-Gray Fund for Marine Research of the American Museum of Natural History).

112.8 TONAL SENSITIVITY OF PRIMARY AUDITORY CORTICAL FIELD (AI) NEURONS IN CATS REARED WITH UNILATERAL COCHLEAR DESTRUCTION. R. A. Reale*, J. Z. Feng*, J. C. K. Chan and J. F. Brugge* (SPON: C.N. Woolsey). Dept. of Neurophysiology and Waisman Ctr., Univ. Wisconsin, Madison, WI 53706.

The effects of mechanical lesions that destroy the organ of Corti on the functional and structural development of auditory cortex were studied by combining microelectrode mapping of neurons' best frequencies with horseradish peroxidase histochemistry for tracing neural connections. Cochlear destruction was aseptically performed during the first postnatal week and cats were older than six months at the time of the combined electrophysiology/anatomy experiment. Best-frequency maps of AI contralateral and ipsilateral to the intact ear were obtained under Nembutal anesthesia.

As expected, the best-frequency distribution formed an orderly and complete tonotopic map in the contralateral AI and the physiology of its neurons were, qualitatively, normal in all respects (except of course that neurons were no longer binaurally sensitive). In control experiments, a large part of AI in normal adult cats is devoted to cells which are excited by a sound delivered through either ear alone, although acoustic intensity thresholds for the ipsilateral ear are markedly higher than those for the contralateral ear. Surprisingly, AI ipsilateral to the intact ear of our experimental animals showed a normal tonotopic representation comprised of single neurons and neuron clusters securely driven by best-frequency tonal stimulation in virtually every electrode penetration examined. Furthermore, the acoustic thresholds where as low as those obtained in AI contralateral to the intact ear in experimental or normal cats. Closely spaced electrode penetrations in AI ipsilateral to the intact ear also revealed that along the isofrequency dimension loci containing neurons with the lowest acoustic thresholds alternated with loci containing neurons with the highest acoustic thresholds.

These findings are surprising because in normal adult cats approximately 30% of AI is devoted to cells that are never excited and only inhibited by sound delivered through the ipsilateral ear. Our observations suggest that some change may have taken place within field AI ipsilateral to the intact ear. Whether this involves changes in connections or some functional unmasking of ipsilateral excitability is not yet known. (HD-03352, BNS 7912939)

112.9 TOPOGRAPHIC DISTRIBUTION OF PRIMARY AUDITORY CORTICAL FIELD (AI) CALLOSAL NEURONS IN CATS REARED WITH UNILATERAL OR BILATERAL COCHLEAR DESTRUCTION. J. F. Brugge*, J. Z. Feng*, R. A. Reale*, and J. C. K. Chan (SPON: R.E. Kettner). Dept. of Neurophysiology and Waisman Ctr., Univ. Wisconsin, Madison, WI 53706.

The effects of unilateral and bilateral cochlear destruction, done within a few days of birth, on the development of auditory callosal connections were studied with horseradish peroxidase (HRP) histochemistry for tracing cortical connections related to the middle ectosylvian auditory cortex (AI). In some animals receiving a unilateral cochlear lesion, microelectrode mapping of neurons' best-frequencies served to guide the HRP injections and to assess the responsiveness of AI neurons both ipsilateral and contralateral to the intact ear. Cochlear lesions were aseptically performed under general anesthesia by exposing the tympanic bulla, opening it and inserting a blunt probe through the round window. Cats were older than six months at the time of the combined electrophysiology/anatomy experiment performed under Nembutal anesthesia. Large injections of HRP were made unilaterally in AI (contralateral to the intact ear in cats with unilateral lesion) one to two days prior to sacrifice. The distribution of callosal perikarya was revealed using TMB as the chromogen and the temporal bones were processed.

In normal adult cats, AI neurons which give rise to callosal axons destined for the contralateral AI are not homogeneously distributed throughout AI but, rather, are found aggregated into clusters separated from each other by regions containing relatively fewer callosal neurons. Furthermore the positions of these callosal aggregations are highly correlated with the binaural representation demonstrated in the high frequency representation of AI. Recently we showed in the kitten (Feng J.Z. & Brugge J.F., J.Comp.Neurol., 214:416-426, 1983) that AI neurons do not form these characteristic adult callosal clusters until the 6th or 7th week of postnatal life. Our present findings indicate that destruction of one or both ears at birth fails to prevent the aggregation of AI callosal neurons into their adult-like cluster distribution even though normal binaural stimulation is obviously absent.

In combined microelectrode mapping experiments, AI ipsilateral to the intact ear showed a normal tonotopic representation and, along the isofrequency dimension, loci containing neurons with the lowest acoustic thresholds alternated with loci containing neurons with the highest acoustic thresholds. Loci containing aggregates of HRP filled callosal neurons also alternated along the isofrequency dimension and, in preliminary experiments, were found in registration with regions where acoustic thresholds of neurons were substantially below (approximately 20 dB or more) thresholds in surrounding areas. (HD-03352, BNS 7912939)

112.10 MIGRATORY STAGES OF THE DORSAL COCHLEAR NUCLEUS PRINCIPAL NEURONS. Willard, F.H., Thompson, D.M. and Martin, G.F., Department of Anatomy, The Ohio State University, Columbus, Ohio 43210.

The postmitotic migration of neurons is a fundamental event underlying the formation of the nervous system, yet little is known concerning the factors which influence these movements. To this end we are examining the migratory patterns of large neurons in the dorsal cochlear nucleus (DCN) of the North American opossum. The opossum DCN consists of four cytologically distinct layers the most notable of which is layer II containing the vertically oriented cell bodies of the principal neurons. The apical dendrites of these neurons extend into layer I while their basal dendrites reach deep into layers III and IV. Two additional features characterize the principal neurons: each principal neuron has a prominent perinuclear cap of Nissl substance seen in no other DCN neuron (Willard et al., 1983); and all principal neurons transport horseradish peroxidase (HRP) from the contralateral inferior colliculus (IC, Willard and Martin, 1983). The only other DCN neurons sharing similar efferent connections are the few giant cells scattered throughout layers III and IV. Given the above criteria, it has been possible to identify principal neurons during early development in pouch-young opossums. These animals are born 12 days post-conception at about 15mm snout-rump length (SRL) and mature to approximately 120mm while residing in an external abdominal pouch.

Horizontally oriented, elongate neurons with perinuclear caps of Nissl substance can be recognized in the presumptive dorsal acoustic stria (DAS) of 18mm (SRL) opossums. In 27mm (SRL) opossums such neurons are present in the lateral extreme of the DAS and in the DCN; these neurons can transport HRP from the contralateral IC. When animals reach 35mm (SRL) most principal neurons are present in the DCN but no systematic orientation of their elongate somata is evident. By 40mm (SRL) the principal neurons are still scattered throughout the nucleus,however,their somata have assumed a vertical orientation. During the 50-60mm (SRL) growth period the vertically oriented principal cell somata form a distinct row in layer II.

Our results suggest a two stage migration of DCN principal neurons. During the 18-35mm (SRL) growth phase these elongate neurons apparently move laterally along the presumptive DAS, tangenital to the radial glial fibers observed in Golgi impregnations. As the animal grows from 35-60mm (SRL) the principal neurons, now contained within the DCN, shift from a horizontal to a vertical orientation and move dorsally, parallel to the radial glial fibers, until their cell bodies reach layer II.

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112.11 ONTOGENY OF AUDITORY FUNCTION IN THE COCHLEA OF THE MONGOLIAN GERBIL: CONTRIBUTION OF THE MIDDLE EAR. N. K. Woolf and A. F. Ryan* Otolaryngology Research Laboratory, University of California at San Diego Medical School, San Diego, CA 92103.

Cochlear microphonic (CM) potentials of mongolian gerbils (Meriones unguiculatis) were examined at 10, 12, 14, 16, 18, and 30 days after birth (DAB), and in the adult. This range of chronological ages included the onset of neonatal hearing through achievment of mature auditory system characteristics. The contribution of the middle ear to CM development was assessed by comparing at each chronological age acoustically driven CM with CM generated by direct mechanical stimulation of the stapes.

Round window CM potential recordings in neonatal gerbils using the two stimulation paradigms revealed similar ontogenetic trends, but different temporal sequences. In both cases, functional onset was followed by a period of rapid threshold improvement, which in turn was followed by a period of slow threshold improvement. The onset of acoustically generated CM occurred at 12 DAB, with thresholds at approximately 115 dB SPL. CM thresholds subsequently improved 65 dB between 12 and 16 DAB. This rapid phase of CM threshold maturation was followed by a slow phase of CM threshold development: CM thresholds improved only 15 dB between 16 and 18 DAB, at which time CM thresholds approximated adult levels.

CM potential recordings generated by direct mechanical stimulation of the stapes revealed a similar trend for thresholds to that seen with acoustic stimulation, but followed a different developmental sequence. The earliest stapes driven CM recordings were observed at 10 DAB, although at this age only a small percentage of the subjects were responsive. CM thresholds at 10 DAB were 85 dB, or more, above adult levels. Stapes driven CM responses were first consistently observed at 12 DAB, with thresholds elevated approximately 65 dB above adult thresholds. With increasing age, mechanically driven CM also exhibited rapid and slow maturational phases: CM thresholds improved 50 dB between 12 and 14 DAB and, thereafter, declined only 15 dB between 14 and 18 DAB. CM thresholds at 18 DAB equalled adult values.

These results indicate that measures of CM development reflect middle ear as well as inner ear maturational factors. The effects of middle ear immaturity are most pronounced during the rapid maturational phases and are responsible for increases in CM thresholds of approximately 40 dB. The small changes in CM thresholds observed during the slow maturational phases are, presumably, the result of terminal cochlear maturational changes.

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112.12 ENHANCED NEURAL REPSONSE FOLLOWING POSTNATAL OLFACTORY EXPERIENCE IN NORWAY RATS. R.M. Coopersmith* and M. Leon. Department of Psychobiology, University of California, Irvine CA 92717.

Norway rat pups will preferentially approach the odor of their mother, a preference acquired by means of postnatal experience (Leon, Galef and Behse, 1977). The pups will also acquire an attraction to an arbitrarily selected odor such as peppermint if given daily eposure to it. In this study, we examined the possibility that this behavioral plasticity is accompanied by an enhanced neural response to pre-exposed odors by the olfactory system.

Rat mothers were given a diet known to suppress their own odor to minimize early olfactory experience for the pups. The pups were pre-exposed for 10 min/day for the first 18 days of life to either the odor of peppermint or to clean air, using a flow-dilution olfactometer. Exposure was accompanied by perineal stimulation, a procedure found to facilitate the acquisition olfactory preferences in neonates (Pederson and Blass, 1982). On day 19, rats previously exposed to either peppermint or air were given a test exposure to either peppermint or air for 45 min after administration of 14C-2-deoxyglucose (20uCi/100g). Autoradiographs of olfactory bulb sections along with reference standards were prepared using standard methods. The autoradiographs were then analyzed with a computer-based image processing and analysis system, which allowed quantitative comparisons between uptake sites to be made.

Rats receiving a peppermint test odor after peppermint pre-exposure showed an enhanced pattern of 2-DG uptake in the glomerular layer of the bulb, relative to rats tested with the identical odor but without previous experience with that odor. Both odor-naive and odor-experienced rats had activity in a dorsolateral complex of glomeruli, approximately 2mm from the rostral pole of the bulb. The intensity and extent of this activity, however, was significantly greater in the experienced rats.

The experienced rats, though, may have sniffed the familiar odor more than the naive rats, and the enhanced neural response may have simply been a function of enhanced stimulus presence. Experienced and naive day 19 rats had their sniff rate to peppermint monitored by means of a sensitive pressure transduction device in a test chamber. No difference in sniff rate was found between the groups, suggesting that early odor exposure can cause functional changes in olfactory neural activity.

112.13 ^{3}H-THYMIDINE-RADIOGRAPHIC STUDIES OF NEUROGENESIS IN THE RAT OLFACTORY BULB, S. A. Bayer. Dept. of Biol., Indiana-Purdue Univ., Indianapolis, IN 46223.

Neurogenesis in the rat olfactory bulb was examined with ^{3}H-thymidine-radiography. For the animals in the prenatal groups, the initial ^{3}H-thymidine exposures were separated by 24 hrs.; they were the offspring of pregnant females given two injections on consecutive embryonic (E) days (E12-E13, E13-E14,..E21-E22). For the animals in the postnatal (P) groups, the initial ^{3}H-thymidine injections were separated by 48 hrs., each group receiving either four (P0-P3, P2-P4,..P6-P9) or two (P8-P9, P10-P11,..P20-P21) consecutive daily injections. On P60, the percentage of labeled cells and the proportion of cells added during either 24 hr. or 48 hr. periods were quantified at several anatomical levels for each neuronal population in the main olfactory bulb (mitral cells, tufted cells, granule cells, interneurons in the external plexiform layer, periglomerular granule cells) and accessory olfactory bulb (output neurons, granule cells, periglomerular granule cells). The total time span of neurogenesis extends from E12 to beyond P20. Output neurons are prenatally generated over 5-9 day periods (with most neurogenesis occurring over 2-4 days) in a strict sequential order beginning with the accessory bulb output neurons (E13-E14) and ending with the interstitial tufted cells lying between the glomeruli in the main bulb (E20-E22). These data indicate that the sequence of neurogenesis in the olfactory bulb is relatable to the pattern of neurogenesis in the amygdala and its olfactory input. With the exception of the granule cells in the accessory bulb (88% generated between E15-E22), the rest of the interneuronal populations are generated postnatally and nearly simultaneously. While most neurons (75-80%) originate during the first three weeks of life, all interneuronal populations, including accessory bulb granule cells, show some neurogenesis beyond P20. Injections of ^{3}H-thymidine in juvenile and adult rats indicates neurogenesis up to P60 in the accessory bulb and up to P180 in the main bulb, especially in the main bulb granule cell population. There is circumstantial evidence for turnover of main bulb granule cells during adult life. This research was supported by the National Science Foundation (BNS 79-21303).

112.14 DEVELOPMENT OF PONTINE PARABRACHIAL NUCLEI TASTE RESPONSES IN RAT. David L. Hill. Ctr. Human Growth & Dev., and Dept. Oral Biol./Sch. Dent., Univ. of Michigan, Ann Arbor, MI 48109.

Developmental changes occur in primary afferent chorda tympani nerve and second-order nucleus of the solitary tract taste responses in the rat. To determine whether there are functional changes in responses from third-order gustatory neurons during development in rats, recordings were made from chemosensitive neurons in the pontine parabrachial nucleus (PBN). Thirty-one single neurons were studied in rats aged 14-25 days, 30 neurons in rats aged 25-35 days and 33 in adults (> 80 days). Chemical stimuli applied to the anterior tongue were 0.1M and 0.5M NH_4Cl, NaCl, LiCl, and KCl, 0.1M citric acid, 0.01N HCl, 1.0M sucrose, 0.1M Na-saccharin and 0.01M quinine HCl. Neural activity was measured for the first 5 sec after stimulation of the tongue; a comparable period of prestimulus spontaneous activity was subtracted to yield response frequencies.

Response frequencies of PBN neurons in rats aged 14-20 days were significantly less than those in adults to all stimuli ($p<0.05$), except responses to HCl ($p>0.20$). Changes in PBN taste response frequencies were also apparent between rats aged 25-35 days and adult rats. Response frequencies to NaCl, LiCl, sucrose, and Na-saccharin increased 50% or more during postweaning development ($p<0.05$). In contrast, PBN responses to all other stimuli were similar between rats aged 25-35 days and adults ($p>0.20$). When neurons were categorized on the basis of responding maximally to 0.1M NH_4Cl, to 0.1M NaCl or LiCl, or equally to all 0.1M salts, most PBN neurons in rats aged 14-20 days, 25-35 days and adults responded equally to NH_4Cl, NaCl and LiCl (58%, 77%, and 64%, respectively), and a relatively small proportion responded maximally to NH_4Cl (16%, 7%, and 3%, respectively).

Similar to the second-order gustatory neurons in the nucleus of the solitary tract (NST), striking developmental changes are found in responses to NaCl, LiCl, KCl, sucrose and saccharin. However, unlike NST responses, developmental changes also occur in PBN neurons to NH_4Cl, citric acid and hydrochloric acid. Further differences include a smaller proportion of PBN neurons maximally responsive to NH_4Cl. Compared to peripheral chorda tympani nerve taste neurons for each of the three age groups, NST and PBN neurons have greater average frequencies to all stimuli, a greater proportion respond equally to monochloride salts, and mature average frequencies occur in older rats to NaCl, LiCl, and KCl. Since responses from NST and PBN neurons alter during development, adult rats may also differ from younger rats in behavioral responses to some taste stimuli. (Supported by NIH Grant NS17404).

112.15 DORSAL ROOT GANGLIA DEVELOPMENT IN CHICKS FOLLOWING PARTIAL ABLATION OF THE NEURAL CREST. Virginia McM. Carr. Dept. Biochem., Molec. and Cell Biol., Northwestern U, Evanston IL 60201

Portions of the brachial neural crest of stage 13 and 14 (Hamburger and Hamilton, '51) chick embryos were partially ablated using an opthalmological cauterization unit. Ganglia developing from the remaining crest material were examined for ganglionic volume, degenerative activity, and neuronal nuclear diameter at stage 35 (9 days of inc.), when neuronal cell growth is becoming pronounced but while cellular degeneration is still occurring (Carr and Simpson, '78a, b; Hamburger et al., '81).

Results showed that the cauterization procedure caused the reduction or absence of dorsal root ganglia (DRG) at the level of ganglia 15, 16, and/or 17 (G15, G16...). In contrast, hypertrophies occurred in remaining ganglia. These hypertrophies were most pronounced, however, not in the ganglia adjacent to those lesioned but rather in nonadjacent ganglia. The mean volume in brachial G14 was 10.4 ± 2.9 $\mu m^3 \times 10^6$ ($\bar{x}$ ± S.D.) in experimental embryos versus 9.9 ± 2.6 $\mu m^3 \times 10^6$ in controls. In contrast, in G13 these values were 8.0 ± 2.0 versus 4.7 ± 1.6 $\mu m^3 \times 10^6$, respectively. G13 is a ganglion that contributes to the brachial plexus but that does not normally innervate the wing (M. Hollyday, pers. commun.) nor show either degenerative or proliferative responses to early wing bud removal (Carr & Simpson, '78a, b). More anterior cervical ganglia also showed slight hypertrophies: 6.0 ± 1.4 versus 4.0 ± 1.6 $\mu m^3 \times 10^6$ for G12 and 5.8 ± 0.9 versus 4.6 ± 0.7 $\mu m^3 \times 10^6$ for G11.

Degenerative activity and the average neuronal nuclear diameter were measured in G11, G13, and G15. Results showed that the mean number of degenerations/unit vol. was reduced by 25% in G11, 30% in G13, and 55% in G15 in experimental embryos. Moreover, within individual embryos these reductions, up to a maximum of 60-70%, were proportional to the degree of ganglionic hypertrophy. In lesioned G15 all ganglia showed reduced degeneration of 50-70%. Enlargements of 6%, 12%, and 16% were found in the average nuclear diameters of both lateroventral and mediodorsal neurons in G11, G13, and G15, respectively. Again, individual changes appeared proportional to the degree of DRG hypertrophy.

These changes indicate an enlargement of DRG neuronal peripheral fields due to reduced peripheral competition. Such conclusions disagree with those of Eide et al. ('82) who found electrophysiological evidence for only limited plasticity of DRG inputs onto lumbosacral motoneurons when chicks subjected to neural crest lesions were examined at 19 to 21 days of incubation. Whether these differences are due to stage of development, axial level, or DRG projections examined remains to be determined.

112.16 CHRONIC VIBRISSA ACTIVATION PRODUCES DECREASED GLUCOSE UTILIZATION IN SI CORTEX BUT NOT IN SUBCORTICAL LEVELS OF RAT VIBRISSA-CORTICAL BARREL SYSTEM. C.L. Hand* and P.J. Hand (SPON: J. Metzler). Dept. of Animal Biology, Sch. of Vet. Med., and Inst. of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

Using the quantitative (^{14}C)-2-deoxyglucose (2DG) method of Sokoloff (J.Neurochem., 1977), we reported that chronic repeated stimulation (5-15 min/day for 60-90 days) of a single vibrissa (#3 of row C; C3) in an intact vibrissa field produces a significant ($p \leq 0.01$) decrease in local cerebral metabolic rates of glucose (LCMRG) in the C3 vibrissa-barrel column in contralateral SI cortex of rat when compared to matched controls(Neurosci. Abst., 1982). In addition, bilateral C3-stimulated naive controls (n=4) show no significant right-left differences in SI C3 labeling (mean diff. of 1.2% in LCMRG in matched pairs; no preferred hemisphere). Based on the conservative criterion that 2DG labeling 20% above background is of significance, we now report that the functional representation in SI of the chronically-stimulated C3 vibrissa not only has a lower LCMRG than the matched control, but also involves less cerebral tissue (mean areal decrease of 15% compared to controls). To determine if this phenomenon is specific to cortex, matched-pair quantitative analysis was extended to subcortical levels of the vibrissal pathway in the same experimental animals (n=13). Concurrently, to determine if receptor adaptation is a factor (and to serve as a check of results at other levels), C3 labeling in both trigeminal ganglia was compared for each animal. Serial autoradiographs were analyzed both densitometrically and by computer-assisted pseudocolor-enhanced image processing routines. Labeled brain structures were identified using the actual Nissl stained sections. Analysis of C3-activated labeling in trigeminal ganglia indicates no significant differences in paired experimental and control LCMRG values. Evaluation of discrete C3 functional labeling in main sensory nucleus and subnuclei caudalis and interpolaris of the trigeminal complex reveals no significant differences in metabolic activity between paired labeling (range=.1%-2% differences in LCMRG). Similarly, analysis of discrete C3 labeling in ventrobasal thalamus of matched pairs indicates no significant change in labeling (1%-2% diff. in LCMRG). There is no consistent tendency for the decreased LCMRG to occur on the experimental side, as it does in cortex.

In conclusion, lack of LCMRG changes at lower levels of the vibrissal pathway, coupled with the observed decreases in cerebral metabolism in the chronically-activated C3 functional column in SI cortex suggests a cortical phenomenon. Additional studies are required to determine if this is the result of an intrinsic cortical mechanism, or whether alternate (as yet unanalyzed) subcortical-cortical mechanisms may be contributing. (Supported by NIMH Training Grant 15092 and NS-14935).

112.17 THE EFFECT OF PERIPHERAL SENSORY DEPRIVATION OR ENRICHMENT ON THE EXTENT OF FUNCTIONAL PLASTICITY IN RAT SI CORTEX PRODUCED BY CHRONIC SUBTOTAL VIBRISSAE DEAFFERENTATION. R.L. Craik, P.J. Hand, C.L. Hand* and B.A. Cozzens*. Moss Rehab. Hosp. and Dept. of Animal Bio., School of Vet. Med., Inst of Neurol. Sci., Univ. of Penna.,Phila.,PA. 19104

The developing rat vibrissa-"barrel" system was selected to examine patterns of functional re-organization in SI in response to chronic subtotal vibrissae deafferentation(n=10). Previous (^{14}C)-deoxyglucose (2DG) studies revealed an enlarged and diffuse pattern of labeling of C3 representation in contralateral SI 90 days after neonatal follicle ablations sparing #3 vibrissa of row C (C3)(Sheu and Hand,Neurosci. Abst.,1982). This study examined the effects of chronic activation or deprivation of the spared C3 vibrissa on the extent and pattern of its altered representation in SI. The intact contralateral C3 vibrissa served as an internal control. An enriched C3 vibrissa received 30 minutes of mechanical stroking daily from postnatal day 3 to 90 (PND 3-90). A deprived C3 vibrissa was maintained clipped from PND 3-83; the vibrissa was allowed to regrow PND 83-90 for stimulation. The 2DG method of Sokoloff(J. Neurochem.,1977) was used to quantitate functional changes in SI. In both enriched and deprived paradigms local cerebral metabolic rates of glucose (LCMRG) of spared C3 vibrissal representation showed a mean increase of 12% compared to the normal C3 vibrissal metabolic column. Both enriched and deprived spared C3 vibrissa representations demonstrated an increased areal extent of labeling in lamina IV (L.IV) compared to normal C3 in 5 of 7 matched pairs. SI areas immediately adjacent to the spared C3 representation have 12% higher LCMRG than control in 6 of 7 matched pairs. To further examine this phenomenon, 3 neonatal rats (PND2) underwent bilateral vibrissal ablations sparing C3. At PND 90 the LCMRG in the enriched spared C3 representation was 7% higher and the areal extent in L.IV 2% larger than its matched spared C3 control. The deprived, spared C3 representation demonstrated a 20% decrease in LCMRG and a 9% decrease in areal extent in L.IV when compared to control spared C3. A bilateral control demonstrated a 4% left-right asymmetry in LCMRG (right higher) but had similar areal extents of labeling. In conclusion, these preliminary results suggest that major features of functional plasticity produced by subtotal vibrissae deafferentation are independent of the level of activation of a spared receptor. However, finer aspects of such plasticity appear affected by peripheral activation: stroking of spared C3 increased LCMRG in SI while LCMRG and SI representation was decreased in deprived spared C3. Ongoing studies involve completion of laminar analyses of the spared vibrissal representation in SI. (Supported in part by the Foundation for Physical Therapy, Inc., and NS-14935.)

112.18 ASCENDING NONVISUAL AFFERENTS TO THE SUPERIOR COLLICULUS IN NEONATAL KITTENS J.G. McHaffie*, K. Ogasawara* & B.E. Stein (SPON: H.P. Clamann), Department of Physiology & Biophysics, Medical College of Virginia, Richmond, VA 23298.

Despite the strong interest in the development of superior colliculus (SC) afferents, almost all work has been restricted to the visual system. Yet nonvisual cells are functional several days before visual cells. The present study was undertaken to determine which of the ascending nonvisual SC afferents are already present at birth or appear during early maturation. Injections of 0.02 µl of 25% HRP were made in the SC of neonatal kittens (1-7 days of age). The animals were sacrificed 24 hrs. later and the tissue was incubated for HRP after the method of Mesulam (1978).

Since SC cells with perioral cutaneous receptive fields have been reported in pre- and early postnatal cats (Stein et al, J. Neurophysiol. 36:667-679, 1973) we were particularly interested in whether labelled neurons are present in the neonatal sensory trigeminal complex, and if so, how they are distributed. Labelled cells were found in this region in even the youngest animal studied; all were contralateral, and the majority were clustered in the rostral part of pars oralis. A few labelled cells were also present in the principal nucleus and pars caudalis, and fewer still in pars interpolaris. Apparently, even in neonatal animals the distribution of trigeminotectal cells is similar to adult cats (e.g. Ogasawara & Kawamura, Okajimas Folia Anat. Jpn. 58:247-264, 1982).

A variety of other subcortical structures from caudal brainstem through diencephalon were found to project to the SC in animals 1-2 days of age. At the level of the medulla, labelling was observed in nucleus paragigantocellularis lateralis and the perihypoglossal nuclei. In the cerebellum sparse numbers of retrogradely-filled cells were found in medialis, interpositus and lateralis nuclei. Auditory structures at pontine levels containing labelled neurons included the dorsomedial periolivary nucleus and the area medial to the medial nucleus of the trapezoid body. In the region of the midbrain, heavy labelling was noted in the substantia nigra, parabigeminal nucleus and locus coeruleus, and in the dienencephalon the zona incerta was densely labelled. A scattering of cells was also seen in the contralateral SC.

These observations indicate that the major ascending nonvisual afferents to the SC are already well-developed at birth. Apparently the major anatomical development of inputs to the SC is a prenatal event; comparatively few changes in the locations and numbers of afferents take place postnatally. This contrasts with the protracted, functional maturation of many of the sensory properties of SC cells. Supported by grants EY04119, BNS 8209857, and the Jeffress Foundation.

113.1 WIDESPREAD LABELING OF AVIAN FOREBRAIN NEURONS AFTER SYSTEMIC INJECTIONS OF ^{3}H-THYMIDINE IN ADULTHOOD. F. Nottebohm and S. Kasparian*, Rockefeller University, New York, N.Y. 10021.

Adult male and female canaries were given 50uCi of ^{3}H-thymidine intramuscularly twice a day for 14 days and sacrificed 26 days after the last injection. Their brains were cut in 6um thick sections treated for autoradiography and stained with cresyl violet. The distribution of labeled cells was mapped in great detail in one male bird, using 8"x10" prints of photographs of the left half of the brain. Labeled cells were identified as falling in either of five categories, based on light-microscopic anatomical criteria, as described in Goldman and Nottebohm, PNAS 80, 1983: endothelial cells, glia, ventricular zone cells, neurons and cells of uncertain identity. A cell was accepted as labeled when the number of silver grains over its nucleus was at least five times that over surrounding neuropil. Labeled glia and endothelial cells were found throughout the brain. Labeled ventricular zone cells occurred profusely bordering the telencephalic ventricle, and were rare or absent elsewhere. Labeled neurons occurred throughout the forebrain, but were very rare or absent in hippocampus, septum, hypothalamus, cerebellum, thalamus, optic lobes or medulla. Extent of label over cells identified as neurons was comparable to that found over glia and endothelial cells. By analogy with the conclusion reached for labeled neurons in nucleus HVc of adult canaries (Goldman and Nottebohm, PNAS 80, 1983), we suggest that label was incorporated into the nucleus of cells during the S-phase of DNA synthesis which normally precedes mitosis. The anatomical correlation between the distribution of of labelled cells in the telencephalic ventricular zone and the occurrence of labeled neurons in forebrain suggests that a process of neurogenesis may occur in the ventricular zone, followed by migration of neuroblasts through adult forebrain tissue. This interpretation, already advanced for nucleus HVc, awaits the ultra-structural confirmation of neuronal identity, and support from time course studies charting the distribution of labeled cells during the first 4 weeks after the last ^{3}H-thymidine injection. Since adult brain weights in canaries occur as early as day 15 after hatching, the hypothesized neurogenesis cum migration would be accompanied by a turnover of the neuronal population. This may be the first suggestion of a constant rebuilding of adult forebrain networks in an adult higher vertebrate. The fact that it seems to occur in a part of the brain thought to be involved with learning may not be coincidental.

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113.2 STEROID INDUCED NEURAL PLASTICITY IS MULTIFACETED. T. J. DeVoogd, B. Nixdorf* and F. Nottebohm. Psychology Department, Uris Hall, Cornell University, Ithaca, N.Y. 14853 and Rockefeller University, 1230 York Ave., New York, N.Y. 10021.

Robustus archistriatalis (RA) is a nucleus in the canary telencephalon involved in song production. This nucleus is smaller in females than in males. Giving testosterone to an adult female canary results in an increase of more than 50% in the volume of RA (Nottebohm, Br. Res. 189:429-436, 1980), and the development of male-like song. The dendritic fields of neurons within RA double in size and there is an increase of more than 60% in the number of synapses contained within the nucleus. We now report that the ultrastructural constituents of RA and the dimensions of synapses within it are also modified by adult exposure to testosterone.

Six adult female canaries were implanted with Silastic tubes of testosterone as reported last year (T.J. DeVoogd, B. Nixdorf and F. Nottebohm, Soc. Neurosci. Abs. 8:140, 1982). After one month, they and matched unimplanted female controls were sacrificed. RA samples were prepared for electron microscopy (sodium cacodylate buffer, 25% Karnovsky fixative). The distribution of tissue constituents was measured on micrographs taken about the center of RA and printed at 7000X. Testosterone treatment was associated with significant increases in the proportion of micrograph area occupied by neuronal somata (13.7 vs 4.8%) and by post-synaptic neurites (predominantly dendrites, 6.4 vs 3.7%). In contrast, no significant differences were seen in the proportion of micrograph area occupied by myelin or capilaries. Micrographs from nucleus rotundus were also measured. This is a thalamic visual nucleus which does not concentrate steroids. Testosterone-treated and control birds did not differ in rotundus area occupied by neuronal somata, dendrites or myelin.

Individual synapses were measured at 32,400X. Maximal cross section of the pre-synaptic area was measured parallel and perpendicular to the contact zone, yielding a mean "diameter" for the pre-synaptic area. Testosterone treatment was associated with an 11% increase in the mean "diameter" of the pre-synaptic area ($p<.02$) and a 10% increase in the number of vessicles seen within this area ($p<.04$). The length of the post-synaptic opaque zone also increased but the mean "diameter" of the post-synaptic area did not differ between the two groups. There were no differences between the groups in the frequency of gaps in the post-synaptic opaque zone. The shift in synaptic dimensions could be due to the properties of the new synapses or result from changes in the existing synapses.

We conclude that sytemic testosterone treatment can affect the anatomy of an androgen sensitive nucleus in several ways: increase in size of neuronal somata, increased dendritic length, a net gain in synapse numbers and a shift in some synaptic dimensions.

113.3 ADULT EFFECTS OF GONADAL STEROIDS ON SEXUALLY DIMORPHIC PREOPTIC/ANTERIOR HYPOTHALAMIC AREA NEURONS OF MALE AND FEMALE FERRETS. S.A. Tobet, D.J. Zahniser* and M.J. Baum, Dept. of Nutrition and Food Science, M.I.T., Cambridge, MA 02139 and Image Analysis Laboratory, Tufts-New England Medical Center, Boston, MA 02111.

We recently reported the existence of a sexual dimorphism in the preoptic/anterior hypothalamic area (POA/AH) of adult ferrets (Tobet et al., Endocrinology 112(suppl.):240, 1983). In males 2 bilateral nuclear groups are discernable in the POA/AH (dorsal and ventral), whereas in females only 1 bilateral nuclear group is discernable (ventral). Furthermore, while dorsal cells are larger in both sexes, the cells within the dorsal nucleus in males are larger than cells in a comparable region in females. We now report that gonadal steroids can increase the somal size of neurons in this sexually dimorphic region of the adult ferret POA/AH. Adult male and female ferrets, castrated or castrated and steroid implanted (estradiol or testosterone in silastic capsules) for at least 2 weeks, were anesthetized and sacrificed via intracardiac perfusion with 0.9% saline followed by 10% neutral buffered formalin. Alternating 40 and 8 μm paraffin sections were cut through the POA/AH and stained with thionin. Boundaries of individual perikarya were determined in 8 μm sections using a DeAnza Image Analysis System with a VAX-11/780 computer. Regions of 128 μm^2 were digitized according to optical density (3 regions from the ventral nucleus of each animal and 3 regions from the dorsal nucleus of each male and the comparable dorsal region of each female) and perikaryal dimensions within these regions were determined automatically (range: 10-28 cells/region). In the dorsal nucleus of males and dorsal region of females, perikarya were significantly larger than in the male and female ventral nuclei. A sexual dimorphism of perikaryal size with larger cells in the dorsal nucleus of males compared to those in the camparable dorsal region in females were evident, as previously reported. Steroid treatment significantly increased the size of dorsal perikarya in males and females by 63% and 104% (somal volume), respectively. However, in the ventral nuclei only the soma of females increased in response to steroid treatment (123%, somal volume). The results indicate that sexually dimorphic neurons of the adult ferret POA/AH are steroid sensitive and respond to adult hormone treatment with an increase in perikaryal size.

Research was supported by NIH grant HD-13634 to MJB and a Whitaker Health Sciences Fund Fellowship to SAT.

113.5 TRANSPLANTATION OF FETAL MEDIAL BASAL HYPOTHALAMIC BRAIN TISSUE INTO THE THIRD VENTRICLE OF ADULT HOSTS: ULTRASTRUCTURAL CHARACTERIZATION. P.W. Coates and H.K. Strahlendorf. Depts. of Anatomy and Med. Surg. Neurol./Physiology, Texas Tech Univ. HSC Sch. of Med., Lubbock, TX 79430.

Fetal medial basal hypothalamic (MBH) grafts in the third ventricle (3V) of adult hosts were examined with scanning and correlative transmission electron microscopy (SEM and SEM/TEM). MBH tissue including the arcuate nucleus region was obtained from late gestation Sprague-Dawley rats and pooled for stereotaxic transplantation into the 3V of adult males. Hosts and controls (age-matched and sham-operated) were sacrificed after 7 weeks and prepared for SEM and SEM/TEM by procedures described previously. SEM revealed that some MBH transplants had lodged at sites distant from the original implantation on the 3V floor. Fractured surfaces of embedded grafts revealed blood vessels. Other graft tissue consisted of small spheres suspended above the 3V floor. These lacked blood vessels when sectioned. All grafts displayed attachment to host tissue via fiber networks and bundles, sometimes strikingly complex. Some thin varicose fibers terminated in small oval endings. Cells were present on the surface of transplants, and along fiber bundles. SEM/TEM revealed that neurons, glia, and nerve fibers were on graft surfaces. A band of mostly glial processes surrounded an inner core of neurons, glia and an intricate neuropil. Synaptic terminals containing small clear and dense core vesicles (DCV) were observed on neurons, within the neuropil, and in plexuses connecting grafts to hosts. Many graft neurons exhibited features suggesting ongoing maturation: indented cell and nuclear membranes, numerous Golgi complexes, some with associated DCV; rough endoplasmic reticulum; mitochondria; prominent nucleoli and synapses. Glial processes displayed filaments. These observations suggest that MBH grafts transplanted to the 3V: 1) may seed to sites other than the floor, possibly via cerebrospinal fluid currents; 2) display massive fiber bundles between grafts in recesses and adjacent median eminence, perhaps indicating a location more compatible to their original position; 3) show evidence of continuing development, suggesting an ultrastructural basis for potential functional interaction between graft and host.

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113.6 DIFFERENTIAL DISTRIBUTION OF CERULOHIPPOCAMPAL PROJECTIONS: ANATOMICAL AND BIOCHEMICAL EVIDENCE. J.H. Haring and J.N. Davis
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We have been studying the possibility that the proliferation of locus ceruleus (LC) fibers in the hippocampus after septal lesions is mediated by branches of the injured axon rather than by collateralization of uninjured axons. Our "pruning" hypothesis requires that branches of a single LC neuron project to the hippocampus by the ventral amygdaloid bundle (VAB) and either the fornix (Fx) or cingulum (Cb). We have recently reported that LC neurons projecting to the dorsal area dentata are localized in the dorsal half of the LC and that certain of these neurons project to both dorsal and ventral area dentata (Exp. Neurol. 79: 785, 1983). We now suggest a differential distribution of the three cerulohippocampal pathways based upon observations obtained using the retrograde transport of HRP in combination with septal lesions and by measurement of norepinephrine (NE) levels after lesions of septum or Cb.

Focal HRP injections of dorsal area dentate made immediately after septal lesion result in no observable LC labeling. Similar injections 16 weeks after septal lesion produce labeling in the dorsal LC but fewer HRP-positive neurons are seen than in normal animals. If large injections are made in dorsal area dentata such that the spread of enzyme includes the mid-septotemporal region, LC labeling is similar to that obtained from ventral injections even though the septum has been lesioned. Septal lesions presumably remove LC fibers destined for both Fx and Cb and produce an approximately 50% reduction in NE levels in both dorsal and ventral hippocampal formation. Lesion of Cb alone, results in negligable NE depletion dorsally however a significant decrement is seen in the ventral hippocampal formation.

The present anatomical and biochemical observations suggest that: 1) LC fibers in VAB project throughout the hippocampus with few terminals in the septal third, 2) Fx NE fibers mainly innervate the septal third, 3) Cb provides a major NE input to temporal hippocampus. Moreover the observation of the return of LC labeling from dorsal area dentata 16 weeks after septal lesion implies the expansion of LC axons from VAB into the denervated region.

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113.7 SURVIVAL OF NONENZYMATICALLY DISSOCIATED RAT AND MONKEY ADRENAL MEDULLARY CELLS IMPLANTED INTO ADULT RAT BRAIN. M.R. Wells and U. Patel. Neurochemical Research Laboratory, Veterans Administration, Washington, D.C. 20422 and N.I.M.H., St. Elizabeth's Hospital, Washington, D.C. 20023.

The implantation of adrenal medullary tissue into the ventricles of ipsilaterally substatia nigra lesioned rats results in survival of the implant and alterations in lesion induced rotational behavior (Freed et al. Nature 292;351, 1981). In order to optimize contact of the catecholamine producing implant with the deafferented caudate nucleus, we have developed a non-enzymatic dissociation technique for adrenal medullary cells of rats and monkeys which allows them to be injected directly into the caudate. Dissected adrenal medullary tissue from adult rats and monkeys were placed in culture medium (Gibco 199) containing 20% fetal calf serum and gently teased through a 250 μ pore nylon mesh. This procedure alone was sufficient to produce viable dissociated cells either as single cells or small aggregates of two or three cells. After dissociation over 90% of the cells could exclude trypan blue (0.1% in medium). Cells were injected into the caudate nucleus of ipsilaterally substantia nigra lesioned adult host rats (2-4 μL Approx. 160-300,000 cells). Both rat and monkey adrenal medullary cells survived for over 14 days in the host brain and showed rapid formation of cell processes. The mechanical dissociation of adrenal medullary cells is a rapid method for obtaining viable cells for implantation. Supported by the Veterans Administration and N.I.M.H.

INVERTEBRATE MOTOR FUNCTION

114.1 IDENTIFICATION OF WIND SENSITIVE INTERGANGLIONIC INTERNEURONS IN THE COCKROACH. R. E. Ritzmann and A. J. Pollack*. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

Interganglionic interneurons are now thought to play an important role in the pattern generation circuitry responsible for flight in locust (Robertson and Pearson, J. Comp. Physiol., 146: 311-320, 1982) and intersegmental coordination in the walking system of the cockroach, Periplaneta americana (Pearson and Iles, J. Exp. Biol., 58:725-744, 1973). In the cockroach both walking and flying can be generated by similar wind stimuli directed at the cerci (Antenna-like sensory structures on the rear of the abdomen). Moreover, a set of identifiable interneurons, the dorsal giant interneurons (dGI's) are capable of initiating either behavior (Ritzmann, et. al., J. Comp. Physiol., 147:313-322, 1982). The choice between walking and running is determined by the presence or absence of leg contact with a substrate. We are interested in determining whether components of these two systems other than the dGI's are also shared. For example, there may be interneurons that serve to coordinate activities between thoracic ganglia in both behaviors.

As an initial part of this study, we have identified over 40 wind-sensitive interganglionic interneurons. Our procedure involves recording intracellularly from axons in the meso-metathoracic connectives and subsequently dye-filling with Lucifer Yellow CH. Identification is based upon three parameters: (1) whole-mount morphology, (2) the tract (Gregory, Phil. Trans. R. Soc. Lond., (B), 267:421-465, 1974) in which the main axon passes (identified in cross-sections of thoracic ganglia), and (3) the response to wind in a flying preparation. For the interneurons we have identified, the last parameter can be further divided into three categories: type 1-excited by wind and/or flight in an unpatterned manner, type 2-excited during flight in a burst pattern in phase with either wing elevator or depressor motor neurons, and type 3-spontaneously active but inhibited during flight. Most of the type 2 interneurons are located in the dorsal lateral tract; an area where interneurons associated with interganglionic coordination have previously been located.

We have also begun to determine connections that are made with these interneurons in the thoracic ganglia. At least one type 1 interneuron appears to be excited monosynaptically by a dGI (GI-7).

We plan to repeat these observations in preparations that make leg contact and are, therefore, capable of eliciting walking motor outputs. These studies will determine how the activity in these interneurons changes with leg contact.

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114.2 Spiking local interneurons are primary integrators of mechanosensory signals in the locust. M. Burrows and M.V.S. Siegler*, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, England.

Two types of local interneurons in the thoracic ganglia of the locust can be distinguished on their ability to spike. One, unable to spike (local non-spiking interneurons), controls sets of leg motor neurons in a graded fashion. The other, able to spike (local spiking interneurons), is involved in the control of local reflexes. Some synapse directly upon motor neurons. A major role for these spiking interneurons is to integrate the information provided by the huge number of receptors on a leg; about 10,000 afferents from a hind leg converge upon some 100 local spiking interneurons. The afferents from external mechanoreceptors synapse directly upon the local spiking interneurons but not upon the non-spiking ones, or upon motor neurons. A spike from a singly-innervated hair is followed at a constant latency (1-2ms) by a chemically-mediated EPSP in a local spiking interneuron. Each interneuron is excited by a specific and restricted array of sensory recptors; for example, some are excited by receptors on the anterior surface of a leg, others by receptors on the posterior surface. Each interneuron has two distinct areas of branches within a ganglion; a ventral area of fine and numerous branches that overlaps with the central projections of the mechanoreceptor afferents, and a dorsal area of sparse and varicose branches that overlaps with those of the leg motor neurons. We infer that the spiking interneurons are involved in two types of local pathways 1) sensory neuron, spiking local interneuron, motor neuron, and 2) sensory neuron, spiking local interneuron, non-spiking local interneuron, motor neuron.

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114.4 SYNAPTIC INTERACTIONS BETWEEN FLEXION-PRODUCING INTERNEURONS IN CRAYFISH. J. Jellies and J.L. Larimer, Department of Zoology, University of Texas, Austin, TX, 78712.

Abdominal posture is produced by segmentally arranged antagonistic muscles in crayfish. Extracellular analysis revealed the presence of "command fibers" (CF's) in the abdominal nerve cord which produce coordinated postures, including a large number of flexion-producing CF's (Evoy and Kennedy, J. Exp. Zool., 165:223, 1967). By combining extracellular stimulation of CF's with intracellular recording, Miall and Larimer (J. Comp. Physiol., 148:159, 1982) discovered that many flexion-producing interneurons received input from the CF's. However, extracellular stimulation of CF's in axonal bundles raises the possibility of a synaptic response contaminated by other neurons, thus making interpretation difficult.

We have succeeded in making simultaneous intracellular, neuropilar microelectrode penetrations in pairs of flexion-producing interneurons. We use high impedance, Lucifer Yellow filled micropipettes (100-400 MΩ) to impale interneurons in the third and fifth ganglia in an isolated abdominal nerve cord of Procambarus clarkii. An extended bridge balance in the preamplifiers allows us to interact pairs of interneurons and view their responses.

Just over half of the pairs of flexion-producing interneurons encountered so far show no evidence of interaction. Of the remaining pairs there are examples where both apparent mono- and polysynaptic interactions are evident. In a smaller number, one flexion-producing interneuron recruited another to spiking and in many pairs synaptic interaction is not obvious but is indicated since one flexion-producing interneuron will influence the firing frequency of the other.

Two general features of all interactions are; when an interneuron is recruited it is activated at a low gain, and, interactions between every pair of interneurons we have studied in these two ganglia are "one-way" interactions. Thus, low gain recruitment and the absence of strong mutual interactions might allow the animal to activate several elements in this system of pre-motor interneurons to produce selected abdominal positions without sacrificing fine control to positive feedback.

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114.5 FUNCTIONAL PROJECTIONS OF ABDOMINAL POSITIONING INTERNEURONS THROUGHOUT THE CNS OF THE CRAYFISH. D. Moore* and J.L. Larimer, Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

Recent evidence (Larimer, J.L. and Jellies, J., J. Exp. Zool., in press, 1983) suggested that many abdominal flexion-evoking interneurons in the crayfish Procambarus clarkii send their axonal projections anteriorly into the thoracic nerve cord. In an effort to determine the organizational relationships between flexion-evoking interneurons found in the abdominal ganglia and those which exist in the circumesophageal connectives (CECs), a semi-intact preparation was used. The ventral nerve cord was dissected from the abdominal region, but left attached to the anterior CNS. Positioning interneurons were found with Lucifer-filled microelectrodes (50-300 MΩ) in the neuropile of abdominal ganglia 2 through 6. Motor outputs evoked by intracellular stimulation of these cells were determined with extracellular recording electrodes placed on flexor and extensor roots along the cord.

A 100 Hz, 100 msec pulse train was delivered separately to each CEC through wire hook stimulating electrodes. The responses of the impaled cells to CEC stimulation could be placed into 4 general categories: (1) Of the total of 82 flexion-evoking interneurons examined, 39% projected directly through the CECs as evidenced by phase-locked spikes occurring one-to-one with the stimulation paradigm. Abdominal flexion-evoking interneurons which course through the CECs may comprise the primary neural elements underlying voluntary control of posture. These "through" fibers apparently do not decussate as they project through the ventral nerve cord to the brain. (2) Some flexion interneurons (13%) were remotely activated to spiking by CEC stimulation, failing to follow the 100 Hz pulse train one-to-one with constant spike latency. In these cases, higher order CEC or intermediary fibers presumably make synaptic connections onto the interneurons at sites distant from the ganglion in which the interneurons were impaled. (3) Approximately 34% of the flexion interneurons were neither directly nor remotely activated, but received strong EPSPs or IPSPs as a result of CEC stimulation. These locally activated interneurons may represent lower order elements for the control of abdominal movements. (4) Approximately 15% of the flexion interneurons were completely independent of CEC stimulation, perhaps reflecting a role largely limited to reflexive control of posture.

The placement of flexion-evoking interneurons into separate categories based on the functional relationships with the anterior nervous system suggests several different levels of neural control for the simple abdominal positioning behavior. (Supported by PHS grant NS 05423).

114.6 THE INFLUENCE OF MOTOR NEURON OUTPUT ON CONTRACTION AND WORK IN VENTILATORY MUSCLES OF THE SHORE CRAB. A. J. Mercier and J. L. Wilkens. Dept. of Biol., Univ. of Calgary, Calgary, Alberta, T2N 1N4, Canada, and Marine Biol. Lab., Woods Hole, MA, 02543.

Gill ventilation in the crab, Carcinus maenas, is achieved by the rhythmic, stereotyped movement of the gill bailer (second maxilla), which pumps water through the gill chambers. As the rate of ventilatory movement increases, the gill bailer works against an elevated gradient of hydrostatic pressure, resulting in an increased load on the ventilatory muscles (Mercier and Wilkens, Amer. Zool. 21:Abst. 23, 1981). Since these muscles receive polyneuronal innervation and possess highly facilitating synapses, it has been proposed that changes in motor neuron recruitment and intraburst impulse frequency compensate for such changes in load by increasing the force and work generated by the muscles (Moody-Corbett and Pasztor, J. Neurobiol. 11:21-30, 1980). Our analyses of electromyographic recordings as well as recordings from a small nerve branch innervating an individual muscle reveal that as the rate of bailer movement increases: (a) the intraburst impulse frequency increases significantly (40 to 120 Hz), and (b) the time interval between bursts decreases notably (2000 to 250 msec). The number of impulses per burst is variable (5 to 30) but does not correlate consistently with ventilation rate.

In order to demonstrate that these changes in patterned output can compensate for increases in load, nerve-evoked contractions of single ventilatory muscles were recorded isotonically at various loads. The motor neurons were given bursts of stimuli resembling the frequency and number of impulses of recorded patterns. Within the physiological range, (a) increasing the stimulus frequency or (b) decreasing the interburst interval increased both contraction and work and also increased the load which could be lifted by the muscle. In each case, muscle EJP's, recorded with floating, intracellular electrodes, were enhanced by the change in stimulus. The effect of increasing stimulus frequency was due mainly to summation of the EJP's, while that of decreasing the interburst interval appeared to result from increased facilitation from one burst to the next. Contraction and work were also enhanced by increasing either the number of stimuli per burst or the number of axons recruited. These treatments also enhanced muscle EJP's. The results support the hypothesis that appropriate changes in motor neuron output compensate for the increased load on the gill bailer muscles at high ventilation rates.

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114.7 INNERVATION OF AUTOTOMY MUSCLES IN THE CRAB CARCINUS MAENAS: LEVATOR UNITS AND A COMMON UNIT SERVING COXAL AND BASAL MUSCLES. Daniel P. Yox* and Stacia Moffett, Dept. of Biological Sciences, SUNY, Buffalo, New York 14260 and Dept. of Zoology, Washington State University, Pullman, WA 99164.

The innervation of muscles in proximal segments of crustacean limbs has not received the intensive study accorded the more distal segments. Our interest in crab basi-ischiopodite levator muscle control relates to the dual role of these two muscles in routine limb elevation and autotomy (Moffett: 1975, J. Comp. Physiol. 96, 285).

Nerve trunk recordings and electrical stimulation showed that three axons innervate the smaller, more posterior levator muscle (PL). The two units which generate the largest and intermediate-sized action potentials exit the ganglion through the RLD (remoter-levator-depressor) nerve bundle and the third small-spike unit joins the RLD nerve through an anastomosis from the CB (coxa-basal) nerve. The basi-ischiopodite depressor, coxal promotor, anterior remotor and the anterior levator are also innervated by this common unit. It produces very small, depolarizing ejp's, but the possibility that it is a common inhibitor has not been ruled out.

The larger anterior levator muscle (AL) consists of three heads. The deep anterior head (AL_a) and the more superficial posterior head (AL_p) originate in the thorax, whereas the small coxal head (AL_c) originates in the coxa close to the origins of some PL fibers.

The nerves that innervate AL_a and AL_p branch separately from the CB nerve trunk and AL_c receives its innervation via a short nerve branch which emerges from AL_p. We were interested in determining if all heads of AL receive the same innervation. We found that AL_c and AL_p are innervated by the same six motoneurons. Dual muscle fiber recordings (Yox and Moffett: 1982, Am. Zool. 52, 950) support the extracellular nerve recording data in showing that four of the six neurons that innervate AL_p are shared with AL_a (one being the common unit) and AL_a has at least one additional unit (with medium-sized spike height) that does not innervate AL_p. Thus there is a limited potential for independent action by the two major muscle heads that could play a role in production or prevention of autotomy. The shared and unshared units have been differentially stained by backfilling one nerve with $NiCl_2$ and the other with $CoCl_2$ and developing the stains with rubeanic acid (Quicke and Brace: 1979, J. Microsc. 115, 161).

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114.8 REFLEX ENHANCEMENT OF MUSCLE STIFFNESS IN HERMIT CRAB ABDOMEN. William D. Chapple. Physiology Section, Biological Sciences Group, University of Connecticut, Storrs, CT. 06268

The longitudinal muscle layer of the ventral superficial muscles (VSM) is the major group of muscle fibers in the abdomen of the hermit crab, Pagurus pollicarus, that is involved in the support of the shell above the substrate. Longitudinal stretch of the posterior abdomen or abduction of the uropods elicits a phasic reflex discharge in the three excitor motoneurons that innervate the muscle in each segment on each side. To determine what muscle variable is controlled in this system, the right VSM and first and third roots of the fourth abdominal segment together with the abdominal nerve cord were isolated. The muscle was mounted in an oxygenated temperature controlled bath between a force transducer and a computer controlled muscle stretcher. It was subjected to ramp stretches of different amplitudes and velocities. Individual motoneuron frequencies were recorded with a floating electrode and interspike intervals, muscle force, and muscle length were stored for off line analysis. Tonic frequency of the motoneurons was not correlated with muscle length. Motoneuron frequency was a function of stretch velocity and the initial length of the muscle, but, above a threshold value, not of stretch amplitude. Static transverse stretch of the underlying cuticle increased frequency during stretch, and transverse stretch of the cuticle was as effective as longitudinal stretch in eliciting the reflex. Thus, the reflex discharge during stretch is a function of the rate of cuticular stress. Since abolition of the reflex with 10^{-3}M atropine sulphate (which blocks acetylcholine, the sensory transmitter) reduces muscle stiffness during stretch to about one half control values, the reflex is effective in increasing muscle stiffness. The reflex appears to compensate for muscle yield but does not control or regulate muscle stiffness.

114.9 LATERALIZATION OF THE NERVOUS SYSTEM TO THE PAIRED ASYMMETRIC CHELIPEDS OF MALE FIDDLER CRABS. C.K. Govind and R.E. Young. Boston Univ. Marine Pgm., Marine Biol. Lab., Woods Hole, MA. 02543

The paired chelipeds in the adult male fiddler crab, Uca pugnax, are asymmetric consisting of a major cheliped which is used for defence and courtship and is about 30X larger than the minor cheliped which is used for cleaning; grooming and feeding. Correspondingly the 1st thoracic ganglion and its nerve root (which serve the chelipeds) is much larger on the side of the major cheliped than on the side of the minor cheliped. (In adult female fiddler crabs the paired chelipeds are symmetrical and of the minor type and there is a corresponding symmetry of their hemiganglia and nerve roots). The asymmetry in size of the hemiganglia in male fiddler crabs was explored by cobalt backfilling of their motoneurons which revealed a constancy in the number and distribution of the somata on the two sides but a significant hypertrophy of the somata and dendritic fields on the major side compared to the minor side. The somata on the major side were approximately 50% larger than their counterparts on the minor side. The asymmetry in the size of the paired nerve roots was explored by sampling the number and diameters of axon profiles from cross-sectional montages of the entire nerve roots which revealed that the major root was 2.3X larger in surface area than the minor root. In random samples, scaled accordingly, the major side had a significantly greater (3.7X) number of axon profiles than the minor side. Conversely in random but equal area (unscaled) samples there was no significant difference in axonal numbers between major and minor sides suggesting a similarity in axonal size between the two sides. This was confirmed directly by measuring axonal diameters which gave almost identical size distribution histograms for major and minor nerve roots. (In both roots the majority (70%) of the axons were less than 1 μm in diameter). Since the number of motoneurons are bilaterally constant in crustacean ganglia, the greater size of the major nerve root must be due to an increase in the number of sensory axons. Neural asymmetry in male fiddler crabs therefore involves hypertrophy of the motoneuron somata in the hemiganglion and hyperplasia of sensory neurons in the nerve root associated with the major cheliped. This demonstration of lateralization in specified neuronal types is a step towards unravelling its ontogeny in the male fiddler crab which displays one of the most flamboyant examples of cheliped asymmetry in crustaceans.

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114.10 CONSERVATION AND CHANGE IN A DECAPOD CRUSTACEAN GANGLION DURING EVOLUTION. D. H. Paul, A. M. Then* and D. S. Magnuson*. Biology Department, University of Victoria, Victoria, B.C. V8W 2Y2.

Our research concerns identification of the sites of evolutionary change in the nervous system that accompanied the acquisition of a new locomotory behavior in the Hippid sand crab Emerita (Crustacea, Anomura). Emerita's use of only the uropods for swimming appears to have evolved from Macruran tailflipping, a behavior in which the entire abdomen is alternately extended and flexed (Paul, J.exp.Biol. 94, 159). The significant differences between Emerita and Macrurans with respect to the different modes of swimming are in the tailfan (TF) and terminal abdominal ganglion (G6). Based on comparisons of the functional morphology of TF neuromusculature in sand crabs and crayfish (a Macruran), muscles in sand crabs have been categorized as 1) conserved, with conserved function, 2) conserved, with altered function and 3) new, with no Macruran homolog (Paul, ibid., 169 and unpublished). We have focussed our attention on a conserved muscle with new function in Emerita that is innervated by the 6th root of G6; in crayfish this root contains motor neurons (MN) that are part of the set of serially homologous fast flexor MNs mediating the power stroke of the tailflip (Dumont and Wine, pers. com.). Preliminary data, tabulated as percentage of neurons in Emerita compared to crayfish, reveal that there are fewer 6th root MNs in Emerita, but that this does not reflect a uniform reduction in numbers of all kinds of neurons in G6.

	G6 neurons	Projection interneurons	MNs, root 7 excluded	MNs in 6th root
Emerita	0.50±.05	0.50	0.75	0.38
crayfish*				

*crayfish data from Reichert et al., J. comp. Physiol. 149, 145

To control for the possibility that some of the differences between G6 of Emerita and crayfish may not be specifically related to the newly evolved function of the TF in Hippids, we are examining the TF and G6 in two tailflipping Anomurans, Munida and Calliannassa. Of interest in each animal are 1) the total number of neurons and the numbers of MNs, projection interneurons and local interneurons in G6, and 2) characteristics of the individual MNs in the 6th root (their central morphology and peripheral distribution and physiology). Comparison of these data from the different genera should reveal to what extent ganglionic architecture and cell numbers are conserved in tailflipping Anomurans, and thus allow recognition of which of the differences between Emerita and crayfish are specifically associated with new functions of the muscles in the TF of Emerita.

Supported by a grant from N.S.E.R.C. of Canada.

114.11 SEGMENTAL HOMOLOGIES IN THE FAST FLEXOR MOTOR SYSTEM IN THE ABDOMEN OF THE CRAYFISH. J.P.C. Dumont* and J.J. Wine (SPON: T. Kilduff). Depts. of Biol. and Psychol., Stanford Univ., Stanford, CA 94305.

Insights into the evolution of neural circuits can be gained by comparing serially homologous ganglia in a segmental animal such as the crayfish. In the abdomen, identified motor neurons innervate the powerful fast flexor muscles used during swimming. The motor neurons in turn receive input from both intra- and intersegmental interneurons, e.g. the Lateral and Medial giants. This pattern is repeated with minor variation through the anterior five segments of the abdomen (Mittenthal, J. and Wine, J., J. comp. Neurol., 177:311-334, 1978). We have extended the study of this system into the sixth segment, the tailfan (telson and uropods), which differs from the anterior segments in that it is capable of a more complex range of movements and is thought to be the fused product of two segments.

We have now identified, by morphology and physiology, all eleven of the motor neurons innervating the fast flexor muscles of the telson, as well as three premotor interneurons. All but one of the motor neurons can be shown (with varying degrees of confidence) to be homologous with motor neurons in anterior ganglia. In particular, two highly idiosyncratic motor neurons have been identified as homologues of Motor Giants on the basis of their limited dendritic branching, soma size and position, axon diameter, input from identified interneurons and size and plasticity of EJP. Since only one pair of Motor Giants exists in each anterior ganglion, their doubling, and the doubling of other similarly identifiable neurons, adds to the evidence that this ganglion was formed by fusion.

The pattern of connections between the premotor interneurons and the other motor neurons shows much more differentiation in the terminal ganglion than in the anterior ganglia. In addition to the previously documented differences between LG and MG (Larimer, J. and Kennedy, D., J. exp. Biol., 51:119-133, 1969; Kramer, A., Krasne, F. and Wine, J., J. Neurophysiol., 45, 550-574, 1981), we find that within the set of motor neurons that are excited by the MGs some are excited at short latency while others are not, and only the directly excited cells produce suprathreshold EJPs in the telson flexor muscles.

Supported by NSF grant BNS 81-12431 (J.J.W.).

115.1 EFFECTS OF INSULIN DEPLETION AND REPLETION UPON INSULIN RECEPTOR BINDING IN DISCRETE CNS REGIONS. D.J.Steel*, R.J.Waldbillig*, and M.S.Kappy* (SPON: G. Hope). Depts. of Psychology and Pediatrics, Univ. of Florida, Gainesville, FL 32611.

Recently much attention has been paid to the action of insulin in the brain. Early reports indicated that the CNS insulin binding system was independent of pancreatic insulin, because CNS insulin binding did not change in streptozotocin-induced diabetes. However, the earlier analysis was performed on whole brain homogenate, and more recent work in this laboratory, using a microdissection technique, revealed that streptozotocin treatment produces regionally specific changes in insulin binding. More specifically it was demonstrated that streptozotocin-induced diabetes leads to an up-regulation of insulin binding in circumventricular brain areas (medial hypothalamus, MH and area postrema, AP) and a down-regulation of binding in the olfactory bulb (OLF). In the lateral hypothalamus (LH) specific binding of I^{125} insulin was unaffected by diabetes.

The purpose of the present study was to determine whether the diabetes-induced changes in specific binding were the result of changes in binding site capacity or affinity. A Scatchard analysis was conducted by incubating brain parts (MH, OLF, AP, LH) from control and streptozotocin-treated (65 mg/kg, I.V.) diabetic male Long-Evans rats with I^{125} porcine insulin and various concentrations of unlabelled porcine insulin. The results of this work replicated our earlier findings and indicated that streptozotocin treatment induces changes in both I^{125} insulin binding site number and affinity. However, these changes are site specific with some brain areas showing changes in binding affinity and not number while other sites changed in both binding site number and affinity.

A second study was conducted to determine whether the changes in specific binding produced by streptozotocin-induced diabetes were reversible with insulin therapy. Diabetic animals were injected with long-acting (PZI) porcine insulin (6 U/kg) and plasma glucose levels were measured. Approximately 6 hours following the insulin injection, animals had recovered from a hypoglycemia, and plasma glucose levels were in the normal range. At this time animals were sacrificed by decapitation. I^{125} insulin binding in the above brain regions was compared to the binding in untreated diabetic and normal control animals. Preliminary results from this work indicate that 6 hours exposure to plasma insulin is sufficient to normalize diabetes-induced changes in CNS insulin binding. The findings presented above indicate that plasma insulin levels are an important determinant of insulin binding in areas both with and without direct exposure to blood-borne insulin.

115.2 MODULATION OF HEPATIC GLUCOSE PRODUCTION BY INSULIN-SENSITIVE NEURONS IN THE AREA POSTREMA. R.J.Waldbillig* and D.J.Steel* (SPON: W.Dawson). Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

It is now known that in the area of the ventromedial hypothalamus blood-borne insulin crosses the vascular wall and suppresses neural activity. Work from our laboratory has shown that in food-deprived rats low-dose (100μU) insulin infusions into this area produce increases in food intake and reductions in plasma glucose (Weider and Waldbillig, Soc. Neurosci. Abst., 8:144, 1982). Because the hypoglycemic effect of these infusions can be blocked by selective hepatic vagotomies, it was suggested the autonomic influences on hepatic glucose output may be modulated by the binding of blood-borne insulin to neurons in the VMH.

More recently it has been demonstrated that pancreatic insulin also binds in the area postrema (van Houten and Posner, Endocrinology, 109:853, 1981). The work presented here investigated the possibility that this region is also involved in modulation of parasympathetic control over hepatic glucose output. Eighteen hour food-deprived, male Long-Evans rats were anesthesized and infused (1μl) in the area postrema with porcine insulin (100μU) or vehicle. Blood samples for plasma glucose determination were collected from the hepatic vein before and 2,5,10,15 and 30 minutes after the infusion.

The results of this work indicated that insulin infusions into the area postrema produced a rapid (latency < 2 min) and long-lasting (duration > 30 min) decrease in plasma glucose levels. This data in conjunction with our previous report indicates that there are multiple sites at which pancreatic insulin can influence plasma glucose levels, by altering the activity in the circumventricular-autonomic axis.

To further examine the conditions under which hepatic glucose output can be modulated by insulin in the area postrema, 12 male Long-Evans rats were tested in the absence of food deprivation. Under these conditions, area postrema insulin infusions were ineffective in changing hepatic vein glucose concentration. The finding that food deprivation is a prerequisite for the hypoglycemia suggests that metabolic state may be an important determinant of insulin's effect in the brain. We suggest that the importance of food deprivation arises from the fact that it places the liver in a glucose-production mode, which is then suppressed by infusions of insulin into the area postrema.

In our current work we are using selective hepatic vagotomies to investigate the role of the parasympathetic nervous system in the circumventricular-hepatic interaction.

115.3 BEHAVIORAL ANALYSIS OF CHOLECYSTOKININ-DOPAMINE INTERACTIONS IN THE NUCLEUS ACCUMBENS, L. K. Blumstein* and J. N. Crawley (SPON: J. Carnahan). Neurobiology Program, E. I. Du Pont Co., Glenolden, PA 19036 and National Institute of Mental Health, Bethesda, MD 20205.

Co-localization of cholecystokinin and dopamine in mammalian brain has been extensively characterized using immunohistological and electrophysiological techniques (Skirboll, et al., Neurosci., 6:2111, 1981). A large percentage of neurons with CCK-DA co-localizations were described in the ventral tegmental area, which contains projections to the medial nucleus accumbens. This dopaminergic pathway has been implicated in several behavioral paradigms, including apomorphine-induced stereotypy and locomotor hypoactivity. The following study was designed to test the hypothesis that CCK modulates the behavioral actions of dopaminergic agents in this region of the nucleus accumbens.

Male Sprague-Dawley rats, 200-250 g, were implanted with 24 gauge stainless steel hypodermic tubing bilaterally at 3.4 mm anterior, 1.2 mm lateral and 5.7 mm ventral to bregma. One week later, behavioral testing began. Animals were first pretreated with either saline or apomorphine 0.1, 0.2 or 0.4 mg/kg i.p., doses which are active postsynaptically. Ten minutes later, saline or CCK_8 (Bachem, Torrance, CA) 20 pg-200 ng was administered bilaterally through a 31 gauge injection tube 0.5 mm below the ventral tip of the guide cannulae. They were injected, with a Sage infusion pump, in a volume of 0.2 μl over a period of 1 minute. Stereotyped behavior was recorded every 60 seconds over the next 15 minutes using a standard scoring system.

CCK_8 20 pg-2 ng into the n. accumbens significantly increased the stereotypy score for systemic apomorphine 0.2 mg/kg. Furthermore, CCK_8 shifted the dose-response curve for apomorphine to the left. CCK alone did not induce stereotypy at doses ranging from 20 pg to 200 ng. These results suggest that CCK potentiates dopamine-mediated behaviors at sites of putative co-existence in the n. accumbens. Further studies on the role of CCK in modulating hypoactivity, induced by a low dose of apomorphine active at the presynaptic dopamine autoreceptor, are in progress.

115.4 STRUCTURE-ACTIVITY ANALYSIS OF CHOLECYSTOKININ FRAGMENTS ON SATIETY-RELATED EXPLORATORY BEHAVIORS. J. N. Crawley, S. St-Pierre, and P. Gaudreau. Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20205, and Dept. Pharmacol., Univ. Sherbrooke, Sherbrooke, Canada.

Cholecystokinin octapeptide-sulfate (CCK_8) binds to a peripheral site to stimulate gall bladder contraction, the release of pancreatic amylase, and to initiate a sequence of behavioral events analogous to an underlying state of satiety[1,2,3]. Autoradiographic and receptor binding studies suggest that smaller CCK peptide sequences may possess many of the properties of the octapeptide[4,5]. To identify the peptide sequence necessary for initiation of the satiety-related behavioral actions of CCK_8, a series of C-terminal and N-terminal fragments were tested in a previously-described exploratory paradigm specific for CCK_8[3].

Male Swiss-Webster mice were administered N-terminal $CCK_{,7,6,5,4,3,2}$ and C-terminal $CCK_{,7,6,5,4,3,2}$ in 0.9% saline, intraperitoneally, immediately before behavioral testing, in doses from 10^{-7} to 10^{-3} moles/kg. Number of investigatory approaches to a novel object, number of pauses of behavioral inactivity, and total pause duration over a five-minute session were recorded. The seven amino acid peptide was active only at much higher concentrations than CCK_8, with smaller fragments showing minimal activity on this behavioral paradigm. There was no evidence for antagonist activity by any of the fragments tested. Addition of a tert-butyl-oxycarbonyl (Boc) group to the N-terminal reduced the potency of CCK_8 one-hundred fold. Unsulfated fragments were inactive at doses as high as 10^{-3} moles/kg.

Comparison of the mouse exploratory behavior bioassay with the pancreatic amylase secretion bioassay reveals major differences in the biological activity of CCK fragments. It is interesting to speculate that the behavioral actions of CCK are mediated through a separate receptor subtype, e.g., the CCK receptors recently localized on the vagus nerve.

1) Gardner et al., First International Symposium on Hormonal Receptors in Digestive Tract Physiology, 1977.
2) Smith and Gibbs, Pharmacol. Biochem. and Behav, 3:1:135-138, 1975
3) Crawley et al., Peptides 3:535-538, 1982
4) Gaudreau et al., Eur. J. Pharmacol., 87:173-174, 1983
5) Innis and Snyder, Eur. J. Pharmacol. 65:123-124, 1980
6) Zarbin et al., Life Sci. 29:697-705, 1981

115.5 EFFECTS OF SINGLE AND REPEATED ELECTROCONVULSIVE SHOCK (ECS) ON REGIONAL BRAIN THYROTROPIN-RELEASING HORMONE (TRH) AND NEUROTENSIN (NT). A. Sattin, J.L. Meyerhoff, G.P. Mueller and M.J. Kubek. Depts. of Psychiat. and Anat., Indiana U. Sch. Med. and VAMC, Indianapolis, IN 46223; Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, DC 20012; Dept. Physiol. Unif. Ser. U. Health Sciences, Bethesda, MD 20814.

We have previously observed large increases of TRH in amygdala-pyriform and hippocampus two days after five alternate-day ECS. In contrast, no effect was seen in subconvulsively shocked compared to sham ECS rats (Neurosci. Abst. 8: 982, 1982). In order to examine the onset of this effect we have now assayed TRH in brain regions following 1, 3 and 5 ECS given on alternate days. Since NT may act physiologically opposite to some effects of TRH we assayed this peptide in similar regions following 5 daily ECS. Tonic-clonic ECS was induced in male S-D rats (150-180g) with 35-60 millicoulombs (a.c.). Sham ECS rats received identical handling but no current. All rats were decapitated 48± 3 hrs after last treatment. Brains were removed immediately, dissected, weighed and frozen on solid CO_2. TRH and NT were assayed by specific RIA following HAc extraction and results expressed as pg/mg tissue (mean ± SEM). Student t-tests (2-tailed) were performed following log transformation of original data. No changes in TRH content were observed following a single ECS and none were seen in septum or s. nigra following 3 or 5 ECS. Significant increases following 3 ECS were seen in pyriform cortex (PFM; 13.1±4.9 vs. 29.2±6.8, $p < 0.005$), amygdala (AY; 34.1±3.9 vs. 47.2±3.1, $p < 0.01$), hippocampus (HC; 9.0±1.4 vs. 18.3±1.2, $p < 0.001$) and hypothalamus (HY; 212.2±7.9 vs. 243.0±6.6, $p < 0.01$). Similar results were observed following 5 ECS except for HY where no significant change was seen as reported previously. Five daily ECS had no significant effect on NT in all regions examined. These results are clinically significant since the antidepressant effect of electroconvulsive treatment (ECT) requires repetitive application and is rarely seen after a single treatment. Furthermore, during ECT significant psychobehavioral improvement is usually seen following 3 to 5 alternate-day treatments. Our failure to detect any prolonged alteration in NT, another behaviorally active peptide, following repeated ECS implies some degree of specificity for TRH as an indicator of functional change induced by ECS. The brain regions demonstrated significant ECS-induced change in TRH are all believed to be involved in affective function and affective disorders. The importance of TRH in PFM and AY is further enhanced by our finding of highest TRH degradation there (T.J. Cavanagh et al., this meeting), the presence of appreciable amounts of TRH, and reported high content of TRH receptors in these regions (J. Neurochem. 38: 1649, 1982). (AM28260 and VA Res. Serv.).

115.6 COMPARISON OF THE PHARMACOLOGICAL EFFECTS OF THYROTROPIN-RELEASING HORMONE (TRH) AND BICUCULLINE. G. R. Breese, T. McCown, G.D. Frye, and R. A. Mueller. Biological Sciences Research Center and Center for Alcohol Studies, UNC School of Medicine, Chapel Hill, NC 27514.

Previous studies have shown that both TRH and bicuculline will antagonize ethanol-induced depression when introduced into brain (Cott, et. al., J.P.E.T., 196:594, 1976; Frye, et. al., J.P.E.T., 233:750, 1982). Based upon observations that GABA-mimetics reduce the effects of TRH against ethanol-induced sleep, Cott and Engel (Psychopharmacology 52:145, 1977) proposed that the effects of TRH were due to inhibition of GABA function. However, the fact that intracisternal administration of the GABA antagonist, bicuculline, results in seizures whereas TRH treatment does not, suggests a difference in their basic mechanism of action. In order to examine the possibility that TRH might antagonize GABA-ergic transmission, TRH and bicuculline were microinjected into two brain sites where bicuculline produces distinctive behaviors not associated with seizures, as well as into a site where bicuculline induces convulsive activity. When injected into the medial septum, bicuculline methiodide (0.45 to 1.9 µg) increased locomotor activity, induced hyperthermia and produced a short duration (30 minute) antagonism of ethanol-induced depression of locomotion. No seizures were observed at these doses of bicuculline. In contrast, microinjection of a dose of TRH (0.5 µg) into the medial septum which antagonized ethanol-induced locomotor ataxia did not alter locomotor activity or body temperature in ethanol-naive rats. Stereotyped behaviors (head bobbing, sniffing, gnawing) and wild running observed when bicuculline (0.6 µg) was bilaterally microinjected into the substantia nigra were not observed when TRH (1 µg) was administered into this site. Bicuculline (10 ng) produced seizure activity when bilaterally injected into the inferior colliculus (see Frye, et. al., Neuroscience Abstracts, 1983), but again behavior was not affected when TRH (1 µg) was administered into this site.

These data are consistent with the view that the medial septum is a critical brain site for the analeptic action of TRH (Kalivas and Horita, J.P.E.T., 212:203, 1980) and may also be an important site of action for bicuculline to antagonize ethanol-induced depression of CNS function. However, differences in the pharmacological profile of bicuculline and TRH suggests that TRH probably does not inhibit GABA function to produce its analeptic action against ethanol. (Supported by USPHS grants AA-02334, AA-05713, NS-17509, and HD-03110 and grants from the N.C. Alcoholism Research Authority.)

115.7 EFFECTS OF CORTICOTROPIN RELEASING FACTOR (CRF) AND GROWTH HORMONE RELEASING FACTOR (GRF) ON PATTERNS OF LOCOMOTION AND BRAIN MONOAMINES IN RATS. E.H.Y. Lee*, M.A. Geyer and A.J. Mandell, Dept. of Psychiatry, Univ. of California at San Diego, La Jolla CA 92093

CRF produces electrophysiological and behavioral activation in rats (Ehlers, C., et al., 1982) in contrast to GRF, which produces sedation. Analysis of their amino acid sequences reveals that CRF exposes more hydrophobic groups to the membrane solvent, with the expectation of higher instability (Davidson & Fasman, 1967), while GRF manifests less exposed hydrophobicity and more potential stability. To test the hypothesis that CRF and GRF would likewise produce opposite effects on the variability of behavior (Mandell, A.J., Int. Rev. Neurobiol. 24, 1983), spatial patterns of rat locomotion were examined using a behavioral pattern monitor.

Sixteen male Sprague-Dawley rats (300-350 g) were implanted with guide cannulae in the right lateral ventricle. The experimental chamber was a 12x24x15-in. box with a 4x8 perpendicular array of photobeams 3/4 in. above the floor. Animals were adapted to the chamber on 2 days and then monitored during continuous infusions of saline (N=5), 0.1 µg/µl CRF (N=6) or 1.0 µg/µl GRF (N=5) at the rate of 20 µl/hr for 30 min. Animals were sacrificed 75 min later, and caudate nucleus and hippocampus were collected for HPLC determination of brain monoamines. Neither peptide significantly affected the amount of locomotor activity, although both group means were below that of controls. However (see figure) animals infused with CRF showed more randomized locomotor patterns; those infused with GRF exhibited reduced variability in their patterns. These differences were confirmed by a statistical measure of the degree of variability in the spatial patterns. CRF elevated 5-HIAA in caudate and hippocampus. Caudate DA and DOPAC were also increased by CRF. Without significantly altering hippocampal NE, both peptides increased hippocampal DA.

These behavioral results support the hypothesis and suggest a possible relationship between dynamics involving sequences of amino acid hydrophobicity in CRF and GRF and the spatial patterns of locomotor behavior under their influences. The monoaminergic system may also play a modulatory role in the physiological functions of the peptides. Supported by a grant from the W. M. Keck Foundation.

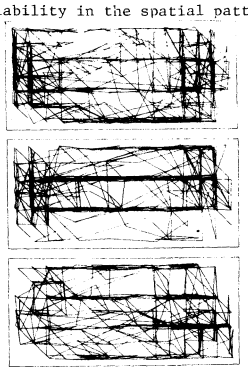

Fig. 1 Top views of representative locomotor patterns induced by saline (top), GRF (center), and CRF (bottom).

115.8 INFLUENCE OF SUBSTANCE P AND FRAGMENTS ON PASSIVE AVOIDANCE BEHAVIOR. O. Gaffori*, J.M. Stewart and D. De Wied. (Spon: European Neuroscience Association). Rudolf Magnus Institute for Pharmacology, Uni. of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands. Health Sci. Center, Uni. of Colorado, Denver, Colorado, USA.

Brain peptides are precursor molecules of neuropeptides with different, opposite and selective CNS activities. Recently, Hall and Stewart (Abstr. Soc. for Neurosci., 8: 369, 1982) reported that the N-terminal SP-(1-7) and C-terminal pyroglutamyl-SP-(7-11) fragments of substance P exert opposite effects in several behavioral paradigms. Glowinski et al. (The Reticular Formation Revisited, Eds. Hobson, J.A. and Brazier, M.A.B., New York, 1980) showed that substance P neurones could play a role in the regulation of the activity of the mesocorticoprefrontal DA pathway. On the basis of these data, the effect of substance P and these fragments was studied in a step through passive avoidance paradigm after micro-injection in the nucleus accumbens

It was found that both SP and SP-(7-11) attenuated passive avoidance behavior, while SP-(1-7) facilited passive avoidance behavior following injections of picogram amount into the nucleus accumbens 1 h prior to the first retention test. The lowest active dose of SP was 3 pg/rat. A dose of 10 pg/rat had a stronger effect. The fragment SP-(7-11) was less potent than SP. SP-(1-7) at a dose of 10 pg/rat attenuated passive avoidance behavior. All 3 peptides showed a U-shaped dose response curve.

The present results agree with observations summarized by Hall and Stewart (Abstr. Soc. for Neurosci., 8: 369, 1982) showing that SP and SP-(1-7) have opposite effects than SP-(7-11). The C-terminal peptide generally acts excitatory while the N-terminal peptide is inhibitory. The present study shows the opposite in that the C-terminal acted inhibitory in passive avoidance behavior. The fact that SP and SP-(7-11) exert the same effect as β-type endorphins (Kovacs and al, J. Neurosci., 2: S121, 1982) suggests that these peptides also may interact with DA-auto-receptors in the nucleus accumbens. The biotransformation of β-endorphin and vasopressin have been shown to generate powerfull neuropeptides with different, opposite and more potent effects (Burbach et al., Nature, 28: 96, 1980). It is possible that SP requires processing by enzymatic cleavage to activate moieties which elicite the opposite behavioral effects found in the present experiments and those reported by Stewart et al. (Peptides, 3: 851, 1982).

115.9 INTRACRANIAL SUBSTANCE P AND SUBSTANCE P FRAGMENTS: EFFECTS ON MOTOR BEHAVIOR IN MICE. M. E. Hall* and J. M. Stewart. Dept. of Biochemistry, Univ. of Colo. Med. Sch., Denver, CO 80262.

Intracranial administration of substance P (SP) has been shown to alter motor behavior in rats and mice. We have examined the effects of SP, SP analogs and SP fragments on motor behavior following freehand intraventricular injection. SP itself produced a dose-dependent increase in grooming behavior, with peak activity at a dose of lug (0.6nmoles)/mouse. Doses above lug led to impairment of motor function and subsequent reduction in ability to engage in coordinated grooming. We also observed reciprocal hindlimb scratching (RHS), a unique response to SP first described by Rackham and Share (S. N. abstr. 5:2092, 1979). RHS exhibited a dose-response curve similar to that for grooming. SP did not significantly alter rearing and leaning behavior (RL), although a trend toward enhanced RL was seen with 100ng/mouse of SP. SP-enhanced grooming and RHS lasted about 3-6min. All behaviors were normal at 15 and 30min after injection. All other peptides were tested at a dose of 0.6nmole/-mouse. SP sulfoxide was as active as SP in enhancing grooming (10.3 vs 9.9 grooming bouts/min) and inducing RHS (3.7 vs 3.3 reciprocal scratches/min). The 7-tyrosine analog of SP showed reduced activity (1.7 RHS/min) as did 9-D-alanine-SP (1.6 RHS/-min). The free acid of SP and l-tyrosine-ll-norleucine-SP were completely inactive. C-terminal fragments of SP were also tested: Pyroglutamyl-SP(7-11) (abbreviated <E-SP(7-11)) was more potent than SP on RHS (5.0/min) and grooming (13.2/min); pyro-glutamyl-SP(6-11) was also quite active. SP(4-11) increased grooming only slightly above controls and produced some RHS (0.6/min), while SP(8-11) was completely inactive. The N-terminal fragments SP(1-4), SP(1-6), SP(1-7) and SP(1-8) failed to induce grooming or RHS. A non-significant trend toward enhanced RL was seen. SP(1-7)-amide did enhance RL behavior ($p<0.03$). To avoid effects of trauma, studies with SP(1-7) and <E-SP(7-11) were repeated in mice with implanted ventricular cannulae. As before, <E-SP(7-11) significantly enhanced grooming and RHS but reduced RL behavior. This reduction was still significant ($p<0.001$) when RL scores were calculated on the basis of time available for RL (total test time minus time spent grooming), demonstrating that this difference was not due to reduced opportunity for RL because of increased grooming time. While SP(1-7) did not alter grooming, it significantly enhanced the number ($p=0.035$) and duration ($p=0.013$) of RL behavior. Thus, N- and C-terminal fragments of SP exert opposite effects on RL behavior in mice following intraventricular injection. They may also exert opposite effects on grooming, but the low level of grooming seen in control animals makes a further reduction difficult to establish. (Supported by NIH grant NS-18531)

115.10 THE EFFECT OF CALCITONINS ON AMPHETAMINE-STIMULATED ACTIVITY AND SPONTANEOUS BEHAVIOR. M.J. Twery, C.W. Cooper*, M.H. Lewis and R.B. Mailman. Depts. of Pharmacology & Psychiatry and Biological Sciences Research Center, Univ. of North Carolina, Chapel Hill, NC 27514.

Centrally administered calcitonins (CT) have been shown to suppress food intake and water consumption. We have previously reported that salmon CT also produces a long-lasting suppression of the locomotor activity stimulated by low doses of amphetamine and the daily spontaneous activity of rats in a vertical running wheel. This study describes an effect of salmon CT on spontaneous behavior, the effect of human and eel CTs on amphetamine-induced locomotor activity, and presents evidence that the effect is not due to gross impairment of motor function. METHODS: Synthetic eel (4100 MRC U/mg), human (100U/mg), or salmon (4200U/mg) CT (Bachem Corp., Torrance, CA) was administered to male Sprague-Dawley rats intracerebroventricularly (ICV, 600ng). Doughnut-shaped cages equipped with photocell detectors were used to estimate the locomotor activity induced by IP administration of 1.5 mg/kg d-amphetamine sulfate. In other experiments, the incidence of amphetamine-induced locomotion, rearing, sniffing, and nose poking was systematically recorded for one hour by observers unaware of treatment assignments. Salmon CT (300 ng) or vehicle was administered two hours prior to treatment with amphetamine. Interobserver reliability, corrected for chance agreements, was greater than 80% for all categories. RESULTS: Amphetamine treatment increased the average number of photocell counts over four fold. Human, eel, or salmon CT decreased the total number of photocell counts produced by amphetamine treated rats 35, 50, and 70%, respectively. The systematic observation of rats revealed that salmon CT reduced the incidence of amphetamine-stimulated locomotor and rearing activity, but failed to block amphetamine-induced nose poking or sniffing. Salmon CT treated rats also exhibited frequent episodes of shaking, tail wagging, and hopping. These dyskinetic movements were observed before and after administration of amphetamine. DISCUSSION: This comparison suggests that centrally administered salmon CT is more potent than other forms of the hormone in suppressing amphetamine-induced locomotor activity. The dyskinesias observed indicate that salmon CT produces effects on spontaneous as well as amphetamine-stimulated behavior. Since the incidence of specific behavioral topographies were not appreciably affected by salmon CT, neither sedation nor general motor impairmant appear to underly the earlier findings with CT. These results are consistent with the concept of CT or a CT-like peptide acting as a chemical messenger (neurotransmitter or neuromodulator) in the CNS.

115.11 SYNTHETIC SCP_B ELICITS PATTERNED NEURAL FEEDING ACTIVITY FROM THE BUCCAL GANGLIA OF TRITONIA. A.O. Dennis Willows, and P.E. Lloyd*. Friday Harbor Laboratories, Friday Harbor WA 98250, and Division of Neurobiology and Behavior, Columbia University C.P.&S., New York, NY 10032.

The neuron pair Bll in the buccal ganglia of the nudibranch mollusc Tritonia contains both the peptide SCP_B which has been isolated, sequenced, and synthesized, and also ACh (P.E. Lloyd, Fed. Proc. 41:2948, 1982; Morris, H.R. et al., Nature, 300:643, 1982). We have reported also that one identified pair of buccal ganglion motor neurons (B5), generates a motor output pattern which is part of the feeding behavior of the intact animal (Willows, A.O.D. Willows, J. Neurophysiol. 44:849, 1980) and which can be modulated by electrical stimulation of Bll or by ganglionic superfusion with SCP_B.

We now report the action of a peptide synthesized according to a sequence derived from Aplysia tissue and compare the responses it produces to those of the native peptide as extracted from Bll in Tritonia. It is an amidated nonapeptide and its structure is H-Met-Asn-Tyr-Leu-Ala-Phe-Pro-Arg-Met-NH2. The synthetic peptide co-elutes on HPLC with SCP_B extracted from Tritonia.

Superfusion of the isolated buccal ganglia with the peptide (either pure synthetic, or extracted and purified from Bll in Tritonia), for 1-2 m. elicits 2-5 m. of cyclic motor output from the motor pattern generator. The dose-response relationship for the peptide perfusion indicates a threshold response below 10-6M. As the peptide concentration is increased, the output of motor neurons increases both in terms of the rate of bursting and the spike frequency during each burst.

Our evidence supports the view that under normal circumstances, Bll modulates the output of the swallowing motor pattern generator by releasing the peptide both from central terminals and onto the esophagus, to co-ordinate and enhance both the central and peripheral aspects of ingestive behavior (Lloyd, P.E., Soc. Neurosci. Abstr. 252, 1979; Lloyd, P.E. and Willows A.O.D., Soc. Neurosci. Abstr. 240.9, 1980). Additionally, we find no differences in the physiological effects produced by superfusion of the synthetic (Aplysia derived) peptide, or extracted from the neurons Bll of Tritonia.

116.1 DIFFERENTIAL REGULATION OF TWO NEUROPEPTIDES IN CULTURED CHROMAFFIN CELLS. R. E. Siegel*, L. E. Eiden*, and R. M. Pruss* (SPON: J. Takahashi). Laboratory of Cell Biology, NIMH, Bethesda, MD 20205.

Previous work from this laboratory has shown that bovine chromaffin cells in culture contain two neuropeptides, vasoactive intestinal polypeptide (VIP) and met-enkephalin (L.E. Eiden, et al., Life Sci., in press). In this study, we have used techniques of radioimmunoassay and immunohistochemistry to both quantitate and localize these neuropeptides and to determine signals important in controlling their expression.

After four days in culture, the level of met-enkephalin in the chromaffin cells was approximately 10-fold greater than the level of VIP. A difference in the number of cells containing the two neuropeptides was also found in immunohistochemical studies. While 80-90% of the chromaffin cells expressed enkephalin-like immunoreactivity, a maximum of 3% of the cells were positive for VIP. When the cells were examined simultaneously for both VIP and enkephalin immunoreactivity, at least 80% of the VIP positive cells were also stained for the enkephalin. Thus, the two neuropeptides coexist in a small population of the chromaffin cells.

The expression of the two neuropeptides was differentially affected by treatment with veratridine. Addition of 10 μM veratridine to the chromaffin cell cultures produced a 10-fold elevation in the amount of VIP within 24 hours. This treatment also caused an increase in the number of cells possessing VIP immunoreactivity; a maximum of 10% of the treated cells appeared positive. The veratridine-induced effect on VIP was both time- and dose-dependent. Surprisingly, it was not blocked by 1.0 μM tetrodotoxin, suggesting that the effect is not mediated by the voltage-sensitive sodium channels. In contrast to the changes in VIP expression, the level of met-enkephalin was virtually unaltered by veratridine. Thus, the levels of neuropeptides in chromaffin cells can be controlled independently. The mechanism underlying the action of veratridine on VIP and the effects of other environmental signals on neuropeptide expression are currently under investigation.

116.2 EVALUATION OF SAPONIN-SKINNED CHROMAFFIN CELLS BY SCANNING ELECTRON MICROSCOPY. S.W. Carmichael and J.C. Brooks. Mayo Med. Sch, Mayo Clinic, Rochester, MN 55905 and Marquette Univ. Sch. of Dent., Milwaukee, WI 53233.

We have previously described a method for chemically skinning (permeabilizing) cultured adrenal medullary chromaffin cells by brief exposure to the detergent saponin (J Neurochem 40:468, 1983). The skinned cells appeared normal by light microscopy but lost membrane-dependent functions. Cytoplasmic enzymes leaked from the cells and catecholamines were released in an apparently exocytotic fashion in the presence of exogenous MgATP and calcium. Both the biochemical evidence and light microscopy indicated limited damage to the cells by the saponin treatment. However, the extent of the membrane discontinuities could not be determined by these methods. Therefore, untreated cells and saponin-skinned cells were examined by scanning electron microscopy to detect changes in surface morphology.

The cells used for these experiments were cultured on polyester film and prepared for microscopy by routine methods. Neither the saponin treatment nor preparation for electron microscopy had an effect on adhesion of the cells to the polyester film. The surface membrane of untreated cells had a globular appearance and nearly complete continuity. The membrane of saponin-treated cells also had a globular appearance but contained holes ranging in size from one micron down to 100nm. The overall morphology otherwise resembled that of the unskinned cells. Skinned cells treated with calcium and ATP to elicit secretion were morphologically indistinguishable from unstimulated, skinned cells.

We conclude that the skinning process causes holes in the plasmalemma of sufficient size for the exchange of large molecules with the cell interior. Either these holes are visualized directly or weaken the membrane and cause the larger holes seen in this study. It also appears that sufficient membrane remains to support an exocytotic mechanism for catecholamine secretion. Access to the cell interior provided by this technique should permit us to identify the components of the secretory system and their role in secretion.

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116.3 IRREVERSIBLE INHIBITION OF CATECHOLAMINE SECRETION BY THIOPHOSPHORYLATION OF SAPONIN SKINNED CHROMAFFIN CELLS. J.C. Brooks and S. Treml* Marquette Univ. Sch. of Dent., Milwaukee, WI 53233

We have previously described a method for permeabilizing chromaffin cells with the detergent saponin (J. Neurochem. 40, 468-473, 1983). This treatment disrupts the continuity of the plasma membrane and gives direct access of exogenous substances to the secretory apparatus. The secretory system of these cells is fully competent, dependent only upon exogenous calcium and ATP to elicit secretion.

There is increasing evidence that protein phosphorylation plays a pivotal role in the control of neurosecretion. We have used chemically skinned chromaffin cells to examine the role of protein phosphorylation in catecholamine secretion using the ATP analog, Adenosine-5'-O-(3-thiotriphosphate)(ATPγS). This analog can be used by cellular kinases to thiophosphorylate proteins. However the thiophosphoproteins are then resistant to dethiophosphorylation by cellular phosphatases. Therefore, use of ATPγS should make it possible to "lock" a phosphorylation dependent process in the thiophosphorylated state.

Skinned cells incorporated thiophosphate from ATPγ(^{35}S) in a calcium-dependent reaction. Thiophosphorylation could be initiated by either calcium or ATP as long as the other was already present in the medium. Use of MgATPγS in place of MgATP abolished secretion. Inhibition of secretion was proportional to the ratio of ATPγS/ATP in the medium. Moreover, cells treated with only ATPγS were subsequently unable to secrete when presented with their normal substrate, MgATP, suggesting that the secretory system was locked in the thiophosphorylated state.

We offer the hypothesis that protein phosphorylation is an obligatory control event in catecholamine secretion by chromaffin cells. Our data suggests that a calcium-dependent protein phosphorylation primes the system for secretion while dephosphorylation is the event required for exocytosis.

Supported in part by a grant from the Marquette University Committee on Research.

116.5 RELATIONSHIP BETWEEN OPIOID PEPTIDE LEVELS AND STORAGE VESICLE SYNTHESIS IN ADRENAL MEDULLARY CHROMAFFIN CELLS. S. P. Wilson[1], O. H. Viveros[2] and N. Kirshner*[1]. [1]Department of Pharmacology, Duke University Medical Center, Durham, NC 27710 and [2]Wellcome Research Laboratories, Research Triangle Park, NC 27709.

Catecholamines (CAs), opioid peptides (OPs) including met- and leu-enkephalin, and larger enkephalin-containing peptides (ECPs) which are inactive at opiate receptors are co-stored in the secretory vesicles of adrenal medullary chromaffin cells and are co-secreted upon stimulation of the gland.

Addition of CA-depleting drugs such as reserpine or tetrabenazine to the medium of bovine chromaffin cell cultures produces an exponential loss of cellular CAs ($t_{½}$=1.0-1.5 d) and a 1 to 2-fold increase in OPs over 2-3 d via increased enkephalin synthesis (Proc. Natl. Acad. Sci. 77: 4364, 1980). These increased OP stores are released in response to secretagogues, establishing their vesicular localization (J. Neurosci. 2: 1150, 1982). If the increase in OPs following treatment with these drugs reflects new vesicle synthesis, then other chromaffin vesicle components including ECPs and dopamine β-hydroxylase (DBH) should increase concomitantly. Analysis of ECP levels, as well as those of membrane-bound and soluble DBH activity, however, showed no such increases preceeding or during the drug-induced increase in OPs. These data suggest that an increase in the net number of vesicles is not required to obtain the increase in OP stores induced by CA-depleting drugs.

Chromaffin cells stimulated by exposure to nicotine to secrete a portion of their CA, OP, ECP and soluble DBH stores recovered all of the lost OPs, ECPs and norepinephrine over the following 3 d. Only 35% of the lost epinephrine was recovered, however, and no recovery of secreted soluble DBH activity was observed. Membrane-bound DBH activity was not altered by exposure of the cells to nicotine and did not change during the following 3 d. These results further suggest that OP and ECP levels may increase independently of other chromaffin vesicle components.

In contrast, addition of insulin (1 nM-1 μM) to chromaffin cell cultures produced concomitant increases in DBH activity and ECP levels from 1-6 d of treatment. Increases in OP and CA contents, however, were not apparent until 3-4 d following insulin addition. Hence, chromaffin cells in culture are capable of an increased rate of vesicle synthesis, although this appears to be part of a general increase in protein synthesis produced by insulin.

This work was supported by NIH grants AM05427 and NS06233 and by grants from the N.C. Affiliate of the American Heart Association and the N.C. United Way.

116.6 INDUCTION OF ADRENAL TYROSINE HYDROXYLASE (TH) BY APOMORPHINE IN RATS RECEIVING α-DIFLUOROMETHYLORNITHINE (DFMO). M. Ekker* and T.L. Sourkes. Departments of Biochemistry and Psychiatry, McGill University, Montreal, Quebec, Canada, H3A 1A1.

Rats exposed to stressors such as cold or bodily restraint, or given the drugs apomorphine or 2-deoxyglucose, exhibit large increases of adrenal ornithine decarboxylase (ODC) and of the putrescine content of that gland. Spermidine is also increased by immobilization (31%), apomorphine (60%), and 2-deoxyglucose (28%). Adrenal spermine is not affected by any of these treatments. The amines are measured fluorometrically after dansylation and separation by reverse-phase HPLC. Pretreatment of the animals with the irreversible ODC inhibitor DFMO, given orally (2% in drinking water) or s.c. (200 mg/kg), almost totally prevents the increase of ODC after apomorphine or immobilization; blocks the increase of putrescine after either treatment; and prevents the increase of spermidine after immobilization, but not apomorphine. This last result suggests that decarboxylation of S-adenosyl methionine, the second step in polyamine biosynthesis, may be rate-limiting. To determine whether the increases in ODC and/or polyamines are necessary for the induction of TH in response to the same type of stimuli, rats were given DFMO in their drinking water for several days during which they also received repeated injections of apomorphine. Administration of DFMO again resulted in effective inhibition of ODC during the experimental period, and blocked the increase in the putrescine content of the adrenals. The induction of TH was not affected. These results question whether increases of ODC activity and of putrescine content are crucial to the induction of TH in the adrenal.

(Supported by a grant of the Medical Research Council of Canada. M.E. holds a "Sciences 1967" Studentship of the Natural Sciences and Engineering Research Council of Canada.)

116.7 SEROTONIN-IMMUNOREACTIVITY IN THE ADRENAL MEDULLA OF THE RAT. M. A. Holzwarth and M. S. Brownfield*, Department of Anatomical Sciences, University of Illinois, Urbana, IL 61801 and School of Veterinary Medicine, Univ. of Wisconsin, Madison, WI 53715.

The adrenal medulla is reported to contain predominently norepinephrine and epinephrine as well as a variety of putative peptide transmitters including substance-P, somatostatin, neurotensin, VIP and met-enkephalin. In this study, we have evidence for the presence of impressive amounts of serotonin-immunoreactivity which is distributed in epinephrine-containing cells of the rat adrenal medulla.

Adrenal glands were stained for serotonin-immunoreactivity using antiserum to serotonin-BSA conjugate (Rabbit) from Immunonuclear (dilutions of 1:1000 and greater) or PNMT (1:5000) and PAP immunocytochemical techniques. Albumin-reactive antibodies were removed from the serotonin antiserum by preabsorption with BSA. We observed serotonin-immunoreactivity distributed in the cytoplasm of clusters of medullary chromaffin cells. PNMT staining in adjacent sections suggests co-localization of serotonin and epinephrine in the same cell. To test the specificity of the serotonin antiserum, preabsorption control studies were run by first incubating antiserum with serotonin, serotonin-BSA conjugate, norepinephrine, epinephrine, dopamine, 5 hydroxyindole-3-acetic acid or 5 hydroxytryptophan (1.0 to 2000 μM). Only the preabsorption with serotonin-BSA conjugate completely blocked staining; a slight reduction was observed with 5-hydroxyindole-3-acetic acid, serotonin, and 5-hydroxytryptophan at concentrations of 100 μM and greater.

The following procedures were tested to determine if the serotonin-immunoreactivity could be altered. The adrenals of rats pretreated with a serotonin synthesis inhibitor, parachlorophenylalanine (i.p., 300 mg/kg at 72 and 48 hr prior to sacrifice) showed a reduction in serotonin staining. Splanchnic nerve ligation also resulted in a decreased serotonin staining. Acute stress or reserpine (i.p., 5 mg/kg, 18 hr previously) also reduced immunostaining. To elevate serotonin, rats were preloaded with L-tryptophan (i.p., 200 mg/kg) 4 hrs previously; this did not increase observable staining, possibly because normal staining was already so intense.

These results provide immunocytochemical evidence for the presence of serotonin-immunoreactivity in adrenal medullary cells of the rat. This immunoreactive serotonin appears to be localized in the catecholamine-containing cells and can be altered by treatments known to affect serotonin levels.

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116.8 α-ADRENERGIC BINDING IN THE RAT ADRENAL MEDULLA: A PUTATIVE SITE FOR THE MODULATION OF EPINEPHRINE SECRETION. M. Bouvier*, J. de Champlain, J. LeGuerrier* and D. Papin* (SPON: L. Descarries). Centre de Recherche en Sciences Neurologiques, Dept of Physiology Université de Montréal, Montréal, Québec, Canada.

Presynaptic regulation of the sympathetic activity has been extensively studied. The existence of α and β adrenergic presynaptic receptors modulating norepinephrine release by sympathetic fibers has been demonstrated "in vivo" and "in vitro". The presence of a similar autoregulatory mechanism in the modulation of adrenal medulla secretion has been recently suggested by "in vitro" experiments. In the present study, plasma epinephrine (E) concentration was used as an index of adrenal medulla secretion to evaluate the functional significance of such a modulatory mechanism "in vivo". Acute administration of the α_2 antagonist Yohimbine (YOH, 0.5 mg/kg i.v.) or of the α_2 agonist Clonidine (CLO 15 μg/kg i.v.) did not modify basal E plasma levels in anesthetized rats. However, YOH potentiated by more than three folds the E increase induced by a bilateral carotid occlusion of one minute in vagotomized animals. Moreover, Clonidine completely abolished the E response to baroreflex stimulation, thus suggesting the involvement of an α_2 adrenergic mechanism in the modulation of adrenal secretion. To determine if that regulatory mechanism could be mediated by an α_2 autoreceptor located on chromaffin cells, binding experiments with tritiated YOH were carried out. These studies demonstrated the presence of a high affinity binding site (Kd ≃ 1nM) for YOH in rat adrenal medulla. The specific binding of YOH to adrenal medulla membranes, observed at 4°C, is saturable, and it is linear over a wide range of tissue concentration (up to 30 mg of tissue). The existence of this high affinity binding site suggests the presence of an α_2 receptor that could be at least partially responsible for the modulation of E release. Since the membrane pellets were prepared from crude adrenal medulla homogenates, it was not possible to determine with certainty whether the binding site is located on chromaffin cells or on pre-ganglionic sympathetic terminals. These studies lead to the conclusion that α_2 receptors presumably located on chromaffin cells can mediate a functional negative feedback on the epinephrine secretion by the adrenal medulla. Supported by Medical Research Council of Canada and Québec Heart Foundation.

117.1 IMMUNOCYTOCHEMICAL LOCALIZATION OF GROWTH HORMONE-RELEASING HORMONE (GHRH) IN HUMAN AND RAT HYPOTHALAMI. I. Merchenthaler*, S. Vigh*, A.V. Schally*, P. Petrusz*, F. Okazaki* and R.V. Randall (SPON: J.D. Mann). Dept. of Anatomy, University of North Carolina, Chapel Hill, NC 27514, Endocrine and Polypeptide Laboratories, V.A. Medical Center and Dept. of Medicine, Tulane University School of Medicine, New Orleans, LA 70146, and Depts. of Endocrinology, Internal Medicine and Pathology, Mayo Clinic, Rochester, MN 55905.

Recent isolation, structural identification and synthesis of human pancreatic GHRH has made possible the generation of specific antibodies against this peptide. Synthetic GHRH, coupled to bovine serum albumin with glutaraldehyde, was used as immunogen. The resulting anti-human pancreatic GHRH serum (SV #96) cross-reacted with rat hypothalamic GHRH. Using this antiserum and vibratome immunocytochemistry we report the presence of GHRH immunoreactive structures in human and rat hypothalamic tissue. All immunostaining was completely blocked by preincubation of the primary antiserum with synthetic GHRH (10 µg/ml). Absorption with glucagon, GIP, VIP, secretin, ACTH, α-MSH, γ-endorphin, GH, PRL, angiotensin, Metenkephalin, neurotensin, somatostatin, GnRH or TRH had no affect on the intensity and distribution of the staining. Immunoreactive GHRH was present in perikarya, neuronal processes and terminals in the hypothalamus of both species. Colchicine treatment in rats dramatically increased the number of GHRH-positive perikarya and terminals as well as the intensity of their staining. Medium-sized bi- or multipolar GHRH-containing neurons were observed in the arcuate and ventromedial nuclei and in the perifornical regions of the anterior and lateral hypothalamus. The number of cells in a 50 µm thick section was 50-60 in rat, and 80-100 in human tissue. Dense accumulation of GHRH-containing terminals was found in the external layer of the median eminence (ME). GHRH-immunoreactive fibers with regularly spaced varicosities were found throughout the hypothalamus, but were most abundant in the dorsomedial nucleus and in the regions where cell bodies were seen. The distribution of GHRH immunoreactivity as reported here is different from that of other neuropeptides or transmitters and is consistent with the results of earlier bioassay studies (Antoni et al., J. Endocr. 91:415, 1981). The localization of GHRH in the ME in both species suggests that human pancreatic GHRH is similar or identical to rat hypothalamic GHRH, and reinforces the view that this peptide plays a physiological role in the control of the release of GH from the anterior pituitary.

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117.2 INDUCTION OF GROWTH HORMONE SYNTHESIS BY GROWTH HORMONE RELEASING FACTOR AND DEXAMETHASONE. S.A. Edwards and W.W. Vale, Peptide Biology Laboratory. The Salk Institute. La Jolla, CA 92037.

Human pancreatic tumor growth hormone releasing factor (hpGRF) was isolated on the basis of its acute effect on growth hormone (GH) secretion. We have now studied the effect of this peptide on de novo synthesis of GH in cultured, dissociated pituitaries taken from 15-day-old, female Sprague-Dawley rats. Cells were incubated for 24 hours under serum free conditions with either 10 nM hpGRF, hpGRF plus 5 nM dexamethasone (DEX), DEX alone, or with no treatment. Postincubation, cells were labelled for 20 minutes with ^{3}H-leucine. Cell extracts were analyzed by SDS polyacrylamide gel electrophoresis and fluorography. ^{3}H incorporation into the GH band was quantitated by densitometric scanning of the fluorogram. ^{3}H incorporation into GH was increased 13.2 ± 2.5-fold over that of untreated cells by co-incubation with hpGRF and DEX, 1.86 ± .34-fold by hpGRF alone, and 1.27 ± .23-fold by DEX alone.

GH was determined by radioimmunoassay for the cell extracts, and for the 24 hour incubation medium. Total (cells plus medium) GH was increased 1.72 ± .06-fold over untreated cells by co-incubation with hpGRF and DEX, 1.47 ± .04-fold with hpGRF alone, while treatment with DEX alone had no significant effect. DEX significantly increased hpGRF mediated GH release. A 72-hour incubation with hpGRF and DEX resulted in a 2.8-fold increase in GH concentration over cultures treated with DEX alone. When initial GH content is accounted for, this represents a 7-fold increase in net GH accumulation. Analysis of the time course indicates that the effects of hpGRF on GH synthesis can be maintained over time. Similar results, although smaller in magnitude, were obtained with pituitaries from adult rats.

Incubation of pituitary cells with hpGRF in the absence of DEX or serum results in a dose-dependent general increase in the incorporation of ^{3}H-leucine or ^{35}S-methionine after 24 hours, an effect that was more prominent in cells taken from adult rats. SDS gel analysis indicates that most major proteins synthesized by the cells were affected. The effects of hpGRF on general amino acid incorporation were similar but not additive to the effects of serum or DEX.

We conclude that incubation of rat pituitary cells with hpGRF results in increased GH synthesis and that this effect is potentiated by DEX.

117.3 HUMAN PANCREATIC GROWTH HORMONE RELEASING FACTOR [hpGRF(1-40)OH] STIMULATES ANTERIOR PITUITARY ADENYLATE CYCLASE ACTIVITY, CYCLIC AMP ACCUMULATION AND GROWTH HORMONE RELEASE IN A CALMODULIN-DEPENDENT MANNER. G. Schettini,* M.J. Cronin, E.L. Hewlett* and R.M. MacLeod. Depts. of Med., Physiol. & Pharmacol., Univ. of Va. School of Med., Charlottesville, VA 22908.

The synthetic hpGRF(1-40)OH stimulates growth hormone (GH) release both in vivo and in vitro and is associated with enhanced cyclic AMP (cAMP) accumulation in pituitary cells. Ca^{2+} involvement in stimulus-secretion coupling and in the activation of the cAMP generating system is often mediated via the Ca^{2+} binding protein calmodulin (CaM). We investigated the role of CaM in the effect of hpGRF(1-40)OH on anterior pituitary adenylate cyclase activity, cAMP accumulation and GH release. hpGRF(1-40)OH stimulates adenylate cyclase activity in a dose-dependent manner (3, 10, 30, 100, 300 nM) both in male and female rat anterior pituitary gland membrane preparations. The inclusion of 100 nM somatostatin reduced 3, 10 and 30 nM hpGRF(1-40)OH stimulation of adenylate cyclase, but had no effect on the highest two concentrations. To determine CaM involvement in hpGRF(1-40)OH stimulation of adenylate cyclase activity, we used a CaM blocker, the naphthalenesulfonamide derivative W7 and the less potent dechlorinated analog W5. W7 (50 and 100 uM) but not W5 (50 and 100 uM) inhibited hpGRF(1-40)OH-stimulated adenylate cyclase activity ($p < 0.05$-0.01) without affecting basal enzyme activity.

Using cultured anterior pituitary cells, we studied the effect of W7 and W5 (5-100 uM) on basal, 10 nM hpGRF(1-40)OH- (4-h exposure) or 10 uM Ca^{2+} ionophore A23187- (15-min exposure) stimulated cAMP accumulation and GH release. W7 dose-dependently inhibited both parameters in all three experimental conditions. W5 did not modify basal or A23187-stimulated cAMP accumulation and GH release; only at concentrations of 50 and 100 uM did W5 reduce hpGRF(1-40)OH-stimulated cAMP accumulation and GH release.

These results indicate that hpGRF(1-40)OH stimulates pituitary adenylate cyclase activity and that somatostatin reduces this effect. CaM participates in the hpGRF(1-40)OH stimulation of adenylate cyclase, cAMP accumulation and GH release.

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117.4 HIGH DOSES OF HEPARIN SUPPRESS PULSATILE GROWTH HORMONE SECRETION IN THE MALE RAT. M.C. Obonsawin* and S.H. Shin* (SPON: H. Dinsdale). Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Growth hormone (GH) is secreted in intermittent bursts at approximately every 3 h. We have noticed that the peak plasma concentrations of GH in our male rats (Sprague Dawley, Charles River, CD, Canadian Breeding Farms and Laboratories) (Life Sci: 31: 547, 1982) was much lower than those obtained by other workers. To see detailed changes in the plasma concentration of GH (half-life of 5-7 min), we collected blood by a high frequency sequential blood sampling technique. When we encountered difficulties in collecting blood because of clotting, we administered an extra amount of heparin to the rats. Bursts of GH secretion in these rats occured less frequently and were of lower amplitude. Because the administration of heparin is a common practice when sampling blood sequentially at small intervals, we felt that it was important to clarify the relationship between the secretion of GH and the dose of heparin. We therefore decided to systematically examine the effects of heparin on GH release. Two days before blood collection, a cannula (Silastic tubing, 0.025" i.d., 0.047" o.d.) was inserted into the right atrium of ether anesthetized rats. On the day of the experiment, polyethylene tubing (PE-60) was connected to the outer end of the cannula, and blood samples (70 µl) were collected from the cannula every 2 min in heparinized microhematocrit tubes. In order not to disrupt the gentle flow pattern of blood in the cannula, there was no backflushing. We tested 4 doses of heparin. First, we injected heparin (200 U/100 g B.Wt., or more) one hour before collection. Most rats did not show any major bursts of GH secretion. Second, we decreased the heparin dose to 150 U/100 g B.Wt. We saw bursts that increased the plasma GH concentration to 200-300 ng/ml. Third, we decreased the heparin dose again to 50 U/100 g B.Wt. We saw greater variation in GH secretion between individual rats, with some bursts as high as 800 ng/ml. In the fourth experiment, we did not inject any heparin into the rats on the day of collection, and used heparin-coated PE-60 tubing to prevent clotting. Peak plasma concentrations of GH rose as high as 1200 ng/ml. The mechanism by which heparin reduces the amplitude of the bursts of GH secretion remains to be elucidated. (Supported by the MRC of Canada).

117.5 HUMAN PANCREATIC GROWTH HORMONE-RELEASING FACTOR-40 STIMULATES GROWTH HORMONE SECRETION AND POTENTIATES THYROTROPIN-RELEASING HORMONE EFFECTS ON GROWTH HORMONE SECRETION IN VITRO. J. L. C. Borges*, D. R. Uskavitch*, D. L. Kaiser*, W. S. Evans* and M. O. Thorner* (SPON: J. A. Jane). Dept. of Internal Medicine, Univ. of Virginia School of Medicine, Charlottesville, VA 22908.

The effects of synthetic hpGRF-40 (courtesy of J. Rivier, J. Spiess, and W. Vale) on GH release from continuously perifused male rat anterior pituitary cells were studied. Pulses (2.5 min.) of hpGRF-40 stimulated GH release in a linear-log dose response relationship. Random hpGRF-40 pulses at doses of 0.03, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, and 100.0 nM elicited a GH response above baseline of 1.2±0.3, 2.4±0.4, 2.8±0.2, 4.3±0.2, 6.2±0.7, 7.0±1.0, 8.7±1.7, and 10.8±0.8 (ug/min/10^7cells; mean±SEM; n=3), respectively; r=0.93. During a 6 h hpGRF-40 infusion, maximal GH stimulation was seen within 5 min and GH levels then waned to near basal by the end of the sixth h. The integrated GH response to 0.03, 0.1, and 0.3 nM of hpGRF-40 were 37.6±7.4, 52.9±8.5, and 66.7±8.2 (ug/min/10^7cells; mean±SEM; n=3), respectively. There was a linear-log dose response relationship with r=0.72. The interaction of TRH and GRF in the control of GH secretion was studied to investigate the mechanism of TRH stimulation of GH seen in GH excess states in humans. Dispersed cells were perifused with either 100 nM TRH for 0.5 h, or 5nM hpGRF-40 for 4 h, or 5 nM hpGRF-40 for 4 h to which TRH was added for 0.5 h at 120 min. GH levels did not change significantly in the presence of TRH alone ($P>0.05$). hpGRF-40 alone stimulated GH release. When TRH was added to hpGRF-40 GH levels rose from 1.4±0.06 to 4.0±1.0; ug/min/10^7cells; n=4 ($p=0.03$). In summary, the dispersed cell system allows comparison of effective doses of hpGRF-40 when given in short pulses and long term infusions. The GH response to TRH seen in acromegaly associated with ectopic GRF secretion as well as the TRH potentiation of hpGRF-40 stimulated GH release in vitro suggest that GRF is involved in the "paradoxical" GH response to TRH seen in vivo in human GH excess states.

117.6 GABA (γ aminobutyric acid) NOT ADRENERGIC MECHANISMS IN ANTERIOR HYPOTHALAMUS FACILITATE GROWTH HORMONE (GH) SECRETION. J.O. Willoughby, P. Jervois*, M. Menadue*. Centre for Neuroscience, Flinders University of South Australia, Bedford Park, South Australia 5042.

Hypothalamic peptidergic neurons regulating GH are influenced by adrenergic mechanisms as well as by GABA, acetylcholine and serotonin, as judged by pharmacologic studies which indicate facilitatory roles for these neurotransmitters in GH regulation. Somatostatin neurons which are inhibitory for GH have been shown by immunohistochemical studies to be located in the periventricular preoptic-anterior hypothalamic area (PO/AHA). The present study examined whether pharmacological agents when injected into the PO/AHA adjacent to a possible site of action on somatostatin neurons, could influence GH secretion, in unanaesthetised unrestrained rats.

Male Porton rats were prepared with indwelling chronic venous cannulae and with bilateral stereotaxic intracerebral guide cannulae directed at the PO/AHA, at co-ordinates A 6200, L ± 0.5 mm (Konig & Klippel). Seven to ten days post-operatively, 15 min serial blood samples for radioimmunoassay of GH (NIADDK) were removed for 90 min after PO/AHA injections. All drugs were administered in 0.5 µl saline via 30 gauge needles inserted through the guide cannulae to a depth of 8.3 mm below brain surface. Animals were accustomed to the injection procedure which necessitated gentle restraint. Drug doses were 0.016, 0.16, 1.6 and 16 nanomoles.

Saline injection resulted in transient inhibition of GH. The effects of epinephrine and salbutamol were no different from saline, extending previous evidence that PO/AHA norepinephrine and dopamine are also without an effect on GH. Acetylcholine, serotonin, glycine and glutamate were without apparent effect. Only muscimol evoked a brisk consistent dose-related GH secretory response (saline 107.5 ± 23.2 ng/ml; muscimol 1.6 nmol, 552.2 ± 81.0 ng/ml; muscimol 0.16 nmol, 561.9 ± 75.7 ng/ml; muscimol 0.016 nmol, 278.3 ± 82.9 ng/ml; $p < .01$ vs saline). Bicuculline was no different from saline in having a slight suppressive effect on GH.

It is concluded that GABAergic mechanisms in the PO/AHA have a facilitatory action in GH regulation, possibly by inhibiting somatostatinergic neurons. Such a possibility is consistent with published GABA immunohistochemical and iontophoretic studies. The site of the facilitatory action of adrenergic mechanisms in GH regulation remains elusive, because neither somatostatin- nor somatocrinin-containing regions of the hypothalamus mediate GH secretion in response to injections of adrenergic agents in the rat.

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117.7 IMMUNOREACTIVE SOMATOSTATIN IN THE HYPOTHALAMUS OF OBESE AND LEAN ZUCKER RATS. L.L. Don Carlos*, R.H. Ho, M. Berelowitz* and J.A. Finkelstein. Northeastern Ohio Univs. Col. Med., Rootstown; Ohio State Univ., Columbus; Univ. Cincinnati, Cincinnati, Ohio.

The content of hypothalamic immunoreactive somatostatin (SOM) has been reported to be significantly lower in the genetically obese vs. lean Zucker rat as measured by radioimmunoassay (RIA) (Sheppard et. al., 1980; Voyles et.al., 1982). Because neuropeptides play a role in the control of food intake and nutrient regulation, altered SOM levels may contribute to abnormal energy homeostasis in obese animals. In the first experiment, we compared immunocytochemical (ICC) localization of hypothalamic SOM in five obese (fa/fa) and lean (Fa/-) littermate pairs of 60 day old male Zucker rats in an attempt to determine if there is a neuroanatomical correlate of the reported decrease in SOM content. Brain sections from each pair were processed simultaneously, using anti-SOM and the PAP technique. The localization of immunoreactive cell bodies in the hypothalamus corresponded closely to previous reports. Appropriate controls confirmed the specificity of immunoreactive staining. SOM-containing neurons in the periventricular area were counted and analyzed at four hypothalamic levels (1: anterior to the suprachiasmatic nucleus [SCN]; 2: through SCN; 3: between SCN and the ventromedial hypothalamic nucleus [VMH]; 4: through VMH). No significant differences in mean number of cells were seen at any level as shown in the table.

Level:	1	2	3	4
Fa/-	31 ± 5	126 ± 21	294 ± 14	31 ± 6
fa/fa	38 ± 4	145 ± 18	293 ± 8	20 ± 4

Fiber density and overall staining intensity in the median eminence did not appear to be different in the two groups of rats. In a second experiment, we used RIA to compare hypothalamic SOM in groups of obese and lean Zucker rats. The difference between groups was not significant. The level of SOM in hypothalamus of obese rats was 1054 ± 115 ng/g wet wt, whereas the level for lean rats was 959 ± 48. Thus, our ICC and RIA data are in agreement and indicate no differences in hypothalamic SOM between obese and lean male rats. The discrepancy between these findings and published reports may be due to variables such as sex, age or antibodies. Supported in part by grants from Sigma Xi, NS14344, 17080, AM30686 and CDA of JDF.

117.8 PERTUSSIS TOXIN ALTERS GROWTH HORMONE RELEASING FACTOR, PROSTAGLANDIN E_2 AND SOMATOSTATIN INFLUENCES ON GROWTH HORMONE RELEASE AND CYCLIC AMP ACCUMULATION. M.J. Cronin, A.D. Rogol*, G.A. Myers* and E.L. Hewlett*. Depts. Physiology, Pediatrics & Internal Medicine, Univ. Virginia Sch. Medicine, Charlottesville, Virginia 22908.

Pertussis toxin (PT) protein, released by the Bordetella pertussis bacterium, has a specific effect on target cells to ADP-ribosylate a cell membrane protein involved in coupling hormone receptors to adenylate cyclase. We have shown that PT affects several hypothalamic releasing and inhibiting hormones at the level of the anterior pituitary gland; PT blocks the inhibition of prolactin release by dopamine agonists and promotes luteinizing hormone release by gonadotropin releasing hormone. Last year at these meetings (abstract 18.57), we reported on the properties of the crude, native human pancreatic growth hormone (GH) releasing factor (GRF) which was subsequently isolated as a peptide, sequenced and synthesized. This year we report on the action of PT relative to synthetic GRF(1-44) as well as another secretagogue, prostaglandin E_2 (PGE_2), and the physiological inhibitor of GH, somatostatin. Primary cultures of male rat anterior pituitary cells were pretreated for 24 hr with or without 70 ng/ml PT. An acute treatment period (± secretagogues, ± somatostatin) was terminated by removing the medium for GH determination and extracting cellular cyclic AMP for radioimmunoassay. GRF and PGE_2 rapidly stimulated cyclic AMP levels and GH release in a dose dependent manner, whereas somatostatin inhibited secretagogue-stimulated cyclic AMP accumulation and GH release. PT amplified the ability of both GRF and PGE_2 to enhance, and reduced the ability of somatostatin to inhibit, these two parameters. Although a low concentration of PGE_2 (1 µM) often had no additive effect on GRF-stimulated cyclic AMP levels, PT treatment induced a synergism between GRF and PGE_2 to markedly increase cellular cyclic AMP. Somatostatin inhibition of basal GH release was blocked by PT and somatostatin inhibition of GRF- and PGE_2-induced effects was attenuated by PT. In a time course study using 10 nM GRF, cyclic AMP levels were significantly greater ($p<0.01$) in the PT-treated vs. control group at the earliest time studied (i.e., 10 min). This PT-induced difference was maintained for at least 6 hr after GRF administration, a time at which the cyclic AMP values were falling back to control (i.e., no GRF) levels. GH release was stimulated by GRF at 10 min and was 3.6-fold greater than control at 2 hr. PT had little or no effect on GRF-stimulated GH release, perhaps due to a maximal GRF stimulation at 10 nM. We conclude that PT modifies a component involved in the transduction of signals from stimulatory (GRF, PGE_2) and inhibitory (somatostatin) receptors that affect cyclic AMP metabolism in the anterior pituitary gland. (The GRF was a gift of Drs. Guillemin and Ling. Supported by RCDA 1K04NS00601, NS18409, AM22125 and 5 S07 RR5431 21)

117.9 CORTICOTROPIN RELEASING FACTOR (CRF) BINDING SITES IN RAT PITUITARY GLAND: AUTORADIOGRAPHIC LOCALIZATION. Errol B. De Souza, Marilyn H. Perrin*, Jean E. Rivier*, Wylie W. Vale and Michael J. Kuhar. Department of Neuroscience, Johns Hopkins Univ., Sch. Med., Baltimore, MD 21205 and Peptide Biology Lab., Salk Institute for Biological Studies, La Jolla, CA 92138

Recently a 41 amino acid peptide that fulfills many of the criteria of a physiological CRF has been isolated from ovine hypothalamic extracts, characterized and synthesized (Vale et al., Science 213:1394, 1981). This peptide is a potent stimulus to the release of ACTH and beta-endorphin/beta-lipotropin from the anterior pituitary, both in vivo and in vitro. In the present study, we have used the radioiodinated CRF analog, NLe^{21}, Tyr^{32}-CRF-I^{125} (^{125}I-CRF), to localize specific binding sites for CRF in rat pituitary by an in vitro light microscopic autoradiographic method (Young and Kuhar, Brain Res. 179:255, 1979).

Before beginning autoradiographic studies in rat pituitary sections, some preliminary biochemical experiments were carried out in slide-mounted bovine pituitary sections in order to determine the optimal binding parameters and to characterize the binding of ^{125}I-CRF in pituitary. The binding of ^{125}I-CRF to bovine pituitary sections was saturable and of high affinity, with an approximate K_D of 0.5 X 10^{-9}M. Ovine CRF and its biologically equipotent analog, NLe^{21}, Tyr^{32}-CRF inhibited ^{125}I-CRF binding with IC_{50}'s of 1.5 and 0.9 nM, respectively. The weak biologically active analog, CRF(1-41)-OH displaced ^{125}I-CRF binding with an IC_{50} of 1 µM and the unrelated peptide, arginine vasopressin did not inhibit ^{125}I-CRF binding.

In autoradiographic studies, slide-mounted sections of rat pituitary were incubated with 0.1 nM ^{125}I-CRF to label CRF binding sites. Adjacent slide-mounted pituitary sections were coincubated with 1 µM ovine CRF in order to determine nonspecific binding. Rat pituitary autoradiograms showed specific binding sites for CRF in anterior lobe and in intermediate lobe; no specific binding sites for CRF were apparent in the posterior lobe. The relative concentrations of specific CRF binding sites (grains/500 μm^2; mean ± SEM) in the anterior and intermediate lobes of rat pituitary were 34.2 + 1.7 and 21.0 + 1.4, respectively. Of interest, the CRF binding sites occurred in clusters in anterior lobe whereas a relatively uniform distribution was observed in intermediate lobe of rat pituitary.

In summary, the results of our investigation confirm the existence of CRF receptors in anterior lobe and demonstrate, for the first time, the presence of specific CRF binding sites in intermediate lobe of rat pituitary.

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117.10 COLOCALIZATION OF BIOTINYLATED CORTICOTROPIN RELEASING FACTOR (CRF) and ACTH. K. N. Westlund, S. Chmielowiec*, T. J. Collins*, and G. V. Childs* Dept. of Anatomy, The University of Texas Medical Branch, Galveston, Texas 77550.

We have produced a biologically active biotin conjugate of CRF (Biotinyl-CRF) using biotinyl-N-hydroxysuccinimide ester (Bayer et al., Methods in Enzymology, 62: 308-315, 1979). CRF-stimulated ACTH release of the Biotinyl-CRF was compared with CRF using three day old dispersed pituitary cell cultures. Biotinyl-CRF was found to be equipotent with CRF producing a 400% increase in secretion of ACTH (ED_{50} 5 x 10^{-10}M). We have also developed stains for the bound Biotinyl-CRF on pituitary cultured cells incorporating the avidin-biotin peroxidase complex and ACTH. Both stains were followed through time with exposure to 10^{-10}M Biotinyl-CRF (1,3,10,30,60 min) and after four hours exposure to various doses of Biotinyl-CRF. The cells were fixed in 1% glutaraldehyde and stained with the avidin-biotin peroxidase complex using nickel intensified diaminobenzidine (black) as the substrate. The cells were subsequently stained immunocytochemically with 1:20,000 anti ACTH using 3-amino-9-ethyl-carbazole as the peroxidase substrate. Both the black Biotinyl-CRF and the bright red ACTH stains were visible in the cells. The percentage of double labeled cells increased with the dose in four hour exposures (7.5 - 14.1%). Greater than 99% of the Biotinyl-CRF stained cells also double labeled for ACTH. The distribution of both the biotinyl-CRF and the ACTH stains in the corticotropes varied with time. Controls displayed only diffuse, light staining for ACTH (8.6% of cells). After one minute exposure to Biotinyl-CRF (10^{-10} M), more intense stain for ACTH and stain for Biotinyl-CRF were observed in 13.7% of the cells. After three minutes exposure to Biotinyl-CRF, the ACTH staining pattern was compartmentalized in "blebs" and "ruffles" in 12.7% of the cells. The black Biotinyl-CRF stain was associated with the compartmentalized ACTH stain in either a broad band, cap, or punctate membrane localization or a diffuse pattern appearing to be within the blebs. Ten minutes after exposure, both stains again appear diffusely over 8.5% of the cells. By thirty min to one hour all variations of the above stains appear in addition to a granular black intracellular stain for Biotinyl-CRF. At thirty min, 7.5% of the cells are double labeled and 11.1% of the cells are double labeled after one hour. The staining patterns and cell percentages suggest a cycling release of ACTH. These studies demonstrate that affinity cytochemistry combined with immunocytochemistry can provide valuable information about cellular events such as interaction of releasing hormones with their target cells and that the CRF described by Vale et al. (Science 213: 1394-1397, 1981) binds specifically to anterior lobe corticotropes. Supported by the Kempner Foundation and RCDA HD00395.

117.11 ANGIOTENSIN II INCREASES ACTH RELEASE IN THE ABSENCE OF ENDOGENOUS ARGININE-VASOPRESSIN (AVP). Eduardo Spinedi* and Andres Negro-Vilar. Department of Physiology, University of Texas Health Science Center at Dallas, Dallas, TX 75235.

Angiotensin II can increase ACTH release in vivo and this effect may possibly occur by two different mechanisms: a) a direct action at the anterior pituitary (AP) level, combined with possible additive effects with the corticotrophin releasing activity (CRA) of other peptides such as AVP and CRF and/or b) by a central mechanism, increasing the release of other neural peptides, such as AVP and/or CRF. Recent studies from our laboratory (Spinedi and Negro-Vilar, in publication) indicate that adult male rats centrally-blocked with chlorpromazine-morphine-nembutal do not show increased ACTH release in response to different i.v. doses of AII, evidence which suggests a primary central site of action of the peptide. To determine the involvement of AVP in the ACTH response to AII, the present studies were designed to test whether AII is able to release ACTH in vivo in a similar fashion in intact, cannulated, freely moving Long-Evans (LE) or AVP-deficient, Brattleboro (DI) female rats. The in vivo response to AII was compared with that elicited by synthetic CRF. Also, the CRA of AII, AVP and CRF was evaluated in vitro using dispersed anterior pituitary cells obtained from both adult female LE and DI donor rats. AII injected i.v. (0.4 or 2 µg 100 g BW) induced a significant, dose-related increase in plasma ACTH values 5 and 15 min after injection, in both LE and DI rats. CRF given to LE and DI rats at 0.4 µg/100 g BW elicited a larger increase in ACTH plasma values than a similar dose of AII, 5 or 15 min after the injection. Moreover, ACTH levels after CRF in DI rats were significantly greater ($p<0.05$) than those obtained in LE controls. The in vitro results indicate that the response of cells from either LE or DI rats to AII or AVP (both at 10^{-9} and 10^{-8}M) was similar. On the other hand, cells from DI donors were hyperresponsive to CRF ($2x10^{-11}$ and 10^{-10}M) in terms of ACTH release when compared with the response of cells from LE rats. Taken together with our previous studies, the present results suggest that: 1) the most important site of action of AII to increase ACTH output is at the central level; 2) the presence of AVP is not essential to mediate the central response to AII and 3) AII may act centrally to stimulate CRF release from the hypothalamus in vivo, which would then enhance ACTH output. The results in the DI rat indicate that the increased response to CRF may be an important compensatory mechanism involved in the regulation of adrenocortical function in the DI rat. (Supported by NIH-09988-06-III and Smith-Kline and French).

117.12 CENTRAL MODULATION OF CORTICOTROPIN RELEASING FACTOR-LIKE IMMUNOREACTIVITY SECRETION BY ARGININE VASOPRESSIN. P.M. Plotsky, T.O. Bruhn* and W. Vale. Peptide Biology Laboratory, The Salk Institute, La Jolla, CA 92037.

Adenohypophysial secretion of ACTH is considered to be predominantly under the control of the recently characterized 41-amino acid corticotropin releasing factor (CRF). Arginine vasopressin (AVP), which may participate in regulation of ACTH secretion, has been shown to have direct stimulatory effects on the corticotrope and to potentiate the ACTH-releasing properties of synthetic CRF. A central effect of AVP on hypothalamic CRF secretion has also been suggested. Due to the interactions between these putative releasing agents, it has proven difficult to determine the site of action of AVP in vivo. We now report direct measurements of CRF-like immunoreactivity (CRF-LI) present in the hypophysial portal circulation following ventricular injection of AVP and an antagonist.

Hypophysial portal blood was collected in sequential 45 min periods from urethane anesthetized male rats by the parapharyngeal technique of Porter. Rats had been implanted with third ventricular guide cannulas 3-6 days prior to portal blood collection. Ventricular injection (1 µl) of one of the following solutions occurred immediately after initiation of the second collection period: (1) 0.1 M phosphate buffered saline, pH 7.4 (PBS); (2) PBS containing 90 pmole AVP; or (3) PBS containing 100 pmole 1-deaminopenicillamine. 2-(O-methyl) tyrosine-AVP, a potent antagonist of the vasopressor and ACTH-releasing properties of AVP. CRF-LI was determined in extracted plasma by RIA. Reported values are not corrected for recovery (60-65%).

The mean concentration of CRF-LI in portal plasma (n=10) was 125.2 ± 19.9 pM (mean ± S.E.M.), while peripheral plasma content (n=10) was <30 pM. Ventricular injection of AVP significantly reduced ($p<0.05$) portal plasma CRF-LI concentration by 50%. In contrast, injection of AVP antagonist resulted in a 15% increase in portal blood CRF-LI concentration. Vehicle alone had no effect on portal plasma CRF-LI concentration.

These observations suggest that AVP is capable of modulating hypothalamic CRF release in an inhibitory manner. The physiological relevance of this effect is unknown. However, the observed weak facilitation of CRF-LI secretion by AVP antagonist supports a physiological tonic inhibitory action of AVP on hypothalamic CRF secretion into the portal circulation. Higher doses of this antagonist, other antagonists or antibody to AVP may have more clearly expressed effects. (Supported by grants from the Mellon Foundation, the DFG (BR 794/1-1), and NIH grant AM26741.)

117.13 PARAVENTRICULAR LESIONS AND ACTH SECRETION IN NORMAL AND ADRENALECTOMIZED RATS. T.O. Bruhn*, P.M. Plotsky, R.S. Sutton* and W.W. Vale (SPON: C. Rivier). Peptide Biology Laboratory, The Salk Institute. La Jolla, CA 92037.

Neurons containing CRF-like immunoreactivity have been localized in the paraventricular nucleus (PVN) of the rat. These neurons project to the median eminence via the paraventriculo-infundibular tract. In this report, we investigate the effect of PVN lesions on CRF-like immunoreactivity in the stalk-median eminence (SME) and on pituitary ACTH secretion.

Normal male rats received bilateral PVN-lesions (1 mA anodal current) 4-6 days prior to experiment. Adrenalectomized rats were lesioned 2 weeks after ADX and used 6 days after the second surgery. Animals were fitted with indwelling femoral artery catheters and blood was collected under low stress conditions. CRF and ACTH were measured by RIA as previously reported. Proopiomelanocortin mRNA levels were quantitated by solution hybridization using a ^{32}P-labeled POMC cDNA probe (in collaboration with N. Birnberg and R. Evans, Molecular Biology Virology Laboratory. The Salk Institute).

PVN ablation caused a 90% reduction of CRF-LI in the SME of normal rats (from control levels of 2040.9 ± 148.5 pg/SME, n=7) and an 80% reduction in the SME of adrenalectomized rats.

Ether stress-induced ACTH secretion was greatly attenuated by 80% in PVN-lesioned normal rats. When these rats were challenged with 0.15 nmol (i.v.) ovine CRF the ACTH response was dramatically increased by 328% at 15 minutes after injection when compared to sham animals.

In adrenalectomized rats ablation of the PVN caused an 80% reduction of resting ACTH levels. Proopiomelanocortin messenger RNA levels in the anterior pituitary were attenuated by 50% indicating a reduction of ACTH synthesis due to PVN ablation.

These data suggest that CRF is an important regulator of ACTH secretion and sythesis in normal and adrenalectomized rats. Pituitary hyperresponsiveness to exogenous CRF results from removal of endogenous CRF.

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117.14 SECRETION OF PROOPIOMELANOCORTIN-DERIVED PEPTIDES IS INHIBITED BY A STRESS-INDUCED RATE-SENSITIVE GLUCOCORTICOID FEEDBACK SIGNAL. G. R. Van Loon and E. B. DeSouza. VA Medical Center and Dept. of Medicine, University of Kentucky, Lexington, KY 40511

Stress produces concomitant secretion of proopiomelanocortin-derived peptides, and their stress-induced secretion is regulated by two distinct phases of glucocorticoid negative feedback inhibition which have been termed fast or rate-sensitive and delayed or level-sensitive feedback respectively. Evidence for rate-sensitive feedback inhibition has been based almost entirely on studies using exogenous glucocorticoid, and the significance of these data has been disputed. In this study, we present physiological evidence for rate-sensitive, fast feedback inhibition of secretion of ACTH and β-endorphin-related peptides. We used a 2 min restraint stress to physiologically increase plasma corticosterone in adult male rats, then examined the plasma responses of immunoreactive ACTH and β-endorphin plus β-lipotropin (βEND/βLPH) to a subsequent restraint stress. After onset of this stress, plasma corticosterone increased from 2.5 to 10 min, then remained at a peak from 10 to 15 min. The rate-rise of plasma corticosterone was 120 nMmin^{-1}, a rate consistent with literature reports using infusions of exogenous glucocorticoids as sufficient to inhibit the pituitary-adrenocortical response to stress during this period. A single 2 min restraint stress produced peak plasma levels of ACTH and βEND/βLPH 2.5 min after onset of the stress, and these plasma concentrations declined after this initial stress at rates of 2.7 and 7.4 pMmin^{-1} respectively. Application of a second restraint stress at the time of the peak corticosterone response produced plasma ACTH and βEND/βLPH responses identical to those following the first stress. Thus, at this time elevated glucocorticoid concentration did not appear to provide a negative feedback signal to inhibit ACTH or βEND/βLPH secretion. Application of a second stress during the period of significant rate-rise of corticosterone in plasma did not result in decreased incremental responses of plasma ACTH or βEND/βLPH. However, the rates of decline of plasma ACTH and βEND/βLPH of 7.6 and 32.0 pMmin^{-1} from peak levels were significantly greater ($p<0.01$ using analysis of covariance) after this second stress applied during the period of significant increase in corticosterone than the corresponding rates of decline observed after the initial stress or after a subsequent stress applied at the peak of plasma corticosterone. In contrast to these data from intact rats, initial and subsequent stresses did not show different rates of decline of plasma ACTH or βEND/βLPH in adrenalectomized rats. In conclusion, the stress-induced rate-rise of glucocorticoid provides a negative feedback signal which serves to terminate and limit the duration but not the peak of the responses of proopiomelanocortin-derived peptides to subsequent stress.

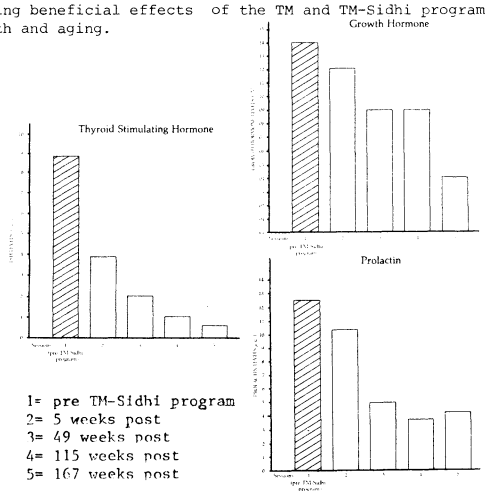
117.15 INHIBITION BY CHOLINERGIC MUSCARINIC RECEPTOR ACTIVATION OF CYCLIC AMP FORMATION AND ACTH SECRETION IN MOUSE PITUITARY TUMOR CELLS. S. Heisler*, L. Larose*, J. Morisset* (SPON: F. Garcin). Unité de Biorégulation cellulaire et moléculaire, Centre hospitalier de l'Université Laval, Québec G1V 4G2 and Centre de Recherche sur les Mécanismes de Sécrétion, Faculté de Sciences, Université de Sherbrooke, Sherbrooke J1K 2R1 (L.L., J.M.)

The AtT-20/D16-16 (AtT-20) mouse pituitary tumor cell line secretes immunoreactive ACTH in response to a wide variety of secretagogues whose activity appears mediated in part by the intracellular accumulation of cyclic AMP. These include corticotropin releasing factor (CRF), β_2-adrenergic agonists, vasoactive intestinal peptide (VIP), and direct activators of the adenylate cyclase complex such as forskolin and cholera toxin. Muscarinic cholinergic receptors are believed to be coupled negatively to adenylate cyclase and in initial studies, using receptor binding techniques, cholinergic muscarinic receptors were identified on AtT-20 cell membranes. The density of muscarinic receptors averaged 120 fmol/mg protein and their affinity constant for [^{3}H]quinuclidinyl benzilate (QNB) was 1.9 X 10^{-10}M. [^{3}H]QNB binding was inhibited completely by atropine (K_i = 1.5 X 10^{-9}M). The effects of carbachol were subsequently investigated on basal and stimulated cyclic AMP formation and ACTH secretion in AtT-20 cells. Carbachol reduced forskolin-stimulated adenylate cyclase activity and markedly lowered the stimulatory effect of forskolin on both cyclic AMP formation and ACTH secretion. The stimulatory effects of (-)isoproterenol on cyclic AMP synthesis and ACTH secretion were blocked non-competitively by carbachol. The ability of carbachol to antagonize (-)isoproterenol-elevated cyclic nucleotide levels and ACTH release was mimicked by oxotremorine, another muscarinic agent. The carbachol effect on the (-)isoproterenol stimulated nucleotide and secretory responses was reversed by the muscarinic antagonist atropine, but not by the nicotinic antagonist gallamine. These findings are consistent with the belief that ACTH secretion from AtT-20 cells may be regulated by activation of cholinergic muscarinic receptors which are negatively coupled to adenylate cyclase.

(Supported by grants from the Medical Research Council of Canada)

117.16 LONG-TERM ENDOCRINOLOGICAL CHANGES IN SUBJECTS PRACTICING THE TRANSCENDENTAL MEDITATION AND TM-SIDHI PROGRAM. O. R. Werner*, R. K. Wallace, E. Arnold*, B. Charles* and R. A. Chalmers*. Dept. of Medicine, Maharishi European Research University, Seelisberg, Switzerland CH6446, and Dept. of Neuroscience and Biology, Maharishi International University, Fairfield, Iowa 52556.

In order to extend previous physiological and biochemical investigations on the TM and TM-Sidhi program, a longitudinal study of a number of hormones was undertaken. Eleven male subjects were examined before and (over three years) after starting the TM-Sidhi program. Measurements of TSH, T3, T4, cortisol, prolactin and growth hormone in serum were performed by radioimmunoassay. A marked and significant decrease in TSH, growth hormone and prolactin levels occurred over three years with almost no change in cortisol, T3 and T4 levels. Furthermore, there was a significant decrease in day to day variation (mean coefficient of variation) in cortisol, prolactin, T3 and T4 levels. These data suggest that the TM-Sidhi program results in an increase in sensitivity of the thyroid-to-pituitary TSH stimulation, and affects the regulation of the pituitary gland. These longitudinal changes are of particular interest in the light of other studies showing beneficial effects of the TM and TM-Sidhi program on health and aging.



117.17 BOVINE SERUM ALBUMIN-GABA-HIS-PRO-NH_2: AN IMMUNOGEN FOR PRODUCTION OF HIGHER AFFINITY ANTISERA FOR TRH, W. W. Youngblood*, L. J. Moray*, W. H. Busby* and J. S. Kizer* (SPON: C. Lifieberry). The Biological Sciences Research Center and the Neurobiology Program, UNC School of Medicine, Chapel Hill, North Carolina 27514.

Dissatisfaction with current methods for the production of immunogens for raising antisera to TRH stimulated us to synthesize the hapten, GABA-his-pro-NH_2. Coupling of this hapten to bovine serum albumin at a molar ratio of 18:1 by means of a water soluble carbidiimide produced an immunogen which stimulated the rapid production in New Zealand white rabbits of antisera with an affinity ($2.42 \pm .3 \times 10^9$ liter/mole) for TRH, some 8-fold higher than that of antisera ($.33 \pm .03 \times 10^9$ liter/mole) raised by immunization with a conjugate produced by the currently accepted bis-diazotized benzidine bridging technique. These higher affinity antibodies when used in a standard TRH radioimmunoassay permitted the detection of less than 1 picogram of TRH per assay tube. Application of this newer radioimmunoassay to the measurement of TRH in brain tissue yielded measurements of TRH content similar to those determined by current RIA methods. Chromatography of whole crude brain extracts revealed one major immunoreactive peak corresponding to authentic TRH. We conclude that immunization of rabbits with hapten rapidly produces antisera with a high affinity for TRH suitable for the development of a very sensitive TRH radioimmunoassay.

This work was supported by NICHD grant HD-14005 and NIMH grant MH-00114. J. S. Kizer is recipient of RSCDA from NIMH.

117.18 REGIONAL HYPOTHALAMIC DISTRIBUTION OF 5' MONODEIODINASES IN THE RAT. P.N. Riskind, J. Kolodny*, J.B. Martin, and P.R. Larsen*. Dept. of Neurol., Massachusetts General Hospital, Boston, MA 02114 and Howard Hughes Med. Institute, Brigham and Women's Hospital, Boston, MA 02115.

The 5' monodeiodinases are enzymes which convert thyroid hormones into their less-iodinated forms. Type I enzyme is widespread in its distribution, while Type II (PTU-insensitive) appears to be mainly restricted to the brain and anterior pituitary. Both can convert thyroxine(T4) into the more potent hormone triiodothyronine (T3), and both enzymes vary in activity with changes in the level of thyroid hormones. These enzymes are thought to perform at least two functions: a) insure a "critical" level of T3 is present for metabolic purposes, and b) provide a mechanism of monitoring the level of T4 in blood. We now report a discrete localization of both Type II and total(Type I + Type II) enzyme activity within the hypothalamus, and characteristic regional responses of enzyme activity to hypothyroidism.

Euthyroid (EU) and longterm hypothyroid (HYPO) adult male Sprague-Dawley rats weighing 250-340 g were decapitated and their brains quickly removed and frozen on CO_2. Brain tissue was kept at -30° until microdissection and assay within 3 weeks. Brains were sliced coronally in 1 mm thick sections, and specific brain areas were dissected with a 1 mm diameter punch; All dissection was done over CO_2. Punches were homogenized and assayed for monodeiodinase activity (reverse T3 to ^{-}I) as previously reported (Science 214: 571, 1981). Results are expressed as femtomoles/mg/hour ± SEM.

Region	Eu: Total	Type II	Hypo: Total	Type II
preoptic area	0	0	5±5	9±5
paraventricular n.	0	0	0	0
arcuate-median em.	103±6	10±10	256±120	249±78
dorsomedial n.	0	0	7±7	0
ventromedial n.	14±2	0	29±8	17±12
lateral hypoth.	0	0	15±6	9±5
cerebral cortex	27±2	0	81±11	67±8
hippocampus	62±7	0	99±2	59±10
amygdala	19±1	0	54±11	34±4

These results demonstrate that high levels of Type II enzyme are present in the arcuate-median eminence area in hypothyroid rats; furthermore, the increase in activity of Type II enzyme and decrease in activity of Type I (Total minus Type II) in that area in hypothyroid rats is considerably greater than in any other brain region measured thus far. Subsequent studies will investigate the source of deiodinase activity within the arcuate-median eminence punch, and the probable role of thyroid hormones in modifying release and/or synthesis of releasing hormones.

117.19 RELEASE OF IMMUNOREACTIVE LUTEINIZING HORMONE AND THYROID-STIMULATING HORMONE FROM RAT HYPOTHALAMUS. N. Emanuele*, J. Anderson*, G. Baker*, D. McDonald*, L. Kirsteins* and A. M. Lawrence* (SPON: J. A. McLane). Biochem. Neuroendocrinol. Lab., VA Hospital, Hines, IL 60141, and Biochem. Dept., Loyola Univ. Stritch Sch. of Med., Maywood, IL 60153.

We have previously validated the wide distribution of luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and growth hormone (GH) in the rodent brain. The presence of LH has been determined by radioimmunoassay (RIA), radioreceptor assay (RRA), in vivo bioassay, and in vitro bioassay; TSH by RIA and in vivo bioassay; and GH by RIA, RRA, and in vivo bioassay. All are present in brain for up to 7 months after hypophysectomy suggesting in situ origin. In these studies we have examined release of immunoreactive LH and TSH from adult male rat whole hypothalamus cultured in vitro as well as from several extra-hypothalamic parts including thalamus, amygdala, caudate, cerebral cortex, cerebellum, hippocampus, pons and medulla. High medium potassium (60 mM) promoted a significant increase in the release rate of both hypothalamic LH and TSH from the stabilized basal rate; TSH increased from 184 ± 23 ng/hypothalamus/30 minutes to 268 ± 30 ng/hypothalamus/30 minutes ($p < .001$), and LH increased from 5 ± 0.8 ng/hypothalamus/30 minutes to 11 ± 1.6 ng/hypothalamus/30 minutes ($p < .001$). In each case, the potassium-induced release was significantly blunted by the omission of calcium from the medium. Sephadex G-100 chromatography of the 30 minute incubation medium revealed that most of the immunoassayable materials co-eluted with pituitary LH and TSH, respectively. In contrast, high potassium was not able to stimulate hormone release from any of the extrahypothalamic brain parts. Conclusions: 1) LH and TSH-like peptides are released from the rat hypothalamus cultured in vitro, 2) Baseline release can be significantly augmented with a depolarizing stimulus such as high potassium concentration and this effect appears to be calcium dependent, 3) The potassium-induced release of brain-based LH seems to be unique to the hypothalamus as it does not occur in any of the extrahypothalamic areas that were studied, and 4) As we have previously shown that brain-based LH and TSH are synaptosomally associated, the data from these studies suggests these peptides can normally be released from pre-synaptic terminals throughout the hypothalamus in response to depolarizing stimuli such as neural impulses.

117.20 EFFECT OF THYROXINE (T4) ON HYPOTHALAMIC, EXTRAHYPOTHALAMIC, AND PITUITARY THYROTROPIN-RELEASING HORMONE (TRH). M.J. Kubek, J.L. Meyerhoff, T.G. Hill,* and A. Sattin. Depts. of Anatomy and Psychiatry, Indiana Univ. School of Med. and VAMC, Indianapolis, IN 46223 and Dept. of Med. Neurosciences, Walter Reed Army Inst. of Res., Washington, D.C. 20012

T4 is known to modulate TSH responses to TRH at the pituitary level. However, its effects on TRH in specific hypothalamic and extrahypothalamic loci have not been clearly elucidated. We therefore sought to examine this question through alteration of the pituitary-thyroid axis.

Male S-D rats were sham (S) or surgically thyroidectomized (Tx) at 4 weeks of age (Tx rats were given 2% Ca lactate daily to drink). Four days after surgery, shams were given NaCl (0.2cc, 0.9% ip) while Tx animals received either NaCl (Tx + S), 0.2ug T4 (Tx + 0.2), or 20ug T4 (Tx + 20) per 100g BW (ip) for 21 days. All rats were decapitated and blood collected 24 ± 3 hrs after the last injection. Brains were removed, immediately dissected, weighed, and frozen on dry ice. Tissues were: anterior (AP) and posterior (PP) pituitary; median eminence (ME); anterior (AHy), middle (MHy) and posterior (PHy) hypothalamus; amygdala (Ay); pyriform (PFM); midbrain (MB); lateral (LM) and medial (MM) medulla; septum; striatum; thalamus; frontal ctx; entorhinal ctx; hippocampus and the n. diagonal band. TRH was assayed by specific RIA following MeOH extraction and the results expressed as pg/mg protein (mean + SEM). Data were log-transformed and analyzed by t-tests (2-tailed). Serum thyroid indices (T4 and TSH) were assessed by specific RIA's.

Thyroidectomy (S vs Tx + S) had no demonstrable effect on TRH except in MB wherein a small ($p<0.05$) decrease was observed. Hyperthyroidism (S vs Tx + 20) however produced marked TRH decreases in AP (198.4 + 66.1 vs 36.4 + 22.2, $p<0.02$), AHy (1326.5 + 103.1 vs 981.9 + 71.7, $p<0.02$), and PHy (2357.5 ± 195.9 vs 1280.9 + 204.2, $p<0.005$). No significant change was observed in either MHy or ME. Of equal importance was the finding of TRH decreases in Ay (187.0 + 12.9 vs 128.5 + 9.4, $p<0.005$), PFM (40.3 + 3.5 vs 26.2 + 2.8, $p<0.01$), and MM (364.9 + 23.2 vs 254.7 + 17.1, $p<0.02$). Partial T4 replacement (S vs Tx + 0.2) also produced TRH decreases in two loci; AP ($p<0.02$) and AHy ($p<0.005$). These results suggest: 1) Hypothyroidism has no significant effect on hypothalamic, pituitary or extrahypothalamic TRH with the possible exception of MB; 2) Experimental hyperthyroidism induced marked decreases in TRH content in specific hypothalamic and extrahypothalamic loci; and 3) The AP and AHy appear to be the sites most sensitive to T4 modulation. The mechanism whereby T4 influences TRH in specific regions is unclear but may include either decreased synthesis or increased metabolism. Supported by NIH AM-28260 (MJK) and VA Research (MJK, AS).

118.1 CORTICAL SPREAD PATTERNS OF THE INTERICTAL SPIKE COMPLEX IN HUMANS ARE REVEALED BY MAGNETOENCEPHALOGRAPHY (MEG). D.S. Barth, W. Sutherling, J. Engel Jr. and J. Beatty. Depts. of Psychology and Neurology, U.C.L.A., Los Angeles, CA 90024.

Interictal spikes recorded in the electroencephalogram (EEG) of patients with complex partial seizure disorders also produce small extracranial magnetic fields. These fields may now be measured using a highly sensitive magnetic sensor, the Superconducting QUantum Interferance Device (SQUID), coupled to superconducting gradiometer coils. To map the magnetic field produced by interictal spikes, the magnetic probe is sequentially positioned at a series of closely spaced measurement points in a rectangular matrix on the scalp over the discharging cortex. At each point, the probes output is averaged over at least 20 interictal spikes. Computer generated magnetic field maps may then be constructed and used to locate the source of the averaged interictal spike in three dimensions (Barth, D.S., et al., Science, 218: 891-894, 1982).

Neuromagnetic recording has frequently revealed the magnetic spike complex to be composed of more than one underlying source. A systematic temporal discharge pattern may often be discerned, revealing a primary source with activity preceding that of secondary sources. In these patients, it has not been possible to adequately characterize these spread patterns in the scalp EEG, probably due to smearing of the electrical potential field by surrounding tissue and the highly resistive skull. In contrast, the MEG is relatively uninfluenced by these factors and thus provides an improved spatial resolution. Work is now under way to determine the importance of these spread patterns in the production of interictal spikes, the manner in which they may influence the timing and morphology of the scalp EEG, their selective susceptibility to antiepileptic drugs, and finally, their relationship to the ictal EEG focus.

118.2 Single unit activity in non-specific and specific thalamic nuclei during generalized spike-wave discharges in feline generalized penicillin epilepsy. R.McLachlan*, M.Avoli and P. Gloor. Montreal Neurological Institute, Dept.Neurology & Neurosurgery, McGill University, Montréal, Québec, Canada, H3A 2B4.

Cats given i.m. penicillin develop absence seizures with generalized spike-wave (SW) discharges similar to those in human primary generalized epilepsy. Penicillin alters the cortical response to thalamocortical volleys from one of spindles to SWs as a result of increased excitability of cortical neurons. Furthermore, neurons in specific thalamic nuclei (lateralis posterior - pulvinar, LP), are entrained to fire in synchrony with the cortical activity and in turn seem to gate this activity. Since stimulation studies suggest that non-specific thalamic nuclei are most intimately involved in thalamo-cortical mechanisms of SW genesis, the EEG and single unit activity in cortex (anterior middle suprasylvian gyrus), specific (LP) and non-specific (nucleus centralis medialis, NCM; centrum medianum, CM; centralis lateralis, CL), thalamic nuclei were recorded simultaneously from cats given i.m. penicillin and the results analyzed by computer.

During SW bursts all cortical neurons exhibited periods of maximum firing probability during the spike of the SW complex alternating with absence of firing with the wave. The same pattern occurred in 91% of the LP neurons, the other 9% firing predominantly during the wave. In NCM only 43% of the neurons fired preferentially during the spike while 20% fired during the wave. The others did not alter their pre-penicillin firing patterns. In CM and CL, 90% fired during the spike and none during the wave while 10% were unaffected. Rhythmic fluctuations in firing probability were much less prominent in the non-specific thalamus than in cortex and LP. Furthermore, those non-specific thalamic neurons which did participate in the SW rhythm always did so well after similar activity had developed in cortex and LP. Unit activity in non-specific thalamic nuclei correlated more with cortical activity than with that in specific thalamic nuclei.

The neuronal activity in both non-specific and specific thalamic nuclei during SW discharge is usually a rhythmic pattern of "excitation" alternating with "inhibition", similar to that seen in the cortex. However, neurons in specific thalamic nuclei seem to be more involved in generalized SW activity than those in non-specific thalamic nuclei.

118.3 Inhibitory mechanisms in feline generalized penicillin epilepsy. D. Giaretta*, M. Avoli, P. Gloor, G. Kostopoulos and R. McLachlan* (SPON: D.W. Baxter). Montreal Neurological Institute and Dept. of Neurology & Neurosurgery, McGill Univ., Montréal, PQ, Canada, H3A 2B4.

Generalized epilepsy with brief periods of "absence" and 4-5 Hz spike and wave (SW) discharges can be induced in the cat by large amounts of i.m. penicillin (300,000 - 350,000 I.U./Kg). Previous work from this laboratory has suggested that the "wave" of the SW complex may result from the activation of an intracortical feedback inhibitory mechanism (Exp.Neurol., 73: 43-71, 1981). Furthermore we also showed that cortical recurrent inhibition (RI) induced in pericruciate neurons by single shock stimulation of cerebral peduncles is not antagonized by these small concentrations of penicillin and thus could participate in this mechanism (Brain Res., 1983, in press). The aims of the present experiments were: (a) to analyze the extracellular responses of pericruciate neurons to single shock cortical stimulation and compare in the same neuron this type of inhibition (Exp. Brain Res., 9: 137-154, 1969) with RI during the development of SW discharges (after i.m. penicillin); (b) to study in intracellular recordings of pericruciate neurons performed with 3M KCl filled glass microelectrodes the hyperpolarization associated with the "wave" of the SW complex.

The extracellular data, studied as peristimulus time histograms, showed that: (a) the duration of the periods of decreased firing probability induced by cortical since shock stimulation as well as RI did not change after i.m. penicillin up to and including the stage of SW discharges; (b) both types of inhibition vanished as EEG tonic-clonic seizures appeared. In the intracellularly recorded neurons we found that: (a) intracellular injections of chloride ions decreased and inverted the early component (first 50-80ms) of the hyperpolarization (duration: 150-200ms) associated with the "wave" of SW complex, but did not clearly affect the late phase of this hyperpolorizing potential; (b) a similar response to chloride injection was observed both before and after penicillin when studying the hyperpolarization (duration: 150-200ms) induced by repetitive stimulation (4-6Hz) of n. ventralis lateralis of the thalamus.

These data demonstrate that at the concentrations of penicillin present in the cortex in feline generalized epilepsy two inhibitory mechanisms (namely those evoked by cortical and cerebral peduncle stimulation) are still preserved during SW discharges. Furthermore, they also show that the early component of the hyperpolarization associated with the "wave" of SW complex of feline generalized penicillin epilepsy results from a mechanism which is chloride-sensitive and thus probably GABAergic in nature.

118.4 TOPICAL COLCHICINE AND FOCAL EXPERIMENTAL EPILEPSY: AN INTRACELLULAR STUDY. A. F. Reynolds, J. C. Oakley. Neurophysiology Lab, Tucson, VAMC, Tucson, AZ 85723.

This is a follow up study to an extracellular study in rat on the generation of experimental focal epilepsy after colchicine application (Oakley, J.C., Neurosci Abstr 7:206.12, 1981). The present study investigates the intracellular events underlying neuronal bursts coincident with surface electrocorticographic (ECoG) spikes. ECoG spikes developed 8-33 minutes following the application of 10^{-1}M colchicine to sigmoid cortex in the locally anesthetized, paralyzed cat. The bursts were coincident with depolarizing potentials (HPs). Membrane potential oscillated 4-5 minutes with very slow depolarization between bursts. These events were seen in a zone 0.5-2 mm away from the point of colchicine application. At the point of application there was electrical silence coincident with the ECoG spike and if the ECoG spikes were frequent additive depolarization was seen.

	N	DP Amp.	DP Dur.	HP Amp.	HP Dur.
Neurons	81	14mv(5-25)	205ms(80-500)	7mv(5-10)	200ms(100-350)
Glia	60	14mv(5-30)	2.1s(25-4)	----	------

Colchicine binds to intracellular proteins involved in intracellular Ca^{++} metabolism and may prove useful in investigating intracellular mechanisms of epilepsy.

DP Amp. = DP Amplitude
DP Dur. = DP Duration
HP Amp. = HP Amplitude
HP Dur. = HP Duration

118.5 CHANGES IN FREE Ca^{2+} RECORDED INSIDE HIPPOCAMPAL PYRAMIDAL NEURONS IN RESPONSE TO FIMBRIAL STIMULATION. M.E. Morris, K. Krnjević & N. Ropert, Depts. Anaesthesiology & Pharmacology, University of Toronto, Toronto M5S 1A8, Ont., and Depts. Anaesthesia Research & Physiology, McGill University Montréal H3G 1Y6, Québec.

In rats, mostly under urethane anaesthesia, double-barrelled microelectrodes that include a Ca^{2+}-sensitive channel (Morris & MacDonald, 1982, Soc. Neurosci. Abst. 8, 909) were used to record simultaneously the membrane potential (V_m) and free Ca^{2+}($[Ca]_i$) inside CA1 and CA3 pyramidal neurons. In 11 cells with V_m better than -50 mV (mean -58.3 mV, S.D. 4.14), mean [Ca] was 3.6 µM, S.D. 1.64, equivalent to a Ca^{2+} activity of about 1.4 µM. Although higher levels of $[Ca]_i$ were often seen in cells with poorer Vm, there was no simple correlation between Vm and $[Ca]_i$, a remarkably low and stable $[Ca]_i$ often being recorded for several minutes after almost complete decay of Vm. This is in contrast to the generally good correlation between Vm and $[K]_i$ that we observed in the same animals when recording with K^+-sensitive microelectrodes, and presumably reflects the high efficacy of Ca^{2+}-buffering by neuronal cytoplasm (Baker & Schlaepfer, 1978, J. Physiol. 276,103).

Repetitive stimulation of the fimbria (10-20 Hz, for 30-60 s), at an intensity sufficient to generate firing in bursts in CA1 and after-discharges in CA3, had the following effects. In the majority of cells there was a moderate fall in $[Ca]_i$ (by 10-50%) that was probably a passive reflection of the sharp fall in extracellular [Ca] frequently evoked by such stimulation (Krnjevic et al., 1980, Can. J. Physiol. Pharmacol. 58, 579). In some cells, a small and progressive increase in $[Ca]_i$ (by 5-20%) was evident during the tetanus and at the onset of the phase of post-tetanic hyerpolarization. In 34 tests on 22 cells, there was a far more conspicuous rise in $[Ca]_i$ - by 2-800 µM (with a median value of 22 µM) - which either started during the stimulation and reached its peak near the end of the tetanus, or was delayed for 10-60 s after the end of the tetanus. The onset was usually marked by increased firing and afterdischarges; but during the 20-60 s period of raised $[Ca]_i$, there was a temporary depression or disappearance of evoked potentials, corresponding to various degrees of post-ictal or spreading depression. These effects were usually fully reversible and sometimes could be evoked repeatedly, even by much briefer tetanic stimulation (100 Hz for 1 second). Thus, strong activation of hippocampal pyramidal cells can cause a remarkably large rise in $[Ca]_i$ - far larger than the <<1 µM changes recorded in spinal motoneurons (Krnjević et al., 1983, Canada Physiol., 13, 108). If unduly repeated during prolonged seizures, such major increases in $[Ca]_i$ could be detrimental for the long-term survival of pyramidal neurons.

(Supported by the Medical Research Council of Canada).

118.6 EFFECT OF NOREPINEPHRINE ON RECURRENT INHIBITION IN THE IN VITRO HIPPOCAMPAL SLICE PREPARATION. P. Leung*, I. Mody, and J.J. Miller (SPON: J. Weinberg). Department of Physiology, University of British Columbia, Vancouver, B. C., V6T 1W5.

Several studies have shown that norepinephrine (NE) produces a slight but significant inhibitory effect on CA1 pyramidal neurons. Following 6-hydroxydopamine depletion of hippocampal catecholamines, NE induces an enhancement of the stratum radiatum (SR)-evoked population spike response and multiple spike discharges characteristic of epileptiform activity. It was suggested that NE, in addition to exerting a direct effect on pyramidal cells, may also act upon recurrent inhibitory interneurons to produce a disinhibition of pyramidal cells (Mody, et al., Can. J. Physiol. Pharmacol., 1983, in press). The present study was undertaken to examine the effect of NE on alveus-evoked inhibition, presumed to be mediated by basket cell interneurons innervating the CA1 pyramidal cells. Experiments were carried out on the in vitro hippocampal slice preparation and inhibition was assessed by the percent reduction of the SR-evoked population spike when preceded by a conditioning pulse applied to the alveus to activate the inhibitory interneurons via the recurrent collaterals of the pyramidal cells. Paired pulse stimulation resulted in inhibition of the SR-evoked test response with conditioning-test intervals up to 60 msec. In order to assess the effect of NE on this inhibition, independent of its influence on the SR-evoked population spike response, the test pulse stimulus intensity was adjusted to maintain a similar amplitude to the pre-NE control response. NE (50 µM) perfusion resulted in a significant and reversible reduction of the alveus-evoked recurrent inhibition (average: 75%; range: 50-90%). These data suggest the possiblity that NE may result in a disinhibition of hippocampal CA1 pyramidal cells due to a direct effect on recurrent inhibitory interneurons.

118.7 PENICILLIN-INDUCED EPILEPTIFORM DISCHARGES IN CA3 HIPPOCAMPAL PYRAMIDAL CELLS: A CURRENT SOURCE DENSITY ANALYSIS. J.W. Swann, R.J. Brady*, R.J. Friedman* and E.J. Smith*. Ctr. for Labs & Research, NYS Dept. of Health, Albany, NY 12201

Current source density (CSD) analysis is a procedure which utilizes extracellular field potential recordings to estimate the anatomical location at which neurophysiological events are generated. We have performed a one dimensional analysis of epileptiform events in CA3 region of hippocampal slices using CSD methods. Slices from rats 25-35 days of age were exposed to 1.7 mM penicillin. Extracellular epileptiform field events, evoked by orthodromic activation of stratum oriens or radiatum were examined. Tips of 3 recording microelectrodes in a triple array were aligned perpendicular to the pyramidal cell body layer and spaced 100µm apart. This array was moved perpendicular to the cell body layer at 25µm intervals across both dendritic trees. For each location of the electrode array, two different types of recordings were averaged (7-10 responses) and analysed on-line with a computer: 1) the field potential recorded by the center electrode in the array and 2) the CSD at that location. Recordings from the three electrodes in the array were used to determine the CSD by an electronic processing system (Nicholson and Llinas, Brain. Res. 100(1975), 418-424).

Cell body layer recordings of penicillin-induced epileptiform bursts consist of a slow positive field potential. Multiple negative-going population spikes ride on the envelope of the slower positive potential. The intracellular correlate of this positive field is a large slow depolarization shift (DS). Our CSD analysis clearly indicates that there are two large current sinks associated with DS generation. These were recorded 75-150µm from the center of the cell body layer in stratum oriens and 100-250µm into stratum radiatum.

Previous studies in our laboratory have shown that when hippocampal slices from rats, 9-19 days of age, are exposed to penicillin, they generate epileptiform bursts each of which is followed in cell body layer recordings by a prolonged negative field potential. Afterdischarges ride on the envelope of this slow field. In slices taken between postnatal days 25-35 afterdischarges were rarely observed. However, a small slow negative field potential was recorded in the cell body layer following the burst. A single current sink for this slow field was recorded 25-75µm from the center of the cell body layer in stratum oriens. Taken together our data suggest that the depolarization shift is not only dendritic in origin but is generated in both the basilar and apical dendrites. In contrast the subsequent slow field potential has its origin close to the cell body layer in stratum oriens.

Supported by Grants from the Epilepsy Foundation of America and NINCDS (NS 18309).

118.8 ADENOSINE BLOCKS NEURAL AFTERDISCHARGES GENERATED IN THE PRESENCE AND ABSENCE OF ACTIVE CHEMICAL SYNAPSES, K.S. Lee, P. Schubert*, Max Planck Institute for Psychiatry, Munich, F.R.G.

Adenosine is a potent depressant in the central nervous system which has recently been shown to exhibit anticonvulsant activity in the hippocampus. The depressive action of adenosine on spontaneous and evoked neuronal activity is generally ascribed to its capacity to depress the release of excitatory neurotransmitters, an effect thought to be mediated by a reduction of calcium influx into the presynaptic terminal. The cellular locus of adenosine's anticonvulsant effect, however, remains unclear. In the present study, extracellularly-recorded afterdischarges elicited in the presence of either penicillin (Pen-G 2,000-5,000 units per ml) or low calcium (0.2 mM Ca++; 4.0 mM Mg++) were examined in the CA1 region of the hippocampal slice. In normal medium (2.2 mM Ca++; 2.0 mM Mg++), antidromic stimulation via an electrode located in the alveus evokes a single negative spike recorded in the pyramidal cell layer. In contrast, in medium containing penicillin or low concentrations of calcium, one or more additional negative deflections are observed which are usually smaller in amplitude than the initial spike. Superfusion of adenosine or certain other adenosine analogues resulted in a reduction or elimination of the secondary negative deflections while apparently not affecting the amplitude of the first spike. The second wave (i.e the first afterdischarge) was reduced by 50% in the presence of 2-5 µM adenosine with the subsequent waves being progressively more sensitive. L-phenyisopropyladenosine (L-PIA) was substantially more potent than D-phenylisopropyladenosine (D-PIA) in attenuating the afterdischarges (IC50s of 60 nM and 2 µM respectively), characteristic of an A1 type adenosine receptor. When adenosine was introduced by pressure microinjections, the proximal apical dendritic region was the most sensitive zone for the reduction of afterdischarges. Again in these experiments the initial antidromic spike was not affected by adenosine. The effect of adenosine (either microinjected or in the medium) was antagonized by the addition of theophylline (50-200 µM) to the bathing medium.

The present studies suggest that the anticonvulsant action of adenosine ocuurs at a postsynaptic site and that this effect is most potent in the apical dendrites. Furthermore the stereospecificity of the actions of L-PIA and D-PIA indicates that this effect may be mediated by an A1 type adenosine receptor.

118.9 KAINIC ACID MODIFIES SOME INPUT-OUTPUT RELATIONSHIPS IN THE IN VITRO RAT HIPPOCAMPUS. P. Aitken. Dept. of Physiology, Duke Univ. Med. Center, Durham, NC 27710.

Slices of rat hippocampus were cut at 400μ and maintained in an interface chamber at 35-36°C in a perfusion medium with ionic composition similar to that of rat extracellular brain fluid (with potassium = 3.5mM and calcium = 1.2mM). Extracellular recording electrodes were placed in the cell body and apical dendrite layers of the CA1 region, and a tungsten stimulating electrode in the stratum radiatum. Trains of 4 constant-current stimulus pulses (100μsec, 0.5Hz, 20-150μA) were delivered and the resulting responses averaged and stored on computer; a series of 10 such stimulus trains, at different intensities, was delivered every 15 minutes before, during, and after a 1 or 2 hour perfusion with medium containing kainic acid (KA; 0.05, 0.1, or 0.2μM). Presynaptic volley and postsynaptic population spike amplitudes were measured as peak-to-peak voltages; population epsp magnitude was measured as maximum slope during the initial stage of the deflection. Input-output (i-o) curves were generated for each stimulation series by plotting (1) The prevolley amplitude vs. the epsp magnitude, and (2) The epsp magnitude vs. the population spike amplitude. For both types of i-o curve, the slope of the linear portion of the curve was determined by a least-squares fit, and taken as a measure of the effectiveness of i-o transfer.

KA had no effect on the slope of either type of i-o curve. There were, however, reversible changes in several other i-o relationships: (1) A decrease in the epsp magnitude required to evoke both single and multiple population spikes, (2) An increase in the prevolley amplitude evoked by a given stimulus intensity, and (3) An increase in the epsp magnitude evoked by a given prevolley amplitude.

The first 2 findings are consistent with suggestions made by others that KA's convulsant action is the result of an increase in the excitability of nerve membranes; the last finding may indicate that KA also has an effect on synaptic function.

(Supported by NIH Grant #17771).

118.10 EXTRACELLULAR POTASSIUM CONTROLS THE FREQUENCY OF SPONTANEOUS INTERICTAL DISCHARGES IN HIPPOCAMPAL SLICES. Paul A. Rutecki* and Daniel Johnston (SPON: F. Pirozzolo). Neurol. Dept. and Neurosci. Prog., Baylor Coll. of Med., Houston, TX 77030.

When applied to hippocampal slices in vitro, many convulsant agents (e.g. penicillin, picrotoxin, bicuculline) produce spontaneous, interictal-burst discharges, which occur at a relatively constant frequency (between 0.05 and 0.5 Hz depending on the agent). The parameters that control the frequency of spontaneous interictal discharges are unknown. The concentration of extracellular potassium ($[K]_o$) has been shown to increase during the interictal discharge, and this increase has been implicated in the transition from interictal to ictal states. The purpose of this study is to test the hypothesis that the frequency of spontaneous interictal discharges is dependent on $[K]_o$ and to quantify the relationship between frequency and $[K]_o$.

400-600 μm thick transverse hippocampal slices were prepared in conventional manner from Sprague-Dawley rats. With $[K]_o$ less than about 4 mM, 10 μM bicuculline methiodide (BMI) in the bath saline produced only irregularly-occurring interictal discharges. The BMI-induced, spontaneous-discharge rate did not become regular until $[K]_o$ was increased to 5 mM or greater. Increasing $[K]_o$ from 5 through 10 mM increased the frequency from about 0.1 to 0.5 Hz, a five-fold change in frequency for a two-fold change in $[K]_o$. The spontaneous-discharge rate, for any given $[K]_o$, was quite consistent and independent of the preceeding $[K]_o$. The pooled results from a number of experiments suggest a sigmoidal relationship between the frequency of spontaneous interictal discharges and log ($[K]_o$).

In the absence of BMI, increasing $[K]_o$ to a value greater than about 6 mM also produced regularly-occurring interictal discharges. Varying $[K]_o$ from 6.5 to 11 mM, increased the frequency from about 0.3 to greater than 1 Hz. At any given $[K]_o$ ($\geq$ 6.5 mM), the frequency of spontaneous interictal discharges was less with BMI present.

These results support the hypothesis that the frequency of spontaneous interictal discharges is dependent on $[K]_o$. If changes in frequency are important for transitions to the ictal state, then these results would suggest a role for $[K]_o$ in those transitions. Our results show that $[K]_o$ levels within the range that have been measured in vivo during seizures or interictal activity can cause interictal type bursting in vitro in the absence of a convulsant agent. The cellular events that are being altered by increases in $[K]_o$ and that are important for controlling frequency are currently being investigated. (Supported by the Grass Foundation and NIH grants NS11535 and NS18295).

118.11 INITIATION OF SYNCHRONIZED BURSTING IN NEOCORTEX. C.P. George* and B.W. Connors. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Isolated slices of neocortex, when exposed to GABA antagonists, display paroxysmal burst discharges which are similar to epileptogenic interictal events recorded in vivo (Gutnick et al., J. Neurophysiol. 48:1321, 1982). Paroxysmal discharges arise from uniquely large excitatory synaptic conductances which occur synchronously in each neuron of a local population. We have now investigated the initiation of synchronized bursts in greater detail.

In vitro slices (500 μm thick, 35°C) of guinea pig somatosensory cortex were exposed to bicuculline (5-50 μM)-containing media. Low intensity electrical stimuli to the white matter elicited all-or-none paroxysmal extracellular field potentials (PFPs) which occurred in synchrony with intracellularly recorded neuronal bursts. Each PFP was also correlated with a glial depolarization (10-15 mV) and an increase in extracellular $[K^+]$ (2-5 mM) in the middle cortical layers. The PFP consisted of an early, brief (15-20 msec) negative spike followed by a more prolonged negative wave which decayed over several seconds. The amplitude was greatest (1-4 mV) at a depth of 400-800 μm from the pial surface. Once initiated, the PFP propagated laterally (0.1-0.3 m/sec), without decrement, to encompass the entire slice. Although spontaneous PFPs were rare in slices treated with low bicuculline concentrations, their frequency was greatly enhanced by high bicuculline levels, addition of 4-aminopyridine (4-AP) or repeated glutamate ejections. Groups of spontaneous PFPs were occasionally followed by spreading depression, which propagated at 4 mm/min.

We hypothesized that synchronized bursts were initiated by a spatially discrete subpopulation of cortical neurons. To test this notion PFPs were evoked by short (5-200 msec), focal pressure ejections of the excitatory amino acid L-glutamate. Thresholds for discharge initiation (i.e. the minimal amount of glutamate evoking a PFP) were measured at various cortical laminae. Threshold minima were consistently centered on layer IV, as determined from histological reconstruction. Conversely, focal GABA was most effective in preventing electrically evoked PFPs when it was applied to the same lamina. Finally, in slices bathed in normal medium, ejection of small amounts of bicuculline yielded evoked PFPs only when applied to the middle cortical layers.

We conclude that neocortical slices contain the circuitry necessary for generating evoked and spontaneous epileptic discharges and spreading depression. The data suggest that certain cellular elements in layer IV, when disinhibited, have a relatively low threshold for burst generation. These cells act as initiators of a synchronous, synaptically driven network burst in other cortical layers.

Supported by BRS grant RR 5353 and NS 06477 from the NIH.

118.12 **EFFECTS OF CONVULSANT AND ANTICONVULSANT DRUGS ON POTASSIUM INACTIVATION** D. Doerner and L.D. Partridge. Physiol. Dept., Univ. of New Mexico, Albuquerque, N.M., 87131.

Spike broadening, a phenomenon common to many repetitively firing neurons, is characterized by the development of a shoulder on the falling phase of successive action potentials. Aldrich et al. (J.P.L. 291:507-530,1979) have shown that broadening is due to cumulative potassium inactivation in the presence of a maintained calcium current.

We have observed that spike broadening is affected by certain convulsant and anticonvulsant drugs. The convulsant drug Pentylenetetrazol (PTZ) causes a slight decrease in the total amount of broadening but decreases the time taken to reach maximal broadening. The anticonvulsant diphenylhydantoin (DPH) significantly depresses the amount of broadening. Both of these effects persist when calcium currents have been blocked suggesting that they result from an effect on potassium inactivation.

Voltage clamp studies were performed in order to assess the effect of these drugs on potassium currents. Two experimental paradigms were used, one being a 6 second maintained pulse and the other a series of 600 msec. pulses at a frequency of 1.5 Hz. Similar results were obtained with both experimental procedures. Potassium currents, in the presence of PTZ, inactivated less but exhibited a shorter time constant of inactivation. DPH caused a marked reduction in total potassium inactivation but showed no apparent change in time constant.

Since transmitter release can be a function of spike duration, spike broadening is a possible mechanism by which synaptic efficacy could be increased. The depression of potassium inactivation by DPH is one possible explanation for this drug's anticonvulsant effects. The more rapid onset of potassium inactivation in the presence of PTZ might facilitate synaptic transmission during short bursts of action potentials. PTZ causes repetitively firing neurons to produce short high-frequency bursts.

This work was supported by NSF grant 8002011.

118.13 MECHANISMS UNDERLYING PICROTOXIN-INDUCED EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPUS. John J. Hablitz, Department of Neurology, Baylor College of Medicine, Houston, Texas 77030

During studies concerning the mechanism of GABA antagonism by various convulsant agents, it was noticed that paroxysmal depolarizing shifts (PDSs) induced by picrotoxin were qualitatively different from those seen after exposure of slices to penicillin. Since PTX had been shown previously to block a Cl- sensitive afterhyperpolarization (AHP), that persisted after penicillin exposure, it was used here for the investigation of spontaneous and evoked PDSs in the absence of demonstrable inhibition.

Intracellular recordings were made in the CA3 region of guinea pig hippocampal slices during the bath application of PTX (50-200uM). Neither the resting potential, input resistance nor the ability of depolarizing current pulses to trigger instrinsic burst responses was affected by addition of the convulsant agent. In contrast, the EPSP-IPSP sequence elicited by mossy fiber stimulation was markedly altered. Within 1-2 minutes PTX reduced the amplitude of the IPSP, which was associated with the appearance of multiple late depolarizations followed by a prolonged hyperpolarization. The initial IPSP then decreased further with concomitant increases in the amplitude of the initial EPSP and late depolarizations. By 5 minutes each depolarizing component was of sufficient amplitude to trigger action potentials, and they gradually merged to form a single epileptiform response consisting of a typical PDS, followed by one or more rhythmic afterdischarges (ADs). Simultaneous intracellular recordings from pairs of CA3 neurons indicated synchronous discharge during the ADs as well as the PDS, suggesting that ADs were synaptic in origin and might result from recurrent excitatory inputs. PDSs could occasionally be triggered by activation of neuronal pairs. Extracellular recordings from the CA1 and CA3 regions showed that spontaneous PDSs originated in the CA3 areas and subsequently appeared in CA1. In many cases, a late secondary discharge was seen in CA3, suggesting a reverberation of excitation between the regions. To better understand the origin of this complex series of events, the two regions were surgically isolated. In contrast to penicillin treated slices, spontaneous PDSs were recorded from both regions. PDSs occurred with remarkable regularity in isolated CA3 sections, while an almost random pattern of significantly longer interburst intervals was seen in CA1. PDSs in both regions were generally followed by an AHP. The AHP duration was not correlated with the interval between bursts.

Further investigation of PTX induced activity should prove useful in studying the mechanism of AD generation and testing the generality of theories developed from the penicillin model.
(Supported by NS-11535 and the Epilepsy Foundation of America)

118.14 INTRACELLULAR STUDIES OF THE EFFECTS OF BACLOFEN ON BICUCULLINE-INDUCED EPILEPTIFORM ACTIVITY IN CA3 PYRAMIDAL CELLS. Robert J. Brady* and John W. Swann (SPON: M. Pierson), Center for Laboratories and Research, NYS Dept. of Health, Albany, NY 12201

Intracellular recordings were obtained from the CA3 neurons in rat hippocampal slices. A second extracellular microelectrode was placed in the cell body layer, close to the intracellular recording site, to simultaneously monitor field potentials. Epileptiform activity was induced by bath application of bicuculline methiodide (BIC) at a concentration of 25, 50 or 100μm. Depolarization shifts (DS) were recorded intracellularly and were coincident with epileptiform bursts in the extracellular field. Our recordings demonstrate that bath application of 10 or 100μm (+) baclofen rapidly (<10 min) blocked all spontaneous epileptiform discharges in CA3 pyramidal cells. The threshold at which stratum radiatum stimulation elicited epileptiform activity rose in response to baclofen application in a concentration dependant manner. A concentration of 10μm baclofen increased the DS threshold approximately 2 fold while 100μm baclofen often completely blocked evoked epileptiform activity even at supramaximal stimulation strengths. In contrast, 1μm baclofen had little effect on DS stimulation threshold and did not alter the frequency of spontaneous epileptiform activity in slices. The time course and extent of baclofen's effect were insensitive to changes in BIC concentration.

Intracellular recordings revealed that application of baclofen at concentrations of 10 or 100μm resulted in a large increase in the conductance of the CA3 pyramidal cell membrane and an associated hyperpolarization of the cell of up to 10mV. These latter effects persisted in slices treated with tetrodotoxin (TTX) which indicate they are mediated by a post-synaptic mechanism. Application of BIC to slices treated with TTX and baclofen did not reverse the conductance change or the hyperpolarization. The effects of baclofen were reversed when the drug was washed from the experimental chamber. Intracellular and field recordings were obtained from CA3 pyramidal cells of slices treated with BIC (25 and 100μm) and subsequently exposed to the GABA agonist, muscimol, (100μm). In contrast to the action of baclofen, muscimol did not block BIC-induced epileptiform activity. We have also bath applied GABA to slices treated with 25 or 100μm BIC. At concentrations of 100μM or 1mM GABA had no apparent effect. At a concentration of 10mM GABA rapidly (<10 min) blocked spontaneous and evoked epileptiform activity and brought about a conductance increase in individual CA3 neurons. This effect seems to be BIC insensitive. Increasing the bath concentration of BIC from 25 to 100μm did not change the time course or magnitude of GABA's effects. Supported by Grants from the Epilepsy Foundation of America and NINCDS (NS 18309).

118.15 EPILEPTIFORM ACTIVITY INDUCED BY BATH APPLICATION OF GABA TO IMMATURE HIPPOCAMPAL SLICES. Thomas J. Chesnut, John W. Swann and David O. Carpenter. Center for Laboratories and Research, NY State Dept. of Health, Albany, NY 12201

Swann and Brady (Soc. Neurosci. Abstr. 8:1016, 1982) reported that repetitive orthodromic stimulation of immature CA_3 hippocampal pyramidal cells can lead to the development of afterdischarges. Ben-Ari et al. (Can. J. Physiol., 57:1462-1466, 1979) reported that tetanic stimulation can result in a loss of efficacy of GABA inhibition in mature hippocampus and suggested that this disinhibition may be due to desensitization. Consequently, we performed the following experiments to test if GABA desensitization can cause seizure activity in the immature hippocampus.

Extracellular field recordings in response to stimulation of stratum radiatum were obtained from the cell body layers of areas CA_2 and CA_3 of transverse slices obtained from hippocampi of immature (10-18 day old) rats. Addition of 1-10 mM GABA to the perfused Ringer induced multiple population spikes and also the appearance of spontaneous seizures culminating in spreading depression. The appearance or augmentation of a slow negative field potential previously reported by Brady and Swann (Soc. Neurosci. Abstr. 8:1016, 1982) to be associated with epileptiform activity in penicillin treated slices was also observed. The amplitude of the population spike was increased as was that of the field positivity recorded in the cell body layer. The positivity occasionally was prolonged to 1000 msec or more in duration. Recordings of the field EPSP from stratum radiatum reflected this prolonged time course. However, the effects at these GABA concentrations were not observed in every slice. The reproducibility of GABA's effects was improved and the GABA concentration needed was decreased by a factor of 100 by the addition of (±)-nipecotic acid (0.1-1.0 mM) to the bath. This compound is a competitive inhibitor of GABA uptake into neuronal elements and was added to produce a calculated 95% block. Perfusion of (±)-nipecotic acid alone occasionally elicited epileptiform activity, but less often and not to the same degree as when GABA was present. These observations are consistent with disinhibition as a result of the GABA perfusion. That desensitization of GABA receptors was responsible for the disinhibition and consequently for the epileptiform activity remains a possibility.
(Supported by NIH Postdoctoral Fellowship NS06930 to Thomas J. Chesnut and NIH grants NS18309 to John W. Swann and NS18435 to David O. Carpenter.)

118.16 ORGANOPHOSPHATE-INDUCED EPILEPTIFORM ACTIVITY IN RAT HIPPOCAMPAL NEURONS. F.J. Lebeda, P.A. Rutecki* & D. Johnston. Dept. of Neurol. & Prog. in Neurosci., Baylor Col. of Med., Houston, TX 77030.

Previous in vitro studies have shown that certain convulsants (i.e., bicuculline, picrotoxin, penicillin and curare) produce regularly occurring, paroxysmal depolarizing shifts (PDSs) in hippocampal neurons, which are due to large, excitatory postsynaptic potentials (Johnston & Brown, Science 211:294, 1981; Lebeda et al., J. Neurophysiol. 48:622, 1982). The amplitudes of these waveforms are monotonic functions of membrane potential and reverse in polarity near 0 mV. Concomitantly, these agents appear to abolish spontaneously occurring inhibitory postsynaptic potentials (IPSPs), which suggests that interference by these compounds with the putative GABAergic system is involved in generating the PDSs. The question we addressed was whether disinhibition is a general mechanism of action for other convulsants.

Rat hippocampal slices were prepared in a standard manner and continuously perfused with physiological media. Extracellular and intracellular recordings were made from the CA3 subfield.

Bath application of the organophosphorus anticholinesterase (anti-ChE), diisopropyl phosphorofluoridate (DFP), at 10 μM, produced spontaneously occurring (.1-.3 Hz) field discharges, which resemble those events caused by the other agents. This DFP-induced activity was not antagonized by the ChE reactivator, pralidoxime (10-100 μM), and ceased upon washout. At higher concentrations of DFP (25-50 μM), these events were accompanied (ca. every 30 sec) by prolonged discharges (1-2 sec). Occasionally, these events were replaced by large (10-20 mV), long lasting (1-2 min), negative-going field potentials during which evoked responses could not be elicited, a phenomenon reminiscent of spreading depression (SD).

Other anti-ChE agents tested (physostigmine, pyridostigmine, 10-200 μM) did not produce repetitive field activity. Two putative K-channel blockers, 4-aminopyridine (4AP, 1-50 μM) and tetraethylammonium (TEA, 1-10 mM), however, were found to generate repetitive and SD waveforms similar to those seen with DFP.

Intracellular recordings in the presence of 4AP or DFP revealed that spontaneously occurring IPSPs were prominent and that the apparent reversal potential of the PDS was more negative than with the previously examined convulsants, a finding that is also consistent with the idea that an inhibitory synaptic input is still functional. These results further suggest that DFP, 4AP and TEA represent a class of convulsants whose principle mode of action is not disinhibition, but instead may involve the modulation of K-currents or some other process.
(Supported by USAMRDC DAMD-17-82-C-2254, the Grass Foundation, and NIH grants NS11535 and NS18295).

118.17 EFFECT OF VALPROIC ACID ON CHOLINERGIC TRANSMISSION. Karim A. Alkadhi. Department of Pharmacology, University of Houston, Houston, TX 77004.

Valproic acid (VPA) is an anticonvulsant drug chemically unrelated to other anticonvulsant agents. The mechanism by which VPA exerts its antiepileptic effect has not been established. Although numerous studies have shown that VPA raises gama-aminobutyri acid (GABA) level, others presented evidence that cast some doubt on the GABA hypothesis (e.g. Anlezark et al. Biochem. Pharmacol. 25:413, 1976). The aim of the present experiments was to obtain information on possible effects of VPA on cholinergic synaptic transmission using frog neuromuscular junction as a model.

In magnesium-blocked intact cutaneous pectoris muscles, VPA in clinically relevant concentrations produced a concentration-dependent decrease in the quantal content of the endplate potential EPP). No apparent change in the resting membrane potential of the muscle fiber was observed. There was a slight decrease (about 20%, 1mM VPA) in the amplitude of miniature EPP (MEPP) but no apparent change in the frequency of MEPP was seen. The effects of VPA were not readily reversible on washing and residual effects of the drug were still present after 20-30 min of continuous superfusion with drug-free Ringer's solution.

Preliminary testing of VPA on release parameters during low frequency facilitation (Maeno and Edwards. J. Neurophysiol. 32: 785, 1969) suggested a parallel downward shift in the frequency facilitation curve.

In voltage-clamped transected cutaneous pectoris muscle fibers, preliminary experiments suggested that VPA (<0.1 mM) had no effect on the time constant of decay (τ) of the endplate current. Investigation of higher, clinically relevant, concentrations is planned.

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118.18 EFFECTS OF CONVULSANT AGENTS ON CULTURED NEUROBLASTOMA CELLS. J. Davenport and R. Rumpf*. VA Hospital and Dept. Neurology, U. Missouri, Columbia, MO 65201.

The synapse-free soma membrane effects of standard experimental epileptogenic agents were studied in cultures of mouse neuroblastoma clone N1E-115. Cells were grown to confluency in DMEM (high glucose, no pyruvate) with 10% FCS, streptomycin and 20 mM Hepes. Morphological differentiation was achieved with 2% DMSO added to growth media in final subculture. After 2-4 weeks, differentiated cells of 65-150 um diameter and possessing 2-9 major neurites were penetrated with 2M KCl-filled micropipettes having DC resistance 15-40 MΩ, using inverted phase microscopy. Standard single-electrode current-clamping techniques yielded reproducible data of somatic resting potential, DC input resistance, Na^+, K^+, and Ca^{2+} currents and repetitive firing behavior. Intracellular recordings were made during constant superfusion with phosphate-buffered saline solution (Tuttle, Richelson. Brain Res 1975;84:129), to which convulsants were added: penicillin 25 mM; picrotoxin 100 uM; pentylenetetrazol 100 mM; bicuculline 100 mM; strychnine 200 uM in final concentration. Specific ionic current behaviors were examined by the use of TTX, TEA and calcium exclusion.

Stable resting membrane potential was 40 ± 16 mV, input resistance was 27 ± 15 MΩ; no repetitive firing was observed in the absence of applied current, which induced decrementing action potentials in 28% of studied cells. Penicillin, picrotoxin, pentylenetetrazol and strychnine had no effect on any examined cell function. Bicuculline produced small changes in action potential shape most consistent with reduced or delayed K^+ currents.

The epileptogenic effects of these standard experimental agents appear to require neuronal mechanisms not present in cultured neuroblastoma soma membranes, possibly including pre- or post-synaptic sites, specially differentiated dendritic trees, or more complex neuronal interactions.

Supported by the Veterans Administration.

118.19 PHENYTOIN AND CARBAMAZEPINE SELECTIVELY LIMIT SUSTAINED HIGH FREQUENCY REPETITIVE FIRING OF CULTURED MOUSE NEURONS. M. J. McLean* and R. L. Macdonald (SPON: G. Siegel). Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

The effect of the anticonvulsant drugs phenytoin,carbamazepine, valproic acid and ethosuximide on sustained high frequency repetitive firing of action potentials was studied.

Mouse spinal cord neurons in primary dissociated cell culture were maintained in vitro for 4-6 weeks prior to experiments. For experiments, the cell cultures were bathed in protein-free buffered salt solutions with elevated magnesium (10 mM) to abolish spontaneous activity. Standard electrophysiological techniques were used to record transmembrane potentials of cells impaled with high resistance (20-50 MΩ) glass microelectrodes filled with 4M potassium acetate. Using the bridge technique to allow simultaneous current passage and recording of potential, long depolarizing pulses (0.5-2.0 sec) were applied to the impaled neurons. In control solution, progressively greater depolarizing pulses evoked sustained trains of action potentials firing at increasing frequency. Anticonvulsant drugs were then introduced by adding aliquots of concentrated stock solution or by superfusion of solutions containing drugs at known concentrations. The range of drug concentrations studied included values equivalent to cerebrospinal fluid concentrations of patients with therapeutic serum levels.

Phenytoin and carbamazepine limited firing to a few action potentials in a concentration-dependent manner. For phenytoin, the effect commenced at 1.0 µg/ml and was maximal at about 2-3 µg/ml. For carbamazepine the effect commenced at about 0.8 µg/ml and was maximal at about 1.5-2.0 µg/ml. The effect was rapid in onset and could be reversed by returning to drug-free solution.

Ethosuximide (25-100 µg/ml) and valproic acid (8-25 µg/ml) did not effect the ability to sustain rapid firing. Repetitive firing in the presence of these drugs did not differ significantly from that in control solution.

Selective limitation of the ability of neurons to sustain high frequency repetitive firing of action potentials at concentrations effective against clinical seizures may represent an important cellular mechanism of anticonvulsant activity of phenytoin and carbamazepine but not for valproic acid or ethosuximide.

118.20 INTERACTIONS OF FLURAZAPAM WITH GLUTAMATE EVOKED EXCITATORY RESPONSES OF CEREBELLAR PURKINJE NEURONS, J.-T. Cheng and B.D. Waterhouse, Dept. of Cell Biology, U. Tx. Hlth. Sci. Ctr., Dallas, TX 75235.

Previous studies from this laboratory have demonstrated that norepinephrine (NE) can enhance both excitatory and inhibitory responses of cerebellar Purkinje (P) cells evoked by microiontophoretic application of glutamate (GLU) and GABA, respectively. Flurazapam (FLU), an anticonvulsant drug, has also been shown to potentiate the inhibitory action of GABA on P-neurons; possibly via a mechanism shared in common with NE. The present investigation was conducted to examine the action of FLU on GLU-evoked excitation of P-neurons and compare these effects with those of NE.

Extracellular activity of cerebellar P-cells was recorded from halothane anesthetized hooded rats. Excitatory responses of P-cells (n=19) to iontophoretic pulses of GLU (13-56 nA) were examined before, during and after FLU microiontophoresis (5-13nA). FLU-induced changes in GLU evoked and spontaneous P-cell discharge were quantitatively assessed by computer based analysis of drug response histograms.

In 9 P-neurons, GLU-evoked excitations were attenuated during FLU application at ejection currents which had little or no effect on spontaneous firing rate. In 2 of these 9 cases, GLU-induced excitations were preserved when FLU began to exert a marked depressant effect on background firing, such that there was a net enhancement of the GLU response. In 7 additional cells, FLU produced a similar net facilitation of the GLU-induced response, when spontaneous discharge was suppressed by 13% or more. FLU had no effect on GLU excitation in 3 cases. Iontophoretic administration of FLU also revealed or potentiated a post-GLU depression of firing in 7 P-cells at doses which suppressed spontaneous discharge.

In summary, these results suggest that flurazapam has a twofold, dose dependent action on P-neuron responsiveness to GLU. At low doses, FLU attenuates GLU-induced excitation. At higher doses which suppress background firing, FLU enhances GLU-induced excitation relative to spontaneous discharge and facilitates post-excitatory depression of activity. A combination of such effects in intact neuronal circuits may contribute to the anticonvulsant actions of this compound. The facilitating actions of FLU on GLU excitation and post-excitatory depression as reported here are similar to those observed for NE; thus the possibility must be considered that these agents share a common mechanism to augment P-cell responsiveness to GLU. (Supported by NINCDS NS 18081 and the Klingenstein Foundation to BDW).

119.1 INNATE ABNORMALITIES IN CNS SEROTONIN CONCENTRATIONS IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs). Phillip C. Jobe, Hugh E. Laird, II and John W. Dailey. Departments of Pharmacology and Psychiatry, LSU Medical Center, Shreveport, LA 71130 and Department of Pharmacology and Toxicology, University of Arizona, College of Pharmacy, Tucson, AZ 85721.

Drug studies support the concept that CNS serotonergic transmission attenuates seizure intensity in the GEPR and that a serotonergic deficit is one determinant of seizure susceptibility in this model. Pathophysiological studies of non-drug treated GEPRs with moderately severe seizures show that, after exposure to a seizure provoking stimulus, deficits in serotonin levels exist in the telencephalon, hypothalamus, midbrain and pons-medulla. The purpose of the present investigation was to determine: (1) whether these deficits exist in GEPRs not exposed to the seizure provoking stimulus; and (2) whether GEPRs with severe seizures have greater deficits than those with moderate seizures. Both the severe seizure and moderate seizure GEPRs were characterized by abnormally low serotonin concentrations in the telencephalon. In midbrain, pons-medulla and cerebellum there were no statistically significant differences in the serotonin concentration when moderate and severe seizure GEPRs were compared with control. In the hypothalamus-thalamus the serotonin concentration was indistinguishable from control in the moderate seizure GEPRs whereas the severe seizure GEPRs had a serotinin concentration that was highly significantly lower than that of control and of the moderate seizure GEPRs. When taken together with the pharmacologic data these pathophysiologic data suggest an innate seizure regulatory role for serotonin in the GEPR (Supported in part by 1R01 NS 16829).

119.2 NEURONAL PROTEIN PHOSPHORYLATION IN A GENETIC MODEL OF EPILEPSY. J.G. Bajorek* and A.V. Delgado-Escueta (SPON:P.Lomax).Dept.of Neurology,Univ. Calif. Los Angeles, and V.A. Hospital Wadsworth,Los Angeles,Calif.

The phosphorylation of specific membrane proteins has been related to several systems which control excitability in neuronal elements. Such proteins including receptors , ionic pump enzyme components and proteins involved in synaptic transmitter release could under conditions which alter their phosphorylative state influence excitability in such a way as to allow epileptic phenomena to be expressed. The approach which we have taken to verify the hypothesis that such proteins are involved in epileptic disorders is to evaluate the state of phosphorylation of specific brain proteins in genetic models of epilepsy. The most important advantage of the models is that they allow differentiation between the effects of seizures from any genetically determined differences in susceptibility.Endogenous phosphorylation was measured in an in vitro assay using P32-ATP incubated for 90 sec. with cortical neuronal membranes. The membrane proteins were separated on SDS-PAGE electrophoresis, and the gels autoradiographed on X-ray film. In the seizure sensitive mongolian gerbil we found that proteins at 16,000,55,000,and 80,000 dalton molecular weight had an increased level of endogenous phosphorylation as compared to seizure resistant control animals. The seizure sensitive animals had been tested in an interictal state. If they were allowed to seize and the phosphorylative state measured, the 16K and 55K values returned to control levels, while the 80K value remained elevated. Other phosphoproteins at 41K,45K,50K,and 65K did not exibit any differences in endogenous phosphorylation using this method. The significance of these changes is being determined by identifying the proteins and evaluating how their phosphorylation is altered by regulatory systems. The genetic differences could be a result of alterations in the proteins themselves, of modulators of the phosphorylation reactions, or of alterations in linked groups of events(such as receptor-depolarization coupling) which may be mediated through phosphorylative steps.

119.3 Ca^{2+} ATPase ACTIVITY IN SUBCELLULAR BRAIN FRACTIONS FROM MICE SUSCEPTIBLE TO AUDIOGENIC SEIZURES. S.T. Palayoor* and T.N. Seyfried, Dept. of Neurology, Yale Univ. Sch. Med., New Haven, CT 06510.

Audiogenic seizure (AGS) susceptible DBA/2J (D2) mice have a significant reduction in brain Ca^{2+} ATPase activity compared to AGS resistant C57BL/6 (B6) mice. We recently showed that this reduction was genetically associated with differences in AGS susceptibility among 21 day-old C57 X DBA recombinant inbred strains (Trans. Amer. Soc. Neurochem. 14: 154, 1983). No genetic association was found, however, between AGS susceptibility and the activity of other ATPase, i.e., total, Na^{+}-K^{+}, or Mg^{2+}. Hence, reduced Ca^{2+} ATPase may be one of the underlying mechanisms responsible for AGS susceptibility in mice. The Ca^{2+} ATPase activity (μ mol Pi/hr/mg protein) was characterized further in several subcellular fractions prepared from fresh whole brain homogenates of 21 day-old B6 and D2 mice. The mean (± SEM) specific Ca^{2+} ATPase activities in the total homogenate and in the microsomol, synaptosomal, and mitochondrial fractions of B6 (n=3) were 8.16 ± 0.55, 19.98 ± 0.88, 11.80 ± 0.48, and 5.98 ± 0.11, respectively. The activities in these fractions from the D2 mice (n=4) were 5.47 ± 0.44, 11.39 ± 0.43, 6.00 ± 0.38, and 5.15 ± 0.28, respectively. The Ca^{2+} ATPase activities of the D2 mice were significantly lower than those of the B6 mice in all fractions except the mitochondrial. The Ca^{2+} ATPase activity was enriched by 45% in B6 synaptosomes (compared to the homogenate), but was enriched by only 10% in the D2 synaptosomes. When the enzyme activity was assayed at pH 9.0 instead of pH 7.4, a two-fold elevation of Ca^{2+} ATPase activity occurred in the microsomal and synaptosomal fractions of both mouse strains. The high pH stimulated mitochondrial Ca^{2+} ATPase activity by 7.5-fold in D2 mice, but by only 5.3-fold in B6 mice. Hence, differences exist between B6 and D2 mice for Ca^{2+} ATPase activity in various subcellular brain fractions. Supported by USPHS Grant (RO1NS17704).

119.4 BENZODIAZEPINE RECEPTOR BINDING DEFICIT IN MIDBRAIN OF SEIZURE-SENSITIVE GERBILS. R.W. Olsen, A.M. Snowman*, J.K. Wamsley, R. Lee*, and P. Lomax*, University of California, Riverside, CA 92521; University of Utah School of Medicine, Salt Lake City, 84132; and UCLA School of Medicine, Los Angeles 90024.

Benzodiazepine and GABA receptor binding were compared in blind studies of brain regions of seizure-sensitive (SS) and seizure-resistant (SR) Mongolian gerbils (Meriones unguiculatus). High affinity (3H)GABA or (3H)muscimol binding to specific synaptic GABA receptors in homogenates showed no difference between SS and SR (6 animals each) in cortex, cerebellum, thalamus/midbrain, or pons/medulla. (3H)Diazepam binding to brain-specific benzodiazepine receptors was similar in cortex, cerebellum, pons/medulla, hypothalamus, and hippocampus, but 25% lower in thalamus/midbrain of SS vs. SR gerbils. Subdivision of this region in another series of animals showed 13%, 17%, and 28% lower binding in membrane homogenates of SS thalamus, midbrain tegmentum and midbrain tectum, respectively. Brain slice autoradiography on (3H)-sensitive film revealed a 20% lower grain density for specific (3H)flunitrazepam binding in substantia nigra zona reticulata of SS vs. SR, 14% in nigral zona compacta, and 12% in the periaqueductal gray area. No differences in nigral pars lateralis, superior colliculus, or hippocampus, and 19% higher binding in SS interpeduncular nucleus were observed. SS animals showed lower benzodiazepine binding in midbrain whether or not animals had seizures just before sacrifice, but binding increased slightly in some regions after seizures. Since benzodiazepine receptors are modulatory sites on the postsynaptic GABA receptor/ionophore complex, the deficit in midbrain benzodiazepine receptor binding may reflect a functional GABA deficit. This could be part of the phenotypic expression of seizure sensitivity in this genetic model of human epilepsy. Such a conclusion would be consistent with recent literature describing a high potency of GABA-related anticonvulsants against gerbil seizures (W. Loscher, personal communication) and a role for the substantia nigra both in the action of anticonvulsants and in the generation and/or spread of seizures of various kinds (e.g. Iadarola and Gale, Science 218: 1237 (1982). These observations are also consistent with numerous other suggestions that impaired GABAergic inhibitory transmission may be involved in certain kinds of seizure disorders.

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119.5 NORADRENERGIC ABNORMALITIES IN THE GENETICALLY EPILEPSY-PRONE RAT: DO THEY CAUSE OR RESULT FROM SEIZURES? John W. Dailey and Phillip C. Jobe. Depts. of Pharmacology and Psychiatry, LSU Medical Center, Shreveport, LA 71130.

The genetically epilepsy prone rat (GEPR) exhibits seizures in response to stimuli that do not cause seizures in normal animals. For example, the GEPR is susceptible to seizures induced by hyperthermia and by acoustic stimulation. In addition, the GEPR exhibits abnormally low electroshock, pentylenetetrazol and bicuculline seizure thresholds. Pharmacologic studies show that drug-induced increments or decrements in noradrenergic transmission cause reciprocal changes in seizure intensity in the GEPR. Some pathophysiologic evidence suggests that the GEPR has innate abnormalities in noradrenergic neuronal tracts. Because most animals used in the pathophysiologic studies have been exposed to a seizure-provoking stimulus in order to ascertain their seizure characteristics, it has not always been possible to ascribe abnormalities in indices of noradrenergic function to the seizure-prone state. That is, the possibility exists that some observed abnormalities are due to the seizure provoking stimulus or to the seizure itself rather than to the seizure prone state.

The purpose of the present study was to determine if selected indices of noradrenergic function in the GEPR represent correlates of the seizure-prone state or if they result from a seizure or the seizure-provoking stimulus. The two indices selected for study were tyrosine hydroxylase activity and norepinephrine concentration. These indices were evaluated in: (1) GEPRs with moderate seizures (GEPR-3) that had been exposed to an acoustic stimulus and had a seizure on three occasions and (2) naive animals whose parents had both been either GEPR-3 or GEPR-9 (severe seizures) and whose previous litters had more than 90% of progeny exhibiting seizures of the same characteristics as the parents. These naive animals were not subjected to a seizure-provoking stimulus and were not known to have had a seizure. In the stimulated GEPRs tyrosine hydroxylase activity was indistinguishable from control in all brain areas except the midbrain where it was significantly elevated. In the non-stimulated progeny of GEPR parents, midbrain tyrosine hydroxylase activity was significantly reduced when compared with control and the mean enzyme activity was lower in the GEPR-9 progeny than in the GEPR-3 progeny. In both stiulated and non-stimulated animals, midbrain norepinephrine was lower than control in the GEPR-3. In naive animals, its was lower in the GEPR-9 than GEPR-3. These data suggest that some noradrenergic abnormalities in the GEPR may be substrates for seizure-prone state whereas others apparently are induced by the seizure provoking stimulus and/or the seizure itself (Supported in part by NIH grant #1 R01 NS16829).

119.6 ABNORMALITIES OF THE AUDITORY RESPONSES OF NEURONS IN THE INFERIOR COLLICULUS OF GENETICALLY EPILEPSY PRONE RATS. C.L. Faingold, M.A. Travis*, P.C. Jobe and H.E. Laird, Dept. of Pharmacology, So. Ill. Univ. Sch. Med., Springfield, IL 62708, LSU Med. Sch., Shreveport, LA 71130 and Univ. Arizona Coll. Pharmacy, Tucson, AZ 85721.

The genetically epilepsy prone (GEP) rat is highly susceptible to seizures especially those induced by high intensity sounds. The inferior colliculus (IC) has been reported to be an important site of epileptogenesis for audiogenic seizures in the GEP rat based on lesion and electrical stimulation studies. Therefore, this study examined the response properties of neurons in this nucleus to pure tone burst stimuli (100 msec duration, 5 msec rise/fall) in locally anesthetized paralyzed or ketamine anesthetized rats. The analysis of IC neuronal responses involved determination of frequency selectivity (tuning curves), characteristic frequency (CF), thresholds (at CF), Q-values and, evaluation of binaural responsiveness. Poststimulus time histograms at CF and at 12 kHz (which will induce seizures in behaving GEP rats) were also examined. Preciseness of tuning was compared for pairs of IC neurons from GEP and normal animals with the same or similar CFs using Q values. The Q values of GEP rats were significantly smaller than normal indicating that they were more broadly tuned. Thresholds at CF were significantly elevated in GEP rats as compared to normal animals. In addition, IC neurons in GEP rats showed a 19% incidence of responses with both an onset and offset peak in response to 80 dB tone bursts. The incidence of this pattern was significantly greater than the 2% level observed in normal rats. This onset-offset pattern may constitute an afterdischarge-like response similar to that observed in many types of seizures. The percentage of neurons responding with an excitatory response to binaural stimulation was significantly higher in IC neurons of GEP rats than that in normals. A recent study (Penny et al., Acta Otolaryngol., 95:1, 1982) has reported that cochlear damage is observed in the GEP rat which may subserve the reduced cochlear microphonic and abnormal auditory nerve responses previously reported and the increased response thresholds observed in the present study. These findings taken with the present data suggest that IC neurons in the GEP rat show patterns of response consistent with hyperexcitability to intense acoustic stimuli which may be the result of compensatory mechanisms to a significant loss of hearing.

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119.7 EVIDENCE FOR GABA IMBALANCE IN SEIZURE-PRONE MUTANT CHICK. J.D. Firman* and M.M. Beck. Dept. of Animal Science, Univ. of Nebraska, Lincoln, NE 68583.

Female chicks carrying the sex-linked recessive paroxysmal (px) gene are susceptible to spontaneous and audiogenic seizures similar to those of Grand Mal epilepsy. Seizure activity begins at 7-9 days posthatching, with anorexia following, and death occurring several weeks later. A dysfunction in the GABA pathway is postulated as a possible cause of the px syndrome. While serum levels of Vitamin B_6, an essential coenzyme of GABA synthesis, are not different ($P>.05$) between px and normal chicks, GABA levels in px brain tissue are elevated considerably over those of normal siblings ($P< .05$). The possibility exists that the increased GABA is not part of the neurotransmitter pool and therefore several anticonvulsants, which are thought to act via the GABA pathway, were administered in order to determine their effects on px seizures and GABA levels. Sodium valproate (300 mg/kg body wt) was administered i.p. to px and normal chicks. Protection against electrically-induced seizures was 100% effective in the px, although GABA levels increased only moderately. Valproic acid (50 and 100 mg/kg) did not affect seizures or GABA levels, indicating that this form of the drug is not effective. Gamma-vinyl-GABA (1500 mg/kg) doubled the px GABA levels, appeared not to affect seizure threshold, but transiently increased duration of stimulation necessary to elicit seizures. Sodium phenytoin (4 mg/kg) did not affect GABA levels, but increased electrical threshold by 60V (to normal level) in 50% of the px chicks. Our results indicate that the px seizures are at least in part the result of a GABA imbalance, but whether through the transmitter pool or the energy shunt remains unclear.

119.8 AGONISTS AND ANTAGONISTS OF THIP INDUCED SPIKE AND WAVE IN RATS. R. G. Fariello, G. T. Golden (SPON: L. A. Marco). Dept. of Neurology and Research, Jefferson Medical College, Philadelphia, PA 19107 and VA Medical Center, Coatesville, PA 19320.

Systemic injections of Muscimol and THIP induce a transient model of Petit Mal epilepsy in rodents. In the present study we have investigated possible agonists and antagonists of the electrobehavorial response to these direct GABA agonists. Male Sprague Dawley albino rats were prepared for chronic EEG recordings under general anesthesia. Ten days after recovery they received i.p. injection of 7.5 mg/kg THIP which induced paroxysmal bursts of S-W discharges lasting 3-5 hours. Either prior to THIP or after the THIP effect on the EEG was evident, several centrally acting drugs were i.p. administered.

EEG effects of THIP were remarkably potentiated by Valproic Acid and Baclofen and suppressed by Diazepam, Clonazepam and Ethosuximide. Other drugs such as Glutamate, Aspartate, Homocysteic acid, GDEE, 2-amino-7-phosphonoheptanoic acid, Atropine, Haloperidol, Naloxone, Bicuculline, Picrotoxin and DABA did not effect the electrobehavioral pattern.

An interaction between the possibly presynaptic bicuculline insensitive GABA receptor and the BZP linked low affinity receptor may be involved in the generation of the observed EEG abnormalities.

119.9 GABA EXACERBATES PETIT MAL LIKE SEIZURES PRODUCED BY γ-HYDROXYBUTYRATE. O.C. Snead. Department of Pediatrics and The Neuroscience Program, University of Alabama in Birmingham School of Medicine, Birmingham, Al. 35233.

Gamma-hydroxybutyric acid (GHB) is a naturally occurring, epileptogenic metabolite of γ-aminobutyric acid (GABA). When administered systemically to animals, GHB produces seizures which bear electrical, behavioral, and pharmacologic resemblance to petit mal epilepsy (Snead, Neurology 28:643, 1978). Because of the structural similarity of this compound to, and its intimate metabolic relation with GABA, it has been postulated that GHB produces seizures via GABAergic mechanisms. Therefore, the object of these experiments was to ascertain what role, if any, GABA might play in the genesis and propagation of GHB seizures.

γ-butyrolactone (GBL), the prodrug of GHB was used to induce seizures. The model was standardized by staging EEG changes at various dosages and determining the brain pharmacokinetics of GHB with each dose of GBL. A dose of 100 mg/kg GBL produced a predictable sequence of electrical and behavioral events in terms of morphology of seizure, time of onset, and duration. Animals were then given either GABA-T inhibitors γ-vinyl GABA, γ-acetylenic GABA, and aminooxyacetic acid, the GABA agonist muscimol, intracerebroventricular GABA, or the GABA antagonists bicuculline, picrotoxin, and DL Allylglycine and the behavioral and electrical response to 100 mg/kg GBL determined. The effect of these compounds on the brain pharmacokinetics of GHB was also determined.

Muscimol, GABA and all the GABA-T inhibitors produced no EEG changes themselves but resulted in a significant prolongation of GBL induced seizure with muscimol being the most potent in this regard. The GABA-T inhibitors prolonged the half life of GHB in brain but muscimol did not. GBL potentiated bicuculline seizures, protected against picrotoxin seizures, and had no effect on seizures produced by DL-Allylglycine.

These data suggest that GABAergic mechanisms may well play a role in the petit mal-like seizures seen in GBL treated animals although such mechanisms are probably not solely responsible for this phenomenon.

119.10 DIAZEPAM PREVENTS LITHIUM-PILOCARPINE NEUROTOXICITY IN RATS. John W. Olney, Michael P. Honchar and William R. Sherman*, Washington Univ, Dept of Psychiatry, St. Louis, MO 63110.

Treatment of adult rats with the cholinergic agonist pilocarpine (Pilo) (30 mg/kg sc) or the cholinesterase inhibitor physostigmine (0.4 mg/kg sc) 24 hr after a single sc injection of lithium chloride (Li) (3 meq/kg) results in an acute seizure-related brain damage (SRBD) syndrome (Honchar et al., Science 200, 323, 1983). At the above doses Li alone or Pilo alone results in a 3-5 fold elevation of brain myoinositol-1-phosphate (MIP) which is potentiated to a 40-fold elevation by Li + Pilo treatment. The repetitive seizures induced by Li + Pilo may contribute to but does not explain the massive MIP elevation since a high dose of Li (10 meq/kg sc) by itself produces a 40-fold elevation of brain MIP in the absence of seizures. The action of Li on MIP is thought to be cholinergically mediated as it is prevented by co-administration of the muscarinic antagonist, atropine. Moreover, in Li-pretreated rats, administration of atropine (150 mg/kg sc) immediately prior to Pilo prevents all aspects of the Li/cholinotoxic syndrome (MIP elevation, seizures, brain damage). Here we have compared atropine and the anticonvulsant diazepam (Dz) for their ability to modify the MIP elevating activity of Li or to either prevent or arrest the SRBD effects of Li + Pilo treatment.

Although atropine, when administered 1 hr after Pilo, substantially reduced the MIP elevation, it did not modify the seizure activity or accompanying brain damage. Dz (20 mg/kg) had no influence on the MIP elevating action of Li, but attenuated the MIP elevating action of Li + Pilo and prevented Li + Pilo from inducing either seizures or brain damage. Moreover, Dz treatment 1 hr following Pilo arrested the seizure activity and decreased the severity of brain damage. The ability of atropine but not Dz to modify the MIP elevating action of Li further supports the assumption that this action is cholinergically mediated. The ability of atropine to block the SRBD syndrome when administered before but not after commencement of seizures probably reflects the confinement of atropine's action to cholinoceptive circuits involved in initiating seizures. The efficacy of Dz when administered either before or after seizures suggests a more general influence of Dz over circuits concerned with the propagation and maintenance as well as initiation of seizure activity.

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119.11 THE ROLE OF SEIZURE ACTIVITY IN THE ACUTE HIPPOCAMPAL PATHOLOGY PRODUCED BY TRIMETHYLTIN. C. Becker*, T.J. Walsh and R.S. Sloviter. Neurology Ctr., Helen Hayes Hospital, W. Haverstraw, N.Y. 10993; U.S. E.P.A., Research Triangle Park, N.C. 27710

Trimethyltin (TMT) causes convulsions and brain damage in experimental animals. The present study was undertaken to evaluate the role of TMT-induced seizure activity in the brain damage caused by this compound and to evaluate the acute hippocampal pathology caused by TMT. Male Sprague-Dawley rats received TMT (7mg base/Kg ip)or saline. Rats were sacrificed at different times after injection (5,7,10,30 days). Brains were embedded in paraffin for subsequent evaluation by light microscopy. Approximately half of the TMT-treated rats displayed convulsions, usually within one week of injection. The latency to seizure onset was 2-3 days. Rats which did not develop convulsions did not exhibit obvious hippocampal damage. In addition, there was a general positive correlation between the severity and frequency of seizures and the severity of hippocampal damage. However, TMT induced different patterns of hippocampal damage in different rats despite the use of the same dose of TMT in one group of rats treated identically. Although some rats displayed acutely necrotic or missing hilar and CA3 pyramidal cells only, other rats displayed damage to the CA1 cells, with or without CA3 damage. Granule cells appeared normal in all rats. A major feature of the TMT-induced hippocampal damage was the appearance of clear, round profiles in many of the dendritic fields of the hippocampus and area dentata during the active seizure state. These profiles have been identified in kainic acid-treated and perforant path-stimulated rats as massively swollen dendritic segments which occur at the sites of excitatory innervation. In severely affected TMT-treated rats, these dendritic swellings were commonly present throughout st. oriens and st. radiatum of CA1 and CA3. This area of altered morphology stopped abruptly at the st. radiatum/moleculare border of CA1 where the commissural/associational input stops. In other rats, dendritic swellings were present only in area dentata. In these rats, the altered morphology was restricted to the inner third of the dentate molecular layer which receives a laminar input from the hilar cells of both hippocampi. These results suggest that: 1)the seizure activity produced by TMT probably mediates the hippocampal pathology induced by this compound; 2)TMT does not produce a highly reproducible pattern of hippocampal damage (as does kainic acid); and 3) the varying patterns of dendritic swelling seen in the hippocampi of TMT-treated rats are the result of seizure activity induced in different excitatory hippocampal pathways. The mechanism by which TMT initiates seizure activity remains to be elucidated.

119.12 SEIZURE THRESHOLDS IN RATS AFTER ACUTE METAL CHELATION OR CHRONIC ZINC DEFICIENCY. John D. Connor, Dept. Pharmacology,Pennsylvania State Univ. Col. of Medicine, Hershey, PA 17033.

Granule cells in the dentate gyrus of the hippocampus have in their terminal boutons high concentrations of divalent metals, mostly zinc with lesser quantities of lead and copper. Responses of granule cells to electrical stimuli via the perforant path have been reported as unchanged after metal chelator injections. This report deals with the functional implications of acute zinc chelation or chronic zinc deficiency on responses to convulsant drugs.

Male Sprague-Dawley rats (325-375 gm) were used throughout. Doses of chelators below threshold for overt sedation or ataxia were as follows: 1,10 phenanthroline (o-phenanthroline), 40 mg/kg i.p.; 2,9 dimethyl-1,10 phenanthroline (neocuproine), 60 mg/kg i.p.; and diethyldithiocarbamate (DEDTC), 1 gm/kg i.p. Vehicle containing PEG and saline was used for controls. Zinc deficient male rats were obtained by feeding a diet low in zinc (0.7 ppm) for 6 weeks. Pair-fed controls were given the same diet, plus 30 ppm zinc in their water.

One hour after chelator injections, 3 rats from each group were taken for Timm's reaction as modified by Sloviter (Brain Res. Bull. 8:771, 1982). Brain slices from control and treated rats were run simultaneously in the same staining solutions. Zinc deficient rats were treated in the same way, except the right femur was also taken for atomic absorption analysis of bone zinc. After 1,10 phenanthroline, the apex of the hilus and the most lateral mossy fibers showed very light staining. The rest of the histochemical reaction was greatly diminished compared to controls. With neocuproine, no Timm's product could be detected. In DEDTC treated rats, metal deposits were discernable in all areas usually reactive, but the intensity was less. Timm's reactions in zinc deficient rats were indistinguishable from pair-fed, zinc supplemented controls. Femur concentrations of zinc in deficient rats were half those of controls.

Controls and treated rats were challenged simultaneously with flurothyl, a convulsant vapor. Clonic convulsion onsets after 1,10 phenanthroline and neocuproine (means of 38 and 52 sec) were significantly different from controls (means of 80 and 98 seconds) as determined by one-way anova at $\alpha = 0.01$. DEDTC and zinc deficient convulsion times (92 and 78 sec) were not significantly different from controls.

Although phenanthroline chelators lower seizure thresholds, results with DEDTC suggest that factors other than simple reduction of hippocampal metal concentrations are involved in the epileptiform response.

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119.13 ZINC BINDING PROTEINS IN BRAINS OF ZINC TREATED AND SEVERELY ZINC DEFICIENT RATS. N. Ebadi, J.C. Wallwork*, S. Swanson* and C. Hebus*. Department of Pharmacology, University of Nebraska College of Medicine, Omaha, Nebraska 68105 and Agricultural Research Service, United States Department of Agriculture (J.C.W.), Human Nutrition Research Center, Grand Forks, North Dakota 58202.

Metallothioneins, low molecular weight, cystein-rich, metal binding proteins, have been isolated from tissues such as kidney and liver. These metallothioneins are inducible following administration of Zn^{++} and other ions. By using Sephadex G-75 column chromatography calibrated with proteins of known molecular weights and by using other techniques, three separate zinc binding proteins with estimated apparent molecular weights ranging from 15,000 to 210,000 daltons were isolated from rat brain (Itoh and Ebadi, J. Neurochem., in press). The zinc binding proteins in brains of rats after 30 days of zinc deficiency remained unaltered in each of the three major proteins studied. It is interesting that zinc deficiency state, did not alter zinc levels in most areas of brain studied with the exception of olfactory lobe (Wallwork, et al., Fed. Proc. 42:3090, 1983). Thus, the steady state concentration of zinc in the brain is firmly regulated. To test this hypothesis further, two other biochemical parameters, one of which is stimulated by zinc and the other is inhibited by zinc, were measured. Free zinc is a potent inhibitor of glutamic acid decarboxylase (GAD) in vitro and in vivo. On the other hand, pyridoxal kinase, which synthesizes pyridoxal phosphate, is preferentially stimulated by free zinc in vitro and in vivo. These parameters were measured in brain of zinc deficient and pair fed rats. The activities of GAD in the brain of zinc deficient and pair fed rats were 581 ± 47 and 567 ± 63 nmoles/CO_2/mg protein/30 mins respectively. The concentrations of pyridoxal phosphate in the brain of zinc deficient and pair fed rats were 1.27 ± 0.04 and 1.31 ± 0.74 µg/g wet tissue respectively. Thus, in the brain, zinc binding proteins not only may function as physiological donors of zinc to zinc-apometalloenzymes, but also may play a decisive role in preventing CNS toxicity by preventing the rise of free zinc ions. Consistent with these views are observations by Itoh and Ebadi (Neurochem. Res. 7:1287-1298, 1982) that intracerebroventricular injection of 0.30 µmol zinc caused epileptic seizures, including tonic-clonic convulsions; whereas the administration of huge doses of zinc (up to 100 mg/kg), either intravenously or intraperitoneally, produced no convulsive seizures or other behavioral abnormalities. (Supported in part from a grant from Am. Eps. Fdn. - NE.)

119.14 ACUTE AND CHRONIC EPILEPTIFORM ACTIVITY INDUCED BY INJECTION OF IONIC COBALT INTO THE RAT NEOCORTEX. S.Veregge* and J.D.Frost,Jr.* (SPON: Y.Clement-Cormier). Program in Neuroscience and Section of Neurophysiology, Department of Neurology, Baylor College of Medicine and The Methodist Hospital, Houston, TX 77030.

The placement of metallic cobalt onto or into the brain of an experimental animal produces epileptiform activity that lasts for days to weeks. In recent studies, however, the iontophoresis of ionic cobalt onto the neocortex of cats produced only short-duration (24 hours or less) epileptiform activity. This observation lead Schwartzkroin et al. (1977) to suggest that the chronic effects of cobalt metal may be due to the continuous release of ionic cobalt from the metallic reservoir, and not to any long-term effects of cobalt itself. The objective of the present study was to further examine the duration and nature of the epileptiform activity produced by ionic cobalt using both surface EEG and single unit recording.

Injection of .2 ul (29 ug) of cobaltous chloride into the right sensorimotor cortex of Sprague-Dawley rats produced interictal spike activity with a rapid onset (less than 2 hours) and a duration of up to 23 days. Seizure activity was also produced and was most frequent during the first week post-injection. In 8 rats, seizures were studied in more detail during this first week and were found to assume a bimodal time-distribution with an early peak of seizure activity on day 1 and a late peak on day 6. Seizures rarely occurred on day 4. The percent of neocortical units that exhibited bursting activity was also bimodally distributed with early and late peaks corresponding to those of seizure activity.

Early and late seizures were quite different. Although both types were focal motor and involved the body musculature contralateral to the cobalt-induced focus, most early seizures were short in duration, lasting less than 60 seconds, while most late seizures were continuous, lasting up to 48 hours. Also, electrocorticographic waves accompanying early seizures were shorter in duration and had a shorter interwave interval than those accompanying late seizures.

These results suggest that, in the rat, ionic cobalt produces both acute and chronic epileptiform activity. The acute activity may, as Schwartzkroin et al. (1977) suggested, be due to the acute presence of the cobalt ion. The chronic activity, however, is more likely due to long-term effects of ionic cobalt, for example, selective neuronal death, gliosis, or changes in neurotransmitter synthesizing enzymes. The distinctly different characteristics of early and late seizures also lends support to the hypothesis that cobalt is producing these two types of seizures by different modes of action.

119.15 ELECTRON MICROSCOPY AND X-RAY ANALYSIS OF RAT CEREBRAL CORTEX EXPOSED TO COBALT AND COPPER. R.G. Frederickson*, C.R. Craig*, J.L. Culberson* and B.K. Colasanti*. Depts. of Anatomy and Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV 26506

Metallic cobalt (Co) is epileptogenic when applied to cerebral cortex; copper (Cu) does not share this property although it evokes a significant gliotic response. The present study compares the structural changes in response to, and the subcellular localization of, these metals. Very small cortical implants of Co wire (10 mil x 0.2 mm) evoke EEG evidence of seizures in 30-35% of treated rats while implants of similar-sized pieces of Cu or glass have no such effects. At 7 to 21 days after implanting Co, swollen neuronal cell bodies are seen in areas devoid of necrosis. Enlarged electron-lucent apical dendrites of pyramidal cells extend through layers II and III. The characteristic feature of the glial response is the appearance of specific granular inclusions within glial cells in paraneuronal, paravascular and subpial locations. These spherical granules appear alone or in clusters and each contains a centrally-placed electron-lucent core. Within the dense rim of each granule, profiles of smaller, electron-lucent bodies are observed.

In non-necrotic cortex adjacent to the Cu implants, glia contain different types of inclusion bodies. These granules are uniformly electron-dense and possess irregular ragged perimeters. Cortex from animals with glass implants shows inflamed and necrotic tissue but no inclusions.

Cu can be identified within the specific glial inclusions of Cu-exposed animals by accumulating X-ray spectra. However, X-ray spectra obtained from the inclusions observed in glial cells of Co-exposed animals, have not yet confirmed the presence of elemental Co. The possibility of identifying Co by other preparatory techniques is suggested by the fact that this element can be demonstrated within subarachnoid macrophages by X-ray spectra and dot mapping following subdural injection of colloidal cobalt. (This research was supported by the WVU Medical Corporation and by NIH Biomedical Research Grant #5S07-RR 05433-18).

119.16 CORTICAL ISCHEMIA CAUSES EPILEPTIFORM DISCHARGES. A. Basil Harris, Dept. of Neurological Surgery, University of Washington, Seattle, WA 98195.

A neuronal differential vulnerability to ischemia has long been suggested. Ischemia can cause seizures in man and has been invoked as a possible cause of experimental epilepsy. Neurons in cortical layers 3, 5 and 6, hippocampal cells and Purkinje cells are thought to be more susceptible. Ischemia studies have primarily used whole brain insult. The present experiments were conducted on the suprasylvian gyrus of the cat. Ischemia was produced in this gyrus by a modification of the technique to isolate the gyrus as described by Gruner, Hirsch and Sotelo (J Comp Neurol 154:1-28, 1974). Cortical ischemia was achieved using the operating microscope and bipolar coagulation to cut free the anterior and posterior limb of the gyrus into the white matter. A vascular clamp was then applied, with the animal heparinized, across the middle cerebral and pial blood supply of the ectosylvian gyrus and another vascular clamp placed across the pial blood supply coming from the marginal gyrus. This produced complete ischemia of the suprasylvian gyrus. Following a selected period of ischemia the heparin was reversed with protamine. Ischemia of this gyrus was 10, 13, 20 and 30 minutes duration in different animals. All animals had normal EEGs prior to inclusion in the study. Thirty days following the ischemic event electrical activity in the cortex was investigated using ECoG under phenobarbital anesthesia followed by perfusion fixation of the brain. Representative blocks of tissue were prepared for Golgi and light microscopic examination. Thirty minutes of ischemia produced a fibrotic scar without surviving neuronal or glial elements and without abnormal electrical discharges. Ten to 13 minutes of ischemia produced spiking, spontaneous epileptic activity in the suprasylvian gyrus. Histological examination showed preservation of cortical volume and cortical cell layers. Golgi studies of this gyrus suggest a reduction in small to medium sized cortical interneurons. A reduction in these sparsely spinous and aspinous neurons, which are thought to be GABA-ergic and may be more susceptible to ischemia, could result in a decreased GABA content which could lead to spontaneous spiking activity.
(Supported by NS 04053)

120.1 BINDING OF ^{3}H-DMCM TO BENZODIAZEPINE RECEPTORS; CHLORIDE DEPENDENT ALLOSTERIC REGULATORY MECHANISMS. T. Honoré*[1], M. Nielsen*[2] and C. Braestrup*[1,2] (SPON: P. Roland). [1]A/S Ferrosan, Research Division, Sydmarken 5, DK-2860 Soeborg, [2]Sct. Hans Hospital, DK-4000 Roskilde.

Benzodiazepine (BZ) receptor ligands comprise a continuum of agents with pharmacological effects ranging from compounds with anxiolytic and anticonvulsant actions via pharmacological neutral compounds to compounds with anxiogenic and convulsant action; tentatively termed agonists-antagonists-inverse agonist.

Specific binding of ^{3}H-DMCM at 37°C (a benzodiazepine receptor ligand with inverse agonistic properties) was influenced by compounds presumed to interact with chloride ionophors associated with the BZ/GABA-receptor complex. Chloride ions (200 mM) enhanced the specific binding of ^{3}H-DMCM four-fold. The effect of chloride ions on specific ^{3}H-DMCM binding was parallel to the observed increase in specific ^{3}H-diazepam binding by the presence of chloride ions (Costa, T., Rodbard, D. and Pert, C.B., Nature, 277:315, 1979; Martin, I.L. and Candy, J.M., Neuropharmacol., 19:175, 1980). On the other hand, iodide ions, which also penetrate chloride ionophors and enhance specific ^{3}H-diazepam binding, have no effects on specific ^{3}H-DMCM binding. In addition specific binding of ^{3}H-DMCM was enhanced by picrotoxinine in the absence of chloride ions.

(+)-Etomidate and pentobarbital reduced the specific ^{3}H-DMCM binding in a partially chloride ion dependent and picrotoxinine sensitive manner. The reduction in specific ^{3}H-DMCM binding is opposite to the observed increase in the binding of BZ-receptor agonists in the presence of (+)-etomidate or pentobarbital (Ashton, D., Geerts, R., Waterkeyn, C. and Leysen, J.E., Life sci., 29:2631, 1981; Ehlert, F.J., Ragan, P., Chen, A., Roeske, W.R. and Yamamura, H.I., Eur. J. Pharmacol., 78:249, 1982).

The results obtained are consonant with a chloride dependent allosteric regulatory mechanism within the BZ/GABA-receptor chloride ionophor complex. Furthermore, the results suggest that the regulation of ^{3}H-DMCM binding by compounds active on chloride ionophor reflect the pharmacological efficacy of DMCM. On the other hand, regulation of ^{3}H-DMCM binding by anions that penetrate chloride ionophors, seems not to be correlated with the pharmacological efficacy of DMCM.

120.2 EFFECT OF PENTYLENETETRAZOL ON MUSCIMOL- AND BARBITURATE-STIMULATED [^{3}H]FLUNITRAZEPAM RECEPTOR BINDING IN MOUSE BRAIN. A. Y. Chweh,* E. A. Swinyard,* and H. H. Wolf* (Spon: J. W. Woodbury). Department of Biochemical Pharmacology and Toxicology, College of Pharmacy, University of Utah, Salt Lake City, UT 84112.

The inhibitory activity of pentylenetetrazol (PTZ) on muscimol- and barbiturate-stimulated [^{3}H]flunitrazepam ([^{3}H]FLU) receptor binding was compared with the inhibitory activity of PTZ on unstimulated (basal) [^{3}H]FLU receptor binding in mouse forebrain and cerebellum. In forebrain, PTZ inhibited both barbiturate-stimulated and basal [^{3}H]FLU receptor binding; however, the inhibitory activity of PTZ on barbiturate-stimulated [^{3}H]FLU receptor binding was greater than that on basal binding. The inhibitory potency of PTZ on barbiturate stimulated [^{3}H]FLU receptor binding was reduced to the basal level by 20 µM picrotoxinin and by the exclusion of chloride ions. PTZ had no effect on muscimol-stimulated [^{3}H]FLU receptor binding in forebrain. In cerebellum, barbiturate had relatively little stimulatory effect on [^{3}H]FLU receptor binding. Therefore, PTZ inhibited barbiturate-stimulated [^{3}H]FLU receptor binding to about the same extent as seen under basal conditions.

Although this study does not reveal the exact site at which PTZ inhibits [^{3}H]FLU receptor binding, the results presented indicate that a chloride ionophore sensitive to barbiturate but not to GABA participates in the PTZ inhibitory activity of benzodiazepine receptor binding. (Supported by NIH Contract No. NO1-NS-1-2347)

120.3 DIFFERENTIAL INTERACTIONS OF DEPRESSANT AND CONVULSANT DRUGS WITH THE COMPONENTS OF BENZODIAZEPINE-GABA RECEPTOR-IONOPHORE COMPLEX. R. Ramanjaneyulu* and M.K. Ticku (SPON: V.S. Bishop). Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284.

The picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex is an important regulatory site, where several classes of convulsant, depressant and anxiolytic drugs appear to interact. In this study, we have investigated the effect of several depressant and convulsant drugs with the [^{35}S]-t-butylbicyclophosphothionate [TBPT], a ligand that binds to the picrotoxinin site. TBPT binds to whole rat brain membranes, to a single site, with an apparent K_D of 25 nM and a B_{max} of 1.404 pmol/mg protein. TBPT binding was displaced by convulsants like anisatin (IC_{50} = 0.1 µM), isopropylbicyclophosphate (IC_{50} = 0.16 µM), picrotoxinin (IC_{50} = 0.40 µM) and pentamethylenetetrazole (PTZ; IC_{50} = 700 µM). TBPT binding was also inhibited potently by GABA, etazolate and stereoisomers of barbiturates. Eleven tetrazole analogues inhibited TBPT binding much more potently than diazepam binding. Inhibition of TBPT binding by tetrazoles was competitive and correlated well with their convulsant potencies. Picrotoxinin also inhibits TBPT binding competitively, whereas GABA inhibits TBPT binding noncompetitively. In contrast, depressants like pentobarbital and (+)etomidate gave a mixed competitive, noncompetitive inhibition of TBPT bindng in whole rat brain membranes. Furthermore, convulsants like picrotoxinin, PTZ and cage convulsant bicyclophosphate esters were equipotent in inhibiting TBPT binding in cortex and cerebellum. In contrast, GABA and depressants like pentobarbital and (+)etomidate were 3- to 6-fold more potent inhibitors of TBPT binding in cerebellum relative to cortex. This difference is not due to differential affinities of TBPT in these regions, since TBPT had similar K_D values in cortex and cerebellum. We have recently demonstrated that pentobarbital and (+)etomidate enhance both GABA and diazepam binding in cortex, to a much greater extent than in cerebellum (Thyagarajan et al, J. Neurochem., in press, 1983). These results further support the notion that the picrotoxinin site is an important site of drug action for a variety of drugs that modulate GABAergic transmission, and that the coupling of various components of the benzodiazepine-GABA receptor complex is different in cerebellum.

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120.4 EFFECTS OF CHRONIC TREATMENT WITH SOME DIRECT GABA AGONISTS ON GABA RECEPTOR BINDING. M.K. Ticku, R.G. Fariello and G.T. Golden. Dept. Pharm., UTHSC, San Antonio, TX 78284; Dept. Neurology, Jefferson College, Phila, PA, 19107; Research and Neurology, V.A. Med. Ctr., Coatesville, PA, 19520.

The effects of chronic treatment (12 days) with the direct GABA agonists THIP (4,5,6,7-tetrahydroisoxazolo (5,4-c)-pyridine-3-ol), Progabide (4-((chlorophenyl)(5-fluro-2-hydroxyphenyl) methylene) amino)-butanamide and 3-APS (3,Amino-propanesulfonic acid) on GABA and benzodiazepine binding was investigated.

A total of 35 male Sprague-Dawley rats (250-300 g) were administered one of three direct GABA agonists or a control vehicle. Nine aminals received THIP (7.5 mg/kg SC) in a normal saline vehicle, eight animals received Progabide (50 mg/kg SC) in a DMSO vehicle, nine animals received 3-APS (100 mg/kg) in a DMSO vehicle and nine animals received 0.2 cc DMSO as a vehicle control. Injections were administered twice a day (9 AM and 4PM) for 12 consecutive days; on the 13th day, animals received the 9 AM injection only. Between 1 and 5 PM, all animals were sacrificed by decapitation, brains removed immediately and dissected on ice into the following areas: cerebellum, brain stem, thalamus, forebrain, occipital cortex and midbrain. Individual areas (bilateral areas pooled where appropriate) were placed in 0.32 M sucrose and immediately frozen in dry ice. Tissue was stored at -80°C until analysis.

The effect of chronic GABA-agonist treatment was investigated on GABA and benzodiazepine binding in six brain regions. The equilibrium binding of GABA to its receptor sites was measured by using [^{3}H]GABA (4 nM) and [^{3}H]muscimol (10 nM). Benzodiazepine binding was measured using 0.2 nM [^{3}H]flunitrazepam and GABA enhancement of [^{3}H]flunitrazepam was compared using 10^{-4} M GABA. All the binding data was compared to vehicle treated controls. THIP treatment decreased GABA binding only in cerebellum. 3-APS decreased GABA binding in forebrain and midbrain, while progabide decreased GABA binding in cerebellum, forebrain, midbrain and thalamus. Thus, the effects of chronic GABA agonist treatment on GABA receptor binding varied with different agonists. Likewise, THIP, Progabide and 3-APS treatments affected [^{3}H]flunitrazepam binding differently in various brain regions, which did not correlate with their effects on GABA binding. None of the treatments altered the enhancing effect of GABA on benzodiazepine agonist binding. These results indicate that chronic treatment with different GABA agonists may not produce identical neurochemical changes at the benzodiazepine-GABA-receptor-ionophore complex.

120.5 Rostrocaudal Concentration Gradient for Conjugated GABA in Human CSF, T.N. Ferraro, R.H. Gerner, T.A. Hare. Thomas Jefferson Univ. Phila., PA 19107 and UCLA Sch. of Med., Los Angeles, CA 90024

Measurement of GABA in sequential aliquots of human CSF obtained via lumbar puncture have revealed the existence of a rostrocaudal concentration gradient supporting the hypothesis that CSF GABA levels reflect central GABAergic activity. Conjugated GABA, predominantly in the form of small molecular weight peptides, is present in CSF at levels 10-100X those of free GABA and may also provide information regarding the central GABA system.

In order to study the normal distribution of conjugated GABA in human CSF, we have determined levels of GABA before and after acid hydrolysis in sequential aliquots of CSF from thirteen neurologically normal adults. The group consisted of seven males (age, X±SD: 32±11 yrs) and six females (age, 31±11: X±SD yrs). CSF was collected on ice via lumbar puncture in two fractions: lst-12th ml (fraction A) and 15th-26th ml (fraction B). Fractions were divided into aliquots and stored at -80°C. CSF was deproteinized in 5% sulfosalicylic acid and subjected to hydrolysis in 6 N HCl at 110°C for 24 hrs. GABA levels were determined using the confirmed ion-exchange/fluorometric procedure.

Results showed that levels of conjugated GABA were significantly higher in fraction B for the population as a whole ($p < .005$, n=13) as well as for both male and female subgroups ($p < .05$, n=7 and $p < .02$, n=6, respectively) when compared to fraction A (paired t-test). Linear regression analysis within the male subgroup revealed a significant correlation between levels of free and conjugated GABA in both fraction A ($p < .01$) and fraction B ($p < .05$); this relationship was not observed in the female subgroup.

Thus, the demonstration of a rostrocaudal concentration gradient for conjugated GABA in CSF suggests that these neurochemicals originate in the brain and reflect metabolic pathways important in GABA system function. Additionally, the data suggests that males and females differ with respect to central GABA metabolism.

120.6 *ANTIBODIES AGAINST SMALL MOLECULES : AN APPLICATION TO GABA*
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The obtention of anti-catecholamine antibodies (Geffard et al.) led us to design a similar immunological system for aminoacids and their derivatives. Two complementary immunological techniques were used for the visualization of GABA by immunocytochemistry on the one hand and on the other hand for the titration of GABA by radioimmunological assay.

Since the GABA molecule alone is unable to stimulate immune responses, carriers and coupling agents have been chosen in function of the desired application of the antibodies.

For immunocytochemical studies (Seguela et al.,submitted for publication) we described a method in which we used glutaraldehyde to couple GABA in such a way as to allow antigenic determinant to be correctly presented to the antibodies. To avoid surface effects specific to each protein, an alternance of immunogen was realized during immunization. Antibody specificity was tested in a RIA system by using the radiolabelled ligand (^{3}H) GABA-G-lysine, which mimicks a structure possessed by the hapten fixed to the tissue and by the immunogen. We obtained good results in immunocytochemical application of this technique.

In order to measure GABA levels, another approach was tested in various biological samples. The RIA developed here used a iodinated radiolabelled ligand reproducing the GABA-succinyl structure. These highly specific antibodies were observed to be suitable for GABA measurements.

Up till now it has been difficult to use immunological approaches for the study of small molecules having a molecular weight of less than 200d. However, it is highly probable that the knowledge and experience gained from the two above-mentioned techniques will be useful for the visualization and titration of other small haptens in the central and peripheral nervous systems, as well as in other tissues.

120.7 PARTIAL PROTEOLYTIC MAPPING OF THE BENZODIAZEPINE BINDING SUBUNIT OF CENTRAL BENZODIAZEPINE RECEPTORS. R.R. Trifiletti and S.H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Partial proteolytic digestion patterns of the (^{3}H)flunitrazepam photoaffinity-labelled benzodiazepine binding subunit of central benzodiazepine receptors for the enzymes trypsin, α-chymotrypsin, S. Aureus V8 protease, papain and carboxypeptidase Y were visualized by SDS/PAGE and fluorography. Preliminary photoaffinity labelling experiments on rat brain membranes indicates that (^{3}H)flunitrazepam labels a single species of apparent moleuclar weight by SDS/PAGE of 52 ± 0.5 kilodaltons. When the SDS/PAGE purified photoaffinity labelled protein is subjected to exhaustive proteolytic digestion and the products analyzed by reverse-phase HPLC, the radioactivity is confined to a single peak for all enzymes tested. This result suggests that the photolabelling process per se does not produce appreciable microheterogenity; the position of the label on the polypeptide chain is thus well-defined and can be used as a "reference point" for partial proteolytic digestion experiments.

Using the results of the partial proteolytic digestion patterns obtained, a physical map of the benzodiazepine binding subunit is proposed. This map localizes the sites of proteolytic cleavage and the label position along the polypeptide chain. The label position localization is useful in the determination of which region of the polypeptide chain might contain a portion of the drug binding site.

Partial proteolytic digestion patterns may also be viewed as "one-dimensional fingerprints" and can be used to compare structure of polypeptides in different samples. Thus, we have compared the structure of the (^{3}H)flunitrazepam-photolabelled polypeptide in different brain regions in a given species and the same brain region in different mammalian species. Tryptic fingerprints were identical in all samples; similar findings were obtained for α-chymotryptic fingerprints. This suggests that there is considerable structural conservation of benzodiazepine binding subunit structure from brain region to brain region and among mammalian species.

120.8 LYSINE ADMINISTRATION DECREASES BRAIN ARGININE AND ORNITHINE LEVELS IN THE RAT. T. J. Maher, B. S. Glaeser and R. J. Wurtman. Laboratory of Neuroendocrine Regulation, M.I.T.,Cambridge, MA 02139 and Department of Pharmacology, Mass. College of Pharmacy and Allied Health Sciences, Boston, MA 02115.

The administration of tyrosine (TYR) or tryptophan (TRP), or the consumption of a meal that increases the ratios of serum TYR or TRP to the sum of the other competing large neutral amino acids, can accelerate the syntheses of the catecholamines and of serotonin, respectively. These increases are associated with behavioral and physiological functions thought to involve monoaminergic transmission, and are especially noted (for catecholamines) among neurons that are firing frequently. Less information is available about the relationships between basic amino acids in serum and brain. We examined the effects of administration of the essential basic amino acid lysine (LYS) on brain levels of LYS, arginine (ARG) and ornithine (ORN). Groups of 5 rats were fasted overnight and, the following morning, given 0, 50, 100, 200, or 400 mg/kg LYS in saline intraperitoneally. One hour later they were decapitated and the brains collected and stored on dry ice until assay for amino acid content with a Beckman Amino Acid Analyzer. Brain LYS levels increased in a dose dependent manner, and brain ARG and ORN decreased, as the LYS dose increased. There was a dose dependent increase in brain GABA reaching significance ($p< 0.05$) in the 400 mg/kg LYS group. In another group of experiments animals were given saline, 100 mg/kg LYS, 100 mg/kg LYS + 100 mg/kg ARG, or 100 mg/kg LYS + 200 mg/kg ARG and sacrificed as described above. Brain levels of ARG increased in a dose dependent fashion as did brain ORN (a 3 fold increase in brain ORN in the 100 mg/kg LYS + 200 mg/kg ARG group). Brain levels of LYS increased to the same extent in all groups given LYS, i.e. the uptake of LYS into the brain was not inhibited by the co-administration of ARG, whereas in the first experiment brain ARG levels were decreased by the administration of LYS (ARG levels decreased by 47% in the 400 mg/kg LYS group).

We have previously shown that the consumption of a 0% protein meal and a 40% protein meal decreases and increases, respectively, brain LYS (similar to TYR) while having no effect on brain ARG or ORN. Brain LYS levels might affect the syntheses of biologically-active metabolites.

120.9 CYCLOPYRROLONES INFLUENCE BENZODIAZEPINE RECEPTOR BINDING AT A NOVEL ALLOSTERIC SITE. J. Nye, R.O. Blaustein*, R.R. Trifiletti and S.H. Snyder, Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

The in vitro inhibition of benzodiazepine receptor binding by several cyclopyrrolones, including the pyrrolopyrazine, zopiclone, and the dithiinopyrrole, suriclone, two novel anxiolytics whose structures differ considerably from classical benzodiazepines, has been characterized in bovine brain. While zopiclone potently (IC_{50} ~ 50 nM) displaces [^{3}H]Ro-15-1788 binding in an apparent mass action fashion, suriclone and its metabolite, 35,489RP are extremely potent (IC_{50} ~ 250 and 500 pM, respectively) and show Hill coefficients of approximately 2.0. Similar to classical benzodiazepines, none of the drugs studied appears to display significant selectivity for Type I or Type II benzodiazepine receptors. All of the drugs studied, however, appear to differ from classical benzodiazepines in several respects: (i) the potencies of cyclopyrrolones at displacing [^{3}H]Ro-15-1788 are unaffected by GABA, Cl^-, pentobarbital/Cl^-, and tracazolate/Cl^-, at concentrations which elicit stimulation of classical benzodiazepine agonist binding; (ii) exhaustive photoaffinity labelling of membranes with unlabeled flunitrazepam does not reduce the potency of cyclopyrrolones at displacing residual [^{3}H]Ro-15-1788 binding, the only exception being zopiclone, whose potency is reduced four-fold; (iii)Scatchard analysis of [^{3}H]flunitrazepam, [^{3}H]ethyl β-carboline 3-carboxylate and [^{3}H]Ro-15-1788 in the presence of all the drugs studied reveals a non-competitive pattern of inhibition of binding in each case; (iv) dissociation of [^{3}H]flunitrazepam from its receptors in bovine cortex is accelerated by cyclopyrrolones.

Using the radiolabeled cyclopyrrolone, [^{3}H]suriclone, we have directly labeled the cyclopyrrolone recognition site in bovine brain. Several cyclopyrrolones appear to be competitive inhibitors of [^{3}H]suriclone binding; classical benzodiazepines inhibit in a non-competitive fashion. The dissociation rate of [^{3}H]suriclone is accelerated by benzodiazepines, but not cyclopyrrolones. [^{3}H]Suriclone binding sites are readily solubilized by 1°/° Triton X-100, and the solubilized binding activity copurifies with benzodiazepine binding activity after sequential DEAE-Sephadex and hydroxylapatite chromatography.

Based on these results, we propose that cyclopyrrolones influence benzodiazepine receptor binding allosterically at a site on the benzodiazepine receptor complex distinct from recognition sites for GABA, chloride ion, and barbiturates.

120.10 CHEMICAL MODIFICATIONS SUGGEST THE PRESENCE OF HISTIDINE AND TYROSINE RESIDUES AT THE BENZODIAZEPINE AND GABA BINDING SITES, RESPECTIVELY. G. Maksay* and M.K. Ticku. Dept. Pharm., Univ. Tx. Hlth. Sci. Ctr., San Antonio, TX 78284.

Chemical modification of the brain GABA-benzodiazepine (BZ) receptor complex by protein group-selective reagents may disclose the chemical topography of these pharmacologically important recognition sites. Selective protection from the inhibitory action of the reagent by the ligands of these sites may support that the modification proceeds at the very site of the binding. Experiments were carried out in rat cortical membrane fraction extensively washed both before and after treatment with protecting and modifying agents. The following reagents were used: tetranitromethane (TNM, pH = 8.1); N-acetylimidazole (pH = 7.5); p-diazobenzenesulfonic acid (DSA, pH = 7.1); and diethylpyrocarbonate (DEP, pH 6.0).

a) BZ binding was inhibited by DEP (which modifies histidine residues), DSA and TNM (which modify tyrosine residues). Protection experiments revealed that DEP inactivation could be selectively and completely protected by its own ligands like flurazepam. In contrast, DSA and N-acetyl-imidazole inactivation could only be partially protected both by its own ligands and by allosteric modulators like GABA, muscimol, pentobarbital and etazolate. TNM inactivation could be partially protected only by GABA and muscimol but not by flurazepam. These results suggest that the BZ binding protein contains a histidine residue at the active site, whereas the data for the location of tyrosine residue were not definitive.

b) GABA binding was inhibited by DSA, N-acetyl-imidazole and TNM, but not by DEP. The inhibition of GABA binding by DSA, N-acetyl-imidazole and TNM could be selectively and completely protected by GABA and muscimol but not by the allosteric ligands like flurazepam and etazolate. Since all these reagents are considered to be selective for tyrosine, the GABA recognition site apparently contains a tyrosine residue. TNM, while having no effect on the high affinity GABA binding sites, decreased the number of the low-affinity GABA sites and also abolished enhancing effect of muscimol on BZ binding. These results are in agreement with a previous study from this laboratory on DSA (Burch et al, Molec. Pharmacol. 23:52, 1983) and support the notion that the enhancing effect of GABA agonists on BZ binding is mediated by the low affinity GABA sites.

In summary, these results suggest (I) the presence of histidine and tyrosine at the binding sites of BZ and GABA receptors, respectively; (II) that GABA and BZs bind to two distinct sites; (III) low affinity GABA receptor sites are involved in the stimulation of BZ binding; and (IV) the topography of the high and low affinity GABA binding sites may not be identical.

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120.11 PERIPHERAL BENZODIAZEPINE BINDING SITES: IN VIVO AND IN VITRO LABELLING BY [^{3}H]PK 11195. G. Le Fur, A. Uzan, C. Guérémy* and J. Bénavidès*. PHARMUKA Lab., 92231 Gennevilliers, France.

Peripheral type benzodiazepine binding sites differ from classical GABA coupled receptors by having a very high affinity for RO5-4864, a benzodiazepine without affinity for the central receptors and a very low affinity for clonazepam. In a search for new ligands of the peripheral type binding sites we found that PK 11195, a isoquinoline carboxamide derivative, displaced with a very high potency ($IC_{50} \simeq 1$ nM) the [^{3}H]RO5-4864 binding to several rat organs and displayed no affinity for the brain receptor labelled with [^{3}H]flunitrazepam. Thus PK 11195 has been tritiated and used in the characterization of the peripheral benzodiazepine binding sites. [^{3}H]PK 11195 binding exhibits a very high affinity (K_D = 1-3 nM), reversibility and specificity. Binding site density was the highest in adrenals (Bm = 34 pmol/mg prot), followed by platelets (40 pmol/mg prot), kidney (9 pmol/mg prot), heart (2 pmol/mg prot) and brain (1 pmol/mg prot). In the brain binding sites were localized by autoradiohistochemistry in olfactory bulb and choroid plexus whereas in the heart they were mainly localized in ventricles, in the kidney, in the medulla and in adrenals in the cortical zone. Thermodynamic studies demonstrated that binding of [^{3}H]PK 11195 was entropy driven, whereas binding of [^{3}H]RO5-4864 was enthalpy driven. These results taken together with the antagonism by PK 11195 of the convulsions induced by RO5-4864 suggest that PK 11195 could be an antagonist of a receptor where RO5-4864 acts as an agonist. [^{3}H]-PK 11195 could be useful to determine the physiological role of "peripheral type" benzodiazepine binding sites because besides its high affinity and specificity in "in vitro" conditions the administration of trace doses of [^{3}H]PK 11195 allow to label specifically its binding sites "in vivo".

120.12 STIMULATION OF ^{3}H-DIAZEPAM BINDING BY GABA AND SQ20009:MODULATION BY THE PERIPHERAL-TYPE BENZODIAZEPINE LIGANDS. R. M. Mangano N. M. Spirt* and R. A. O'Brien. Department of Pharmacology II, Hoffmann-La Roche Inc., Nutley, NJ 07110.

The high affinity benzodiazepine (BZ) binding site appears to be one constituent of a macromolecular complex that is functionally linked with the gamma-aminobutyric acid (GABA) recognition site and the Cl^- ion conductance channel in the mammalian brain. GABA, in μM concentrations, has been reported to enhance the binding of [^{3}H]-BZs to central nervous system (CNS) recognition sites. This effect is due to an increase in affinity rather than a change in the number of binding sites (Nature, 274:383, 1978). This GABA effect is bicuculline-sensitive, concentration dependent and can be observed in several brain regions. In addition to GABA, the pyrazolopyridines have been reported to facilitate the binding of BZ agonists to CNS recognition sites. The pyrazolopyridine etazolate (SQ 20009; SQ) enhances BZ binding through an increase in receptor affinity which is both bicuculline and picrotoxin sensitive (Pharmacol. Biochem. Behav., 9:849, 1978). In a previous study, we reported that the GABA enhancement of [^{3}H]-BZ binding in the mammalian CNS could be inhibited in a dose-dependent manner by a convulsant BZ, Ro 5-3663 (Life Sci. 26: 1441, 1980). In the present study we have investigated the effect of Ro 5-3663 as well as several "peripheral-type" BZs on the enhancement of [^{3}H]-BZ binding by GABA and/or SQ.

Binding studies were performed with membranes prepared from a crude P_2 fraction of rat cortex. The membranes were washed a total of five times to remove endogenous GABA and resuspended in 50 mM Tris-HCl containing 100 mM NaCl (pH 7.4 at room temperature).

Under these conditions, GABA (10 μM) and SQ (10 μM) enhanced specific [^{3}H]-diazepam (DIAZ) binding by 95% and 31% respectively. GABA plus SQ (10 μM each) enhanced specific binding in a additive manner to 131% of control values. At 10 μM, neither Ro 5-3663, Ro 5-4864 nor Ro 5-5115 had any effect on DIAZ binding. These BZs, at 10 μM, variably attenuated both the GABA (10 μM) and SQ (10 μM) enhancement of specific binding by 10%-20% and 20%-60%, respectively. However, these compounds appear to have only a minor effect on the enhancement of DIAZ binding produced by the combination of 10 μM GABA plus 10 μM SQ. Although the additive nature of the enhancement of BZ binding in the presence of both GABA plus SQ indicates that these two compounds are acting at different sites, the modulation of the GABA and SQ effect by the convulsant and peripheral BZs suggests that the GABA recognition site does play some role in SQ enhancement of BZ binding.

120.13 EFFECT OF BUFFER AND FREEZING (STORAGE) ON BENZODIAZEPINE BINDING AFTER PHOTOAFFINITY LABELING. M.T. Spoerlein, R.M. Mangano and R.A. O'Brien. Department of Pharmacology II, Hoffmann-LaRoche Inc., Nutley, N.J. 07110.

Möhler et al. (PNAS 77: 1666, 1980) reported that, when irradiated with UV light, the benzodiazepine (BZ) receptors could be photoaffinity labeled with flunitrazepam (FLU), which is then irreversibly bound. Gee and Yamamura (Eur. J. Pharmacol. 82: 239, 1982) and Thomas and Tallman (J. Neurosci. 3: 433, 1983) showed that photoaffinity labeling of BZ receptors alters agonist binding but not antagonist binding. This report presents some methodological considerations of this technique, i.e., differences in results with storage time and with Na_2HPO_4 buffer, pH 7.4 and Tris-HCl buffer, pH 7.4.

Rat cerebral cortices were homogenized in cold 0.32 M sucrose and the P_2 pellet prepared. The pellet was resuspended in 90 vol of 50 mM Na_2HPO_4-HCl buffer, pH 7.4. This was either 1) stored by freezing at -20°C in aliquots for future photolabeling and binding studies or 2) was photolabeled and after 2 washes with Na_2HPO_4 buffer or 2 washes of 50 mM Tris-HCl buffer, stored in their respective washing buffers at -20°C for future binding studies.

Photoaffinity labeling was performed by exposure of membranes to UV light at 254 nm for 20 min. at 0°C in the presence of 30 nM FLU. Filtration binding assays were performed using ^{3}H-diazepam (DIAZ) and ^{3}H-Ro-15-1788.

When Na_2HPO_4 buffer was used to store the membrane preparation, a decline in binding activity of all of the controls for ^{3}H-DIAZ and for ^{3}H-Ro-15-1788 binding occurred rapidly, with a decrease to 80-90% of the day "0" activity in 24 hrs.; a decrease to 40-50% in 48 hrs. and to 10-15% after 7 days. The ^{3}H-DIAZ binding with photolabeled membranes showed an initial increase to 150% of day "0" results at 24 hrs.; a decrease to 90% by 48 hrs. and to 40-45% after 7 days. These results (the increase in binding) were not seen with the antagonist, ^{3}H-Ro-15-1788; only a decrease as seen with the controls was observed.

Storage for 7 days in Tris-HCl buffer resulted in no significant decrease in binding of ^{3}H-DIAZ or of ^{3}H-Ro-15-1788. Again a marked increase (185%) in binding was observed after 24 hrs. of freezing. After 48 hrs. the increase was only 140% of that on day "0" (unfrozen); this was also seen after 7 days of freezing.

Stability of the membranes is evident with Tris-HCl buffer as compared to Na_2HPO_4. Freezing may, however, change the available binding sites or influence the irreversible photolabeled sites.

120.14 BIOCHEMICAL EVIDENCE THAT PK 8165 A QUINOLINE DERIVATIVE WITH PURE ANTICONFLICT PROPERTIES IS A PARTIAL AGONIST OF BENZODIAZEPINE RECEPTOR. J. Bénavidès*, A. Uzan, C. Guérémy* and G. Le Fur.

The atypical pharmacological profile of PK 8165, a quinoline derivative with pure anticonflict properties (Le Fur et al., Life Sci. 28:1439-1448, 1981) seems to be due to the fact that this compound interacts with benzodiazepine receptor in a different way that classical benzodiazepine structure ligands. PK 8165 is a competitive inhibitor of [^{3}H]flunitrazepam binding to brain cortex membranes with a Ki value of 80 nM. Inhibition curves by PK 8165 of [^{3}H]flunitrazepam binding present a Hill slope close to unity. Possible discrimination by PK 8165 of BZ_1 and BZ_2 receptor subtypes has been tested by measuring the displacement of [^{3}H] flunitrazepam binding to brain sections. PK 8165 presents the same potency in BZ_1 rich areas as in BZ_2 rich areas. These results have been confirmed by quantitative analysis of autoradiograms of the binding to tissue sections, where opposite to CL 218,872 PK 8165 is unable to discriminate between cerebellum (BZ_1) and dentate gyrus (BZ_2). GABA and flunitrazepam photolabelling produce potency shifts which are intermediate between full agonists and antagonists. Thermodynamic analysis shows that PK 8165 binding opposite to RO15-1788 is associated with a decrease in entropy but smaller than that of clonazepam. Moreover PK 8165 by itself is unable to modify the cerebellar cGMP level, but potentiates the lowering of cGMP by diazepam and does not present antagonistic properties of this effect. Thus PK 8165 pharmacological properties seem to be due to the fact that this compound is a partial agonist of benzodiazepine receptors.

120.15 SENSITIVITY OF THE GABA RECEPTOR TO CHANGES IN ANIONIC COMPOSITION OF THE BUFFER, Paul Madtes, Jr. Lab. of Vision Research, NEI, NIH, Bethesda, MD 20205.

Γ-aminobutyric acid (GABA) has been established as a major inhibitory neurotransmitter in the retina. Two sodium-independent binding sites for GABA are present in the retina, one of high affinity and one of low affinity. These sites are thought to represent postsynaptic GABA receptors. Little is known, however, about what regulates the sensitivity of GABA receptors to the transmitter, although in vitro characterization of GABA receptors in nervous tissue shows a sensitivity of the receptors to the presence of various ions. The presence of sodium, for example, masks the appearance of the high-affinity binding site; however, the addition of bicarbonate ions, in the presence of sodium, renders the site detectable again. In addition, the presence of chloride ions modulates the inhibitory action of picrotoxin on the GABA receptor complex. It is reported here that the number of high-affinity binding sites for ^{3}H-GABA is preferentially decreased in the presence of TRIS-HCl buffer, compared to the values found in the presence of TRIS-citrate buffer.

Bovine eyes were obtained from a local supplier and synaptosomal membranes were prepared from the retinas. The tissue was assayed for ^{3}H-GABA binding according to a modification of the method of Enna and Snyder (Brain Res. 100(1975) 81-97). The samples were incubated either in 50 mM TRIS-citrate buffer, pH 7.4, or in 50 mM TRIS-HCl buffer, pH 7.4. No difference was found between the osmolaric strengths of the two buffers. Scatchard analysis of the specific binding data showed that the samples assayed in TRIS-citrate had a high-affinity binding site (K_D values ranging from 10 to 30 nM; B_{max} values ranging from 1.3 to 1.5 pmol/mg protein) and a low-affinity binding site (K_D: 50-130 nM; B_{max}: 2.3-3.0 pmol/mg protein). The samples incubated in TRIS-HCl also had two binding sites - a high-affinity site (K_D: 5-25 nM; B_{max}: 0.3-0.5 pmol/mg protein) and a low-affinity site (K_D: 60-120 nM; B_{max}: 1.7-2.3 pmol/mg protein). There was a 3-4 fold decrease in the number of high-affinity binding sites (B_{max}) for the samples incubated in TRIS-HCl, compared to the values for those incubated in TRIS-citrate. Also, a slight decrease (< 20%) in the number of low-affinity sites was noted. No significant differences were found between the values for the apparent affinities, comparing the two buffering conditions. This dramatic decrease in the number of high-affinity binding sites may indicate that the GABA receptor is sensitive to the presence of anions in its environment. This finding is especially interesting in light of the well-known effect of GABA on chloride permeability.

120.16 ANALYSIS OF BENZODIAZEPINE RECEPTOR TURNOVER IN CELL CULTURE BY SDS-GEL ELECTROPHORESIS. C.M. Czajkowski* and D.H. Farb*. (SPON: C. Lance-Jones). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr. Bklyn.,NY 11203

The results of previous experiments indicate that photoaffinity binding of flunitrazepam(FNZM) to embryonic chick brain and spinal cord cultures (prepared from 7d. embryos) is directed toward the functional benzodiazepine receptor (BZD-R): We have shown that there is a good correlation (r^2=0.96) between the binding affinities of a series of BZD's and their EC50's for potentiation of GABA conductance. Secondly, irreversible photoaffinity binding of FNZM to cells decreases by 75% both reversible (3H)FNZM binding and the ability of chlordiazepoxide to potentiate GABA conductance. Two rates (half-times 4 & 32 hrs) for BZD-R degradation have been measured in culture. These rates may reflect multiple receptor types or different pools of receptor. The purpose of this study was to investigate the molecular nature of the functional BZD-R in chick brain cultures and to determine whether receptor heterogeneity could account for the observed biphasic kinetics of degradation. In the present study, 7 and 20 day chick brain homogenates were photolabeled by exposure to 5nM (3H)FNZM and UV light. Separation of the labeled proteins by SDS-PAGE revealed two labeled bands at 51K and 48K. In culture, intact neuronal cells were photolabeled and two labeled proteins of 51K & 48K were also observed. These results are consistent with those obtained in photolabeled rat brain homogenates (Nature 286: 285,1985). After photolabeling, the disappearance of the two bands was examined to determine if their degradation rates were different: Cultures were photolabeled, washed and returned to the incubator for various times. Cells were scraped, homogenized, washed and SDS-PAGE performed. The two labeled proteins disappeared concomitantly with a half-life of about 24 hrs (n=4), indicating that they are not degrading at different rates. By SDS-PAGE it is unclear whether the rate is biphasic. While the amount of radioactivity decreased the pattern of radioactivity was unchanged, suggesting that the 48K protein is not a result of processing of the 51K protein. Subsequent to photoblockade BZD-R replacement was also followed. Cultures were photoblocked (100nM FNZM), washed and then photolabeled with 5nM (3H)FNZM immediately or after a recovery period of 24 hrs. SDS-PAGE following immediate photolabeling revealed an equal decrease in the labeling of both the 51K & 48K proteins. After photolabeling at 24 hrs.there was a 30% recovery of labeling in both species compared to labeling immediately after photoblockade. The results, taken together, suggest coordinate synthesis and degradation of both BZD-R proteins in cell culture. (Supported by NIH NS-18536 & New York Heart).

120.17 REGULATION OF BENZODIAZEPINE RECEPTOR DEGRADATION IN EMBRYONIC BRAIN CULTURES. L.A.Borden,* D.Mierlak,* and D.H.Farb.* (SPON: J.Jakway). Dept. of Anatomy & Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203

High-affinity benzodiazepine binding sites have been identified in brain membrane homogenates, and probably represent the functional benzodiazepine receptor (BZD-R). To investigate the mechanisms involved in receptor regulation we have examined BZD-R turnover in living cell cultures derived from 7d. embryonic chick brains. BZD-R appearance and degradation can be monitored in cells maintained in culture for 7d. by using flunitrazepam (FNZM) as a photoaffinity label. Half-times for BZD-R degradation of 4 and 32 hrs have been determined (submitted). In an attempt to gain an understanding of the mechanisms involved in BZD-R degradation, we have examined a number of drugs and conditions for their ability to alter the fast component of BZD-R degradation. Brain cultures were photolabeled with (3H)FNZM (5nM, 0°C, UV 1 hr), washed, and returned to the incubator (37°C) following the addition of complete medium. Medium was removed at various times and the radioactivity determined. BZD-R degradation was inhibited 20% by the inhibitors of ox. phos. NaN_3 and 2,4-DNP(5mM) and by 2-deoxyglucose(10mM). When degradation was carried out under step down conditions in Earle's BSS, a 20% decrease in degradation rate was also observed. BZD-R degradation was acutely dependent on temperature, as greater than 90% inhibition was observed when degradation was conducted at 0°C. Degradation appears to occur via a non-lysosomal pathway, as the lysosomal protease inhibitors E-64, pepstatin, chymostatin, and bestatin (each 50µg/ml) and the lysosomotropic agent NH_4Cl (10mM) were without effect. These results are similar to those obtained for the insulin receptor in cultured hepatocytes (J.B.C.257:1372,'82), but unlike those obtained on the ACh receptor in myotubes (J.Cell.Phys.107:185,'81).

To determine if BZD-R degradation and synthesis are coordinately regulated, we examined the effect of protein synthesis inhibitors on BZD-R levels and degradation. Cycloheximide (CH,20µg/ml) inhibits protein synthesis 95%, and blocks BZD-R recovery following photoinactivation. A 5 hr incubation with CH decreased BZD-R levels 11% but did not affect degradation. However, tunicamycin (Tm,0.5µg/ml,5hr) decreased protein synthesis 38% and BZD-R levels 44%. The large decrease in BZD-R levels compared to CH probably results from Tm's ability to inhibit glycosylation (44% inhibition of (3H)mannose incorporation into TCA precipitable material). No effect of Tm on BZD-R degradation was observed. These results suggest a role for glycosylation as well as protein synthesis in the maintenance of BZD-R levels and suggest that BZD-R synthesis and degradation can be separately modified. (Supported by NIH NS-18536 & New York Heart).

120.18 DETERMINATION OF AMINO ACID NEUROTRANSMITTERS IN BRAIN VIA PRE-COLUMN DERIVATIZATION AND LIQUID CHROMATOGRAPHY WITH ELECTRO-CHEMICAL DETECTION (LCEC). R. D. Greenland*, S. M. Lasley, and I.A. Michaelson. Dept. Environ. Health, Univ. Cinti. Coll. Med., Cincinnati, Ohio 45267

Recent studies have demonstrated that the reaction of o-phthaldialdehyde (OPA) with free amino acids forms fluorescent compounds that are also electrochemically active (Joseph and Davis, J. Liq. Chromatogr., in press). Experiments were undertaken to determine the optimum conditions for measuring content of the putative amino acid transmitters in small regions of the rat brain employing LCEC.

Amino acids were derivatized by mixing 25 ul of standard and 100 ul of derivatizing solution containing OPA and β-mercaptoethanol at alkaline pH. Exactly 2.5 minutes later 20 ul of the mixture was injected into a liquid chromatograph consisting of a 5 um reverse-phase Biophase C_{18} column and a TL-8A glassy carbon detector cell coupled with an LC-3 amperometric controller. Once gamma-aminobutyric acid (GABA) had been eluted, a washout was initiated by switching to 90% methanol and removing those amino acids with longer retention times. All amino acids of neurotransmitter interest (aspartic acid, glutamic acid, taurine, and glycine)were eluted prior to GABA. Plots of the peak response currents vs. applied potential indicated a common oxidation mechanism among the derivatives. On the basis of these curves a potential of +0.7 V was selected for subsequent work. Standard curves were observed to be linear in the range up to 8 nanomoles, and limits of detection at twice background were 5-10 picomoles. Breakdown of several of the amino acid derivatives occurred rapidly, resulting in decreased peak responses at longer reaction times than 2.5 minutes.

Long-Evans hooded rats were sacrificed by near-freezing, brains removed and sectioned at -15°C for dissection of discrete regions. Tissue was stored at -80°C until extraction. Brain regions were homogenized in 10 volumes of methanol, centrifuged briefly, and 25 ul of supernatant diluted with 25 ul of methanol and reacted with 200 ul of derivatizing solution before injection of 20 ul. Content values are comparable to those previously reported. This method therefore provides a simple and rapid means to assay putative brain amino acid neurotransmitters. (Supported by NIH grant ES-01566).

120.19 SATURABLE BINDING OF [^{35}S]T-BUTYLBICYCLOPHOSPHOROTHIONATE ([^{35}S]TBPS) TO SPECIFIC SITES COUPLED TO RECEPTORS OF γ-AMINO-BUTYRIC ACID (GABA). D. T. Wong[1] and R. F. Squires[2]. [1]Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and [2]Rockland Research Institute, Orangeburg, NY 10962.

Specific binding of a new ligand, [^{35}S]TBPS (Squires et al, Mol. Pharmacol. 23, 326, 1983), is demonstrated in membrane fractions of rat cerebral cortex and accounted for 84 to 94 percent of total binding dependent upon concentrations (1-40 nM) of [^{35}S]TBPS. The optimal temperature for [^{35}S]TBPS binding is near 25°C, with none at 0°C and 60°C. [^{35}S]TBPS specific binding reaches an equilibrium after 90 min of incubation at 25°C. Sodium chloride or bromide is required and is less effectively substituted with sodium iodide or fluoride, suggesting the dominating role of anion. GABA agonists, including GABA and muscimol, inhibit 50 percent of [^{35}S]TBPS binding (IC_{50}) at 1700 and 565 nM, respectively, while GABA antagonist, picrotoxin, but not bicuculline, inhibits with IC_{50} of 257 nM. The diaryltriazine LY81067 (F. P. Bymaster et al, Neurosci. Abst. 1982, p. 579) and the two pyrazolopyridines cartazolate and tracazolate inhibit [^{35}S]TBPS binding with IC_{50} values of 243, 500 and 2000 nM, respectively. GABA (3 µM) inhibits [^{35}S]TBPS binding in a noncompetitive fashion with decreasing the number of binding sites (B_{max}) from a control value of 2.1 to 0.8 pmole/mg protein, whereas picrotoxin (50-250 nM) inhibits competitively by increasing the dissociation constant (K_D) from 32.4 to 49.8 nM. LY81067 at 500 nM inhibits in a pseudocompetitive way by increasing the K_D value from 29.8 to 52.4 nM and lowering the B_{max} value from 1.79 to 1.36 pmole/mg protein. Based on these findings, GABA appears to interact allosterically with [^{35}S]TBPS binding sites while picrotoxin appears to occupy at least a portion of these sites (Squires et al, 1983). We, therefore, conclude that [^{35}S]TBPS binds to specific sites which are coupled to GABA and ion recognition sites in the macromolecular complex of GABA receptor in rat cerebral cortex and similar to those sites which mediate the enhancement of [^{3}H]GABA and [^{3}H]flunitrazepam binding by LY81067 (F. P. Bymaster et al, 1982).

121.1 RELATIONSHIP BETWEEN ^{3}H-FLUNITRAZEPAM BINDING SITES AND DISTRIBUTION OF GAD-LIKE IMMUNOREACTIVITY WITHIN THE RAT BRAIN.
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There have been accumulated data on interaction between benzodiazepine binding sites and GABA receptors. The authors performed autoradiography of ^{3}H-flunitrazepam(flu) binding sites and immunohistochemistry of glutamic acid decarboxylase(GAD) alternatively on serial sections of the rat brain and thus compared distribution of benzodiazepine receptors with that of the GABA marker.

Wistar strain rats were perfusion-fixed by 0.1% paraformaldehyde and serial cryostat sections of 10um thickness were prepared. For GAD immunohistochemistry, the indirect enzyme antibody technique with use of GAD antiserum was applied(PAP method). The autoradiography of benzodiazepine binding sites was done on a tritium sensitive film after 90 min incubation at 0°C with 2 nM ^{3}H-flu. As the results, distribution of GAD immunoreactivities was almost consistent with that of ^{3}H-flu binding sites except in the cerebellum. Nevertheless, high density of ^{3}H-flu binding sites was observed in the cerebral cortex and the density was less in the caudoputamen, while vice versa in GAD immunoreactivity. In the cerebellar cortex GAD immunoreactivity was richest in the Purkinje cell layer with poor distribution in the both molecular and granular layers. In contrast, ^{3}H-flu binding sites were evenly distributed in the whole layers of the cerebellar cortex.

In addition, we performed combination of autoradiography of ^{3}H-flu and GAD immunohistochemistry on the same section at EM level. It is an established fact that ^{3}H-flu binding sites are closely related with GABA receptors on the cell membrane. Möhler et al. pointed out that only one-third of the photolabeled benzodiazepine receptors were found to be associated with immunocytochemically GAD stained nerve endings. In our studies, benzodiazepine binding sites were more densely and widely distributed than GAD immunoreactivities especially in the cerebral and cerebellar cortices. Above-mentioned results were considered to be important as characteristics of benzodiazepine receptors in the brain.

121.2 STUDY OF GABAERGIC NON-PYRAMIDAL NEURONS IN PLEXIFORM LAYERS AND DEEP WHITE MATTER OF RAT HIPPOCAMPUS.
B.G. Vickrey*, D.E. Schmechel*, and J.H. Haring (SPON: A. Roses). Dept. of Medicine (Neurology), Duke University Medical Center, Durham, NC 27710.

Many non-pyramidal neurons in rat hippocampus are immunoreactive for glutamic acid decarboxylase (GAD), the biosynthetic enzyme for gamma-aminobutyric acid (GABA), and are presumably GABAergic neurons (Ribak, C., Brain Res., 140:315, 1978). Some non-pyramidal neurons in hippocampus are also immunoreactive for other neurotransmitter candidates such as cholecystokinin (CCK-8), enkephalin, and other neuropeptides. Not all non-pyramidal neurons are local circuit neurons since some project to medial septum (Chronister, R.B. and DeFrance, J.F., Exp. Neurol., 66:509, 1979). The purpose of the present study was to determine the proportion of GABAergic neurons in the plexiform layers and deep white matter of rat hippocampus, their possible content of other neurotransmitters, and whether some might project to medial septum.

The proportion of GAD immunoreactive to total neurons was very easy to visualize in vibratome sections of perfusion-fixed rat hippocampus with two color double immunocytochemistry (PAP method). Non-GAD immunoreactive neurons were labeled with antiserum to the specific neuronal marker, neuron-specific enolase (NSE). Approximately 90% of the neurons in stratum lacunosum-moleculare, 85% of the neurons in stratum radiatum, and 2/3 of the neurons in stratum oriens and the dentate molecular layer are GAD immunoreactive and presumed GABAergic non-pyramidal neurons.

Double immunocytochemistry (two color) for GAD and CCK-8 showed that the high proportion of GABAergic neurons in these layers is accompanied by overlap of GAD and CCK immunoreactivity. About 3/4 of neurons immunoreactive for CCK were also GAD(+). Such cells were especially numerous in stratum radiatum as well as in the pyramidal and dentate granule cell layer. CCK pre-absorption supported the specificity of CCK immunoreactivity.

A series of rats were injected with horseradish peroxidase (HRP) in the medial septum to yield a population of back-labeled non-pyramidal neurons in hippocampus, as previously reported. Using a different chromogen, GAD immunocytochemistry revealed that some of the HRP-labeled cells in stratum oriens were also GAD(+). Such presumed GABAergic neurons projecting to medial septum represented a small subgroup of both GAD(+) cells and HRP-labeled neurons.

The present results show that (1) a high percentage of neurons in the superficial or deep layers of rat hippocampus are GABAergic, (2) a subset of GABAergic non-pyramidal neurons also display CCK-like immunoreactivity, and (3) a few GABAergic neurons in stratum oriens appear to project to medial septum. The relationship of these findings to development and function of hippocampus will be discussed. (Supported by grant from Mather Foundation).

121.3 AUTORADIOGRAPHY OF ^{3}H-FLUNITRAZEPAM BINDING IN GLOBUS PALLIDUS, ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA AFTER STRIATAL KAINIC LESIONS IN THE RAT.
H. S. Pan*, J. B. Penney and A. B. Young. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

Striatal kainate lesions in the rat provide a biochemical model for Huntington's disease (HD). HD brains show marked decreases in striatal GABA, ACh, and various peptides. Neurotransmitter receptors are also altered. In the rat model, GABA receptor changes in striatum and its projection areas parallel those observed in HD. Benzodiazepine (BDZ) and GABA receptors are linked functionally and anatomically in the basal ganglia. BDZ receptor numbers in striatum are reduced in HD and its animal model. However, this agreement does not hold for distal BDZ receptors. Decreased BDZ binding affinities with no change in receptor numbers have been reported in the deafferented nigra 3 to 4 weeks after striatal lesions (Biggio et al, Brain Res. 220, 344-9; Shibuya et al, Europ. J. Pharm. 62, 243-4). In HD the substantia nigra shows increased BDZ receptor numbers and decreased binding affinities. A major difference between HD and its animal model is chronicity. We have studied BDZ receptors in rat brain acutely and chronically after striatal kainate lesions.

Twenty micron frozen sections of brain were thaw-mounted on subbed slides. Slides were washed 3 times for 5 min in 50 mM Tris.HCl buffer (pH 7.4 @ 4°C) and incubated in 0.5-50 nM ^{3}H-flunitrazepam (^{3}H-Flu) for 30 min at 37°C with or without various BDZ analogs. After incubation, sections were rinsed twice in 4°C buffer for 2 min and blown dry. GABA stimulated ^{3}H-Flu binding was studied using similar incubation conditions and 10^{-4} - 10^{-7} M GABA. Nonspecific binding was determined in adjacent sections co-incubated with 5 μM nonradioactive clonazepam. Autoradiograms were processed and analyzed as previously described (Pan et al, J. Neurosci., in press).

The effects of unilateral striatal kainate lesions on BDZ receptors in the striatum and its immediate projection areas in 6-8 rats were investigated 7 days and 8-12 weeks after the lesion. Acutely lesioned animals showed a 42% loss ($p<.001$, 2 tailed paired Student t-test) of BDZ receptors in the lesioned striatum. No changes in baseline or GABA stimulated BDZ binding were found in other areas. In chronically lesioned rats, a 53% decrease ($p<.001$) in BDZ receptor number was seen in lesioned striatum. B_{max} was increased in deafferented striatal projection areas globus pallidus (29%, $p<.02$), entopeduncular nucleus (27% $p<.02$), and the substantia nigra pars reticulata (33%, $p<.02$). Binding affinities were decreased on the deafferented side. The data suggest that BDZ receptor changes which resemble those seen in HD do occur in chronic striatal kainate lesioned rats.

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121.4 γ-AMINOBUTYRIC ACID STIMULATES THE RELEASE OF ENDOGENOUS ASCORBIC ACID FROM RAT STRIATAL TISSUE.
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γ-Aminobutyric acid (GABA) was found to induce the release of ascorbic acid from rat striatal homogenates and minces. This release was studied with the use of a rapid superfusion system with an on-line amperometric detector that monitors for the presence of easily oxidized substances (i.e. ascorbate, 3,4-dihydroxyphenylethylamine (dopamine)). Such a superfusion system allows the rapid application and removal of chemical substances to a rat striatal tissue sample. Application of GABA (10^{-4} M) in the presence of elevated potassium (40 mM) stimulated the release of an electroactive substance while application of GABA alone (10^{-3} M to 10^{-5} M) had no effect. This substance has been identified to be ascorbic acid by liquid chromatography with electrochemical detection. The release was found to be calcium independent and could be replenished through nonstereospecific uptake. The releasing action of GABA was mimiced by the GABA agonists, muscimol and was completely inhibited by the GABA antagonist, picrotoxin. The structural analogues of GABA, β-alanine and γ-hydroxybutyric acid had no effect. These data indicate that ascorbate release is GABA-receptor mediated and synaptically localized. The presence of a releasable pool which is resistent to depletion by the superfusion stream indicates there is a tightly bound storage compartment for ascorbate in the rat caudate.

121.5 IDENTIFICATION OF BINDING SITES FOR ^{3}H-γ-AMINOBUTYRIC ACID IN SCIATIC NERVE OF THE FROG. A. Barolet*, S. J. Kish* and M. E. Morris (SPON: O. Hornykiewicz). Departments of Pharmacology and Anaesthesia and the Human Brain Laboratory, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada.

The inhibitory neurotransmitter γ-aminobutyric acid (GABA) has previously been shown to increase the excitability of myelinated fibers of the isolated, desheathed frog sciatic nerve *(Morris et al., Neurosci. Abs. 4: 582, 1978; Brain Research IN PRESS, 1983)*. This depolarizing action (reflected by changes in the stimulus-evoked half-maximal A-fiber compound action potential) has an ED_{50} value of 0.09 mM, is chloride-dependent and can be reversibly antagonized by bicuculline and picrotoxin. Fibers forming the dorsal roots show markedly greater sensitivity to GABA than those which contribute to the ventral roots *(Barolet & Morris, Proc. Can. Fed. Biol. Soc. 26, 1983; Morris et al., Brain Research IN PRESS, 1983)*. In the comparison of potencies of agonists muscimol > 3-aminopropanesulfonic acid (3-APS) > GABA > β-guanidinopropionic acid (β-GP) > guanidoacetic acid (GuAc) > δ-aminovaleric acid (δ-AVA) > β-alanine.

This initial characterization of peripheral extrasynaptic receptors on myelinated sensory axons is now followed by studies which identify saturable, Na^+-independent binding sites for GABA in the frog sciatic nerve. The binding of ^{3}H-GABA was carried out on ice under Na^+-free conditions with fresh tissue homogenate. Incubation mixtures contained 2 mg tissue and 25 nM ^{3}H-GABA in 50 mM TRIS citrate (pH 7.6). Non-specific binding was determined in the presence of 6 μM unlabelled GABA. Following a 20 min incubation, samples were centrifuged and the pellets rinsed superficially and counted for radioactivity. Preliminary analysis of saturation data suggests the existence of a population of sites which bind ^{3}H-GABA with an apparent dissociation constant (K_d) of 85 nM. Hypotonic treatment of the sciatic nerve homogenate, followed by multiple washings with buffer, did not significantly alter the amount of specific binding. Incubation at 37°C resulted in a slight decrease in binding, whereas freezing at -80°C caused a marked decrease. The rank order of potencies of agonists that displaced bound ^{3}H-GABA was muscimol > GABA > δ-AVA > β-alanine. However 3-APS, β-GP, and GuAc, as well as bicuculline and picrotoxin (all in concentrations up to 0.1 mM) had no discernible effect on the binding of GABA.

Thus, under our assay conditions the ^{3}H-GABA binding site which has been identified does not appear to have characteristics similar to those of either the receptor mediating GABA's depolarizing effect in the peripheral nerve or the classical ^{3}H-GABA binding site present in the CNS.

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121.7 GABA AGONISTS POTENTIATE NOREPINEPHRINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN RAT BRAIN FRONTAL CORTEX SLICES. E.W. Karbon* and S.J. Enna. Depts. Pharmacol. and Neurobiol., Univ. Texas Med. Sch., Houston, TX 77025.

It has been suggested that there are at least two pharmacologically and functionally distinct GABA receptor populations in brain. Whereas $GABA_A$ receptors appear to be directly coupled to a chloride ion channel and are blocked by bicuculline and picrotoxin, the $GABA_B$ sites are not. Unlike some other neurotransmitters, there is no evidence to support a direct link between $GABA_A$ receptors and a second messanger. The present study was undertaken to determine whether the $GABA_B$ site may be associated with a cyclic nucleotide generating system.

Slices of rat brain frontal cerebral cortex were incubated with ^{3}H-adenine and adenylate cyclase activity determined by measuring the production of ^{3}H-cAMP. Adenylate cyclase activity was increased 12-15 fold in the presence of 100 μM norepinephrine. When the samples were incubated with baclofen, a $GABA_B$ receptor agonist, 100 μM norepinephrine increased activity 40-fold. The EC_{50} for baclofen to enhance the stimulated response was approximately 20 μM. Baclofen alone produced a small (2-fold), but statistically significant, increase in adenylate cyclase activity. Kojic amine and GABA, both of which are $GABA_A$ and $GABA_B$ receptor agonists, also potentiated norepinephrine-stimulated adenylate cyclase activity in this system. Muscimol, a relatively selective agonist for the $GABA_A$ site, was relatively weak in comparison with baclofen. Baclofen (100 μM) enhanced the ability of norepinephrine to stimulate cyclase activity at all concentrations (0.1 to 100 μM) of catecholamine studied. The selectivity of this interaction is indicated by the finding that other amino acids, such as glycine and glutamate, had no effect on either basal or norepinephrine-stimulated cyclase activity. These findings suggest that there may be a functional link between GABA receptors and the adrenergic receptor-coupled adenylate cyclase system in brain. The initial data suggest that this associateion may be a characteristic of $GABA_B$ receptors. (Supported, in part, by USPHS grants NS-00335 and GM-07405, and by NSF grant BNS-8215427).

121.8 MULTIPLE BENZODIAZEPINE BINDING SITES IN NEURONAL CULTURE: FUNCTIONAL SIGNIFICANCE AND DEVELOPMENTAL CHANGES. G.D. Schiller*, R. Romaine* and D.H. Farb* (SPON: M. Halpern) Dept. of Anatomy and Cell Biology, SUNY Downstate Med. Ctr., Brooklyn, NY 11203

Two binding sites of high (A) and low (B) affinity for benzodiazepines (BZD) have been found in 7-d old chick CNS, but site-B disappears by 20-d. Electrophysiological evidence from cultured spinal neurons established a good correlation betweeen the EC50s of various BZDs for potentiation of GABA conductance and their in vitro binding affinity to site-A, but not site-B. The physiologically observed EC50s are 38 and 5 fold lower affinity compared to binding at 0 °and 37° respectively. This raises the question of whether there is a difference in binding affinities between BZD-R in membranes prepared from embryonic brain and those from brain cell cultures. The present study sought to examine whether or not multiple BZD binding sites also exist in cultured neurons and if they are subject to developmental change. Homogenates were prepared from 7-d brain cultures (derived from 7-d embryos) and the binding affinities for clonazepam (CZ), flunitrazepam (FNZM), diazepam (DZ), flurazepam (FZ) and chlordiazepoxide (CDPX) determined by competition binding studies. Multiple binding sites similar to those of 7-d CNS were observed: The respective site-A (nM) and site-B (uM) affinities were: CZ 2 & 19.5, FNZM 2.6 & 1.61, DZ 13.6 & 23, FZ 23.6 & 21.3, CDPX 103 & 13.5. A good correlation was found between the affinity of site-A and the EC50 determined electrophysiologically (slope=1.21, r^2 =0.93). This was not so for site-B (slope=0.49, r^2=0.098). The former correlation supports the hypothesis that site-A is physiologically relevant. Site-B can be distinguished from the peripheral type BZD site since the latter binds FNZM and DZ with high affinity (<20nM; Life Sci.28,991,'80) but to site-B with low affinity. A transition from site-A and -B to only site-A also occurred with time in culture. This suggests that the factors that determine the expression of BZD binding sites also exist in culture. Comparisons of the Bmax for FNZM binding to site-A and -B reveals that site-B predominates in a ca. 35 fold excess in 7-d cultures, becoming ca. 5 fold by 14-d and is undetectable in 20-d cultures. Results suggest that both sites are neuronal: Thus, cultures nearly free of neurons do not exhibit irreversible photoaffiniy labelling of site-A; further, the loss of site-B with time in culture, and its abundance in young cultures (treated with ARA-C to inhibit growth of non neuronal cells), points to site-B also being neuronal. Future research will aim to study the relationship of site-B to site-A and the binding properties of BZDs to intact cultured cells with regard to the physiological significance and possible regulation by chronic BZD exposure. (Supported by NIH NS-18536 & New York Heart).

121.9 BETA-CARBOLINES AND BENZODIAZEPINES: INTERACTIONS BETWEEN NEGATIVE AND POSITIVE MODULATORS OF GABA-INDUCED CONDUCTANCE CHANGES ON CULTURED CHICK NEURONS. C.Y. Chan* AND D.H. Farb* (SPON: D. Soifer). Dept. of Anatomy & Cell Biology, S.U.N.Y. Downstate Med. Ctr., Brooklyn, NY 11203.

We have previously shown that methyl beta-carboline (BCCM) inhibits GABA-induced conductance increases (gGABA) and that benzodiazepines (BZD) potentiated gGABA in perfused cultures. Biochemical studies show that beta-carbolines (BCC) and BZD compete for binding to rat brain membranes. BCC and BZD may either compete for the same functional site or exhibit independent actions mediated by separate sites or different domains of the BZD site. Thus, we examined if BZD reverse the inhibition of gGABA by BCC: If methyl 6,7-dimethoxy-beta-carboline (DMCM) and clonazepam (CZ) act independently, then CZ would not reverse the ability of DMCM to inhibit gGABA, but would simply shift the inhibited GABA dose-response curve to the left.

Drugs were applied from pressure pipets and the responses recorded intracellularly. DMCM inhibited gGABA in a Michaelis-Menten like fashion, with a potency of 10±4nM and an efficacy of 66±8% inhibition (n=4). The innibition of gGABA by DMCM was not surmountable by maximal GABA: 30nM DMCM inhibited gGABA at 500UM GABA (EC50=17μM) by 32±7% (n=2). However, the inhibition of 500uM GABA by 30nM DMCM was reversed to control by a mixture of 30μM CZ and 30nM DMCM. No potentiation of gGABA past the control level was seen, consistent with the fact that BZD do not potentiate maximal GABA response. In experiments using 50μM GABA, DMCM inhibition was reversed and net potentiation was produced by 30μM CZ and 30nM DMCM, as expected for competitive interaction between the two ligands. UV-flunitrazepam photoinactivation did not seem to affect this inhibition of gGABA by DMCM. The same reversal of DMCM inhibition of the response to 500μM and 50μM GABA was observed (n=2) on photoinactivated cultures; however, the CZ/DMCM mixture did not cause net potentiation of 50μM GABA responses. This result is consistent with blockade of binding and electrophysiological action of BZD by photoinactivation, but indicates that competition by BZD at the DMCM site was unaffected. RØ15-1788 caused a dose-dependent potentiation of gGABA with an EC50 of 130nM comparable to that of CZ (EC50=138nM) but a maximal potentiation of about 72% as compared with 450% potentiation by BZD agonists. Maximal Ro15-1788 applied with maximal BCCM also reversed the BCCM inhibition of submaximal gGABA and caused a small net potentiation, suggesting competition of the BCC site with a partial agonist. The hypothesis that BCC and BZD are negative and positive modulators at a common receptor is supported by a good correlation (r^2=0.97,slope 1.7) between GABA ratios (Braestrup, et al. Science 216:1241,'82) and maximal potentiation or inhibition of gGABA. (NIH NS-18536 & N.Y. Heart & NHDA Fellowship).

122.1 $GABA_A$ AND $GABA_B$ RECEPTORS IN MYENTERIC NEURONS: AN INTRACELLULAR STUDY. E. Cherubini and R. A. North. Neuropharmacology Laboratory, M.I.T. Cambridge, MA 02139

The effects of γ-aminobutyric acid (GABA) applied both by ionophoresis and perfusion to myenteric neurons of the guinea-pig ileum were investigated by intracellular recording techniques. Ionophoretic application of GABA (10 pC - 30 nC) caused membrane depolarization of AH neurons but not S neurons. This depolarization was associated with a conductance increase, and reversed polarity at a membrane potential of -18 mV with KCl electrodes and -39 mV with K acetate, citrate or sulphate electrodes. This depolarization was antagonized by bicuculline in an apparently competitive manner. During prolonged or repeated ionophoretic application of GABA, both the depolarization and conductance increase desensitized.

Superfusion of GABA (1-100 μM) caused a membrane depolarization in AH neurons, associated with an increase in membrane conductance, The increase in conductance was much smaller than that evoked by ionophoresis of GABA. Bicuculline only partially depressed the depolarization induced by superfusion of GABA, particularly reducing its rate of rise. β-p-chlorophenyl GABA (baclofen) (10 μM-1 mM) mimicked the effect of GABA perfusion. The depolarization induced by GABA superfusion did not decline during prolonged applications. The bicuculline resistant effect of GABA superfusion was reversibly blocked by cobalt (2 mM). Since the bicuculline insensitive, non-desensitizing depolarization may result from reduction of an inward calcium current, we examined the effect of GABA on two calcium dependent events: synaptic transmission and the calcium spike in AH neurons.

GABA reduced the amplitude of the fast excitatory postsynaptic potential (e.p.s.p.) without changing the amplitude of the nicotinic response to ionophoretic application of acetylcholine (ACh). This effect was mimicked by baclofen (10-100 μM) and not blocked by bicuculline (10 μM). It did not desensitize during prolonged applications. Cholinergic and non-cholinergic slow e.p.s.p.s evoked by single or repetitive presynaptic nerve stimulation were also reduced by GABA. GABA reduced the duration of the action potential in AH neurons in concentrations that did not affect the membrane potential or conductance. The effect was very marked when electrodes were filled with CsCl and tetrodotoxin was in the superfusing solution. This effect was also mimicked by baclofen, was insensitive to bicuculline and did not desensitize. Thus, GABA inhibits release of ACh and the transmitter mediating the slow e.p.s.p. and this may result from the inhibition of an inward calcium current; the receptor appears to be $GABA_B$. GABA also opens chloride channels in the same cells by acting on a $GABA_A$ receptor.

122.2 SUBSENSITIVITY TO GABA FOLLOWING CHRONIC BENZODIAZEPINES: RECEPTOR BINDING STUDIES. S. L. Rauch* and D. W. Gallager. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

A number of clinical and behavioral studies have demonstrated reductions in drug response following chronic benzodiazepine administration. We have recently found a functional subsensitivity to GABA following chronic exposure to benzodiazepines (Gallager et al, Neurosci. Abst. 1983). In order to investigate whether receptor alterations accompany these changes in GABA sensitivity, we examined various binding-site components of the GABA postsynaptic receptor complex.

As in the electrophysiological studies, adult male rats were treated daily with vehicle or diazepam (DZ; 5mg/kg,ip) for 3 weeks. In these studies we utilized ^{3}H-flunitrazepam (^{3}H-FLU) binding and GABA stimulation of ^{3}H-FLU binding as probes of the GABA receptor complex. ^{3}H-muscimol was used as a measure of the high affinity GABA recognition portion of the GABA receptor complex. In agreement with a number of previous studies, our treatment regimen did not produce measurable alterations in benzodiazepine receptor number or affinity as measured by saturation binding of ^{3}H-FLU (0.5 to 16nM; 37°C) to cerebral cortical membranes (VEH: B_{max}= 3932 ± 283 fmoles/mg protein, K_D= 21.8 ± 7.0nM; DZ: B_{max}= 3737 ± 252 fmoles/mg protein, K_D= 21.5 ± 0.6nM). In addition, GABA was found to enhance ^{3}H-FLU binding in membranes from both the VEH and DZ treated animals (EC_{50}GABA= 4μM). However, the magnitude of the GABA enhancement was found to be significantly reduced in membranes from chronic diazepam treated rats (VEH= 43.0±5.0%; DZ= 21.5 ± 2.3%). These effects do not appear to be due to decreases in GABA recognition sites since neither GABA receptor number nor affinity were affected by chronic DZ treatment as measured by ^{3}H-muscimol binding (VEH: B_{max}= 1538 fmoles/mg protein, K_D= 11.9nM; DZ: B_{max}=1539 fmoles/mg protein, K_D= 8.8nM).

These results suggest that rather than a change in receptor number or affinity, chronic DZ exposure may produce a more subtle change in interactions between various elements of the GABA macromolecular complex. (Supported by Klingenstein Foundation and the State of Connecticut.)

122.3 SUBSENSITIVITY TO GABA FOLLOWING CHRONIC BENZODIAZEPINES: ELECTROPHYSIOLOGICAL STUDIES. D.W. Gallager, S.F. Gonsalves*, S.L. Rauch* and J.M. Lakoski. Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06508.

Benzodiazepines exert many of their pharmacological effects by a selective facilitation of the postsynaptic actions of GABA. Electrophysiological studies of serotonin(5HT)- containing neurons in the dorsal raphe (DR) nucleus indicate that immediately following benzodiazepine injection, GABAergic inhibition is potentiated without altering sensitivity to other neurotransmitter substances. While several clinical and behavioral studies have reported a reduction in drug response following chronic benzodiazepine treatment, physiological studies of neurotransmitter function following chronic exposure are lacking. We now report a selective decrease in microiontophoretic sensitivity to GABA in DR neurons following chronic benzodiazepine administration.

Microiontophoretic sensitivity to the neurotransmitters 5HT and GABA was tested in DR neurons of adult male rats treated daily for 3 to 6 weeks with either vehicle (VEH) or diazepam (DZ;2.5 or 5 mg/kg/day,ip). As is observed following acute DZ administration, chronic DZ treatment did not alter the spontaneous firing rate of DR neurons (DZ:0.8 ± 0.1 vs VEH: 1.0 ± 0.1 spikes/sec). Neuronal sensitivities to GABA and 5HT were calculated as the product (IxT_{50}) of the iontophoretic current (nA) and time (sec) required to decrease spontaneous firing of a recorded cell by 50% of its basal rate. DR neurons in animals chronically treated with 5 mg/kg/day DZ were significantly less sensitive to GABA ($p<0.001$) than their corresponding controls (DZ: IxT_{50}=22.2±2.8; VEH: IxT_{50}=10.7±1.4); a similar trend was observed in the 2.5 mg/kg/day DZ group. These effects were selective for GABA as sensitivity to 5HT was not significantly altered (DZ:IxT_{50}=182±15; VEH:IxT_{50}= 164±24). To determine whether the subsensitivity to GABA was mediated through a benzodiazepine receptive site, neuronal sensitivities to GABA and 5HT were tested before and after the iv injection of the specific benzodiazepine antagonist RO15-1788 (2 mg/kg). Following RO15-1788, sensitivity to GABA in chronic DZ-treated animals increased to the range observed in vehicle-treated rats. However, RO15-1788 failed to alter GABA sensitivity in vehicle-treated animals or to significantly modify 5HT responses of DR neurons in either treatment group.

These studies indicate that 5HT neurons in the DR become subsensitive to GABA following chronic exposure to diazepam. This effect may be correlated with the development of tolerance to benzodiazepines following chronic drug exposure. (Support: Klingenstein Fund, USPHS MH 14276, State of Connecticut)

122.4 NEUROTRANSMITTER-ACTIVATED CHLORIDE CHANNELS IN CULTURED MOUSE SPINAL NEURONS ARE ALSO VOLTAGE-REGULATED. R.E. Study* and J.L. Barker (SPON: R. Aldrich). Lab. of Neurophysiology, NINCDS NIH, Bethesda, MD 20205.

The inhibitory neurotransmitters GABA, glycine, and beta-alanine, as well as the anesthetic barbiturate (-)pentobarbital, have been previously shown to increase membrane chloride conductance in cultured mouse spinal neurons (Barker, J.L. and Ransom, B.R., J. Physiol. 280, 331-354, 1978; Huang, L.Y.M. and Barker, J.L., Science 207, 195-197, 1978). We have found that the magnitude of the conductance change produced by each of these agents is highly voltage-dependent, showing up to a 15-fold increase over the range of -90 to +10 mV, as measured using voltage steps with a two-electrode voltage clamp. The voltage-dependence is similar with all of the four agents, even though the channels activated by each differ in terms of their kinetics and conductance. Plots of net conductance change vs. potential were non-linear, with minimum slope near resting and at hyperpolarized potentials, and increasing slope at depolarized potentials. This voltage-dependence of neurotransmitter activated chloride channels appears to be the rule rather than the exception in cultured spinal neurons, although the steepness of the conductance-voltage relationship varies somewhat. For GABA and glycine, the changes in chloride channel behavior underlying this voltage-dependence were studied using fluctuation analysis. For the range of -90 to +10 mV, there was no change in the average single-channel conductance for either neurotransmitter. In both cases, however, there was an increase in average channel open lifetime which was insufficient (about 2-fold) to fully account for the large increase in net conductance. This indicates that there was also an increase in open channel lifetime underlying the net effect. Since the inhibitory action of the activated chloride channels is much enhanced at depolarized potentials, this behavior may be an important means by which such inhibitory transmitters stabilize the post-synaptic membrane in the presence of excitatory input.

122.5 SOMATIC AND DENDRITIC ACTIONS OF GABA AGONISTS AND UPTAKE BLOCKERS IN THE HIPPOCAMPUS IN SITU. C. Rovira*, Y. Ben-Ari and E. Cherubini. (SPON. Anthony M. Adinolfi) Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif sur Yvette, France.

GABA agonists and GABA uptake blockers were ionophoretically applied to the soma and apical dendrites of CA_1 hippocampal neurones of intact urethane anaesthetized rats. A twin set of multibarrelled pipettes was used to enable concomitant recordings of somatic and dendritic field potentials elicited by commissural stimulation and iontophoresis of drugs at either site. Application of GABA, isoguvacine or muscimol on the pyramidal layer produced a biphasic effect: an inhibition of the population spike that showed a rapid "fading", followed by an enhancement with the appearance of a second and occasionally a third population spike ("off" response). The "off" response produced by muscimol was consistently greater than that produced by isoguvacine and GABA. The "fading" and the "off" response were exclusively restricted to the immediate vicinity of the pyramidal layer and were not associated with significant changes in the concomitantly recorded dendritic field EPSP. In contrast application of GABA and its analogs to the stratum radiatum, induced a reduction of the field EPSP and the somatic population spike. No "fading" and "off" response were observed even during long periods of application of these drugs. Somatic application of GABA uptake blockers nipecotic acid or guvacine consistently enhanced GABAergic inhibition, induced a decrease in its latency to peak, and an increase in the recovery time. The fading and the "off" response were completely blocked by these drugs. Dendritic application of the uptake blockers reduced the field EPSP and the somatic population spike, but failed to produce prominent changes in the action of GABA and its analogs. Somatic application of nipecotic acid and guvacine blocked also the enhancement of the somatic population spike induced by increased frequency of stimulation. These data suggest that in the pyramidal layer of CA_1 hippocampal neurones removal of GABA is highly efficient and may be in part responsible for the lability of inhibition.

122.6 INTRACELLULAR RECORDINGS FROM NEURONS IN THE LATERAL SEPTAL NUCLEUS AND THE EFFECTS OF DRUGS INTERACTING WITH GABA-RECEPTOR IONOPHORE COMPLEXES. David R. Stevens, Joel P. Gallagher and Patricia Shinnick-Gallagher. Department of Pharmacology and Toxicology, Univ. of Texas Med. Br., Galveston, TX 77550.

Intracellular recordings were made from an in vitro rat brain slice preparation to study inhibition at the cellular level in the lateral septal nucleus. The preparation was similar to that described previously by Miller (1981). We have recorded from over 100 neurons of the dorsolateral nucleus which had stable resting potentials averaging 67mV (range: 55-78mV). These neurons had input resistances of 40-100 megohms and long time constants (~40 msec) compared to other CNS neurons. Stimulation of the medial septal area resulted in antidromic action potentials (APs) with short latencies whereas stimulation of the fimbria/fornix area produced excitatory postsynaptic potentials (epsps) and/or orthodromic APs.

Fast sodium APs of 2-4 msec duration could also be elicited by direct stimulation via the intracellular microelectrode. These APs were characterized by a brief afterhyperpolarization followed by a longer lasting hyperpolarization (50-200 msec). Most cells had a depolarizing after-potential which became more evident at slightly hyperpolarized membrane potentials.

Spontaneous epsps were frequently present. Epsp amplitudes were enhanced by membrane hyperpolarization. Spontaneous APs were observed in about 50% of the cells and could usually be attributed to epsp activity.

GABA superfusion (10^{-4} M) resulted in either a small hyperpolarization (2-5mV) or a depolarization (10mV); occasionally, large GABA-induced depolarizations (30mV) were recorded. All responses were associated with a conductance increase. Iontophoretic GABA application resulted in depolarizing or biphasic responses with concomitant conductance increases. Biphasic responses could be converted to depolarizing responses by decreasing the GABA ejection current. Picrotoxin superfusion (10^{-5}M) resulted in an increase in AP frequency and the onset of bursting activity. The sedative/hypnotic benzodiazepine, flurazepam (10^{-6} - 10^{-5} M), hyperpolarized the membrane potential and decreased spontaneous activity. This membrane hyperpolarization was associated with a conductance increase. Flurazepam at these concentrations converted biphasic GABA responses to monophasic depolarizing responses.

The excitation observed during picrotoxin suggests the presence of tonic, GABA-mediated inhibition. It is possible that the effects of flurazepam are due to enhancement of GABA's action.

122.7 PHYSIOLOGICAL EVIDENCE FOR TWO PHARMACOLOGICALLY DISTINCT, BICUCULLINE-INSENSITIVE ACTIONS OF GABA IN THE RAT HIPPOCAMPAL SLICE. Brian Ault* and J. Victor Nadler. Dept. Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

It has been proposed that the anti-spastic drug baclofen acts at a subpopulation of GABA receptors that is insensitive to bicuculline (BIC). Our previous studies demonstrated that GABA could depress synaptic responses of CA1 hippocampal pyramidal cells evoked by stimulating Schaffer collateral-commissural fibers, even in the presence of BIC, and that baclofen reproduced these actions. Similar actions of the GABA analogue 3-aminopropanesulfonic acid (3-APS), which has a low affinity for BIC-insensitive receptors, were more readily antagonized by BIC than were actions of GABA. We have now studied the effects of these compounds upon perforant path-evoked responses of dentate granule cells.

The perforant path fibers were stimulated at a rate of 2/min in vitro and just-maximal population spikes were recorded from the granule cell body layer. The lateral perforant path extracellular EPSP was recorded in the distal dendritic region of the fascia dentata, while stimulating at an intensity just below that required to elicit a population spike.

Bath-applied GABA reduced the amplitude of the perforant path population spike with an EC_{50} of 3.5 ± 0.4 mM and usually abolished this response at a concentration of 8 mM. BIC (100 µM) rather weakly reversed the action of GABA (concentration ratio of 1.5 ± 0.1). 3-APS depressed the population spike with an EC_{50} of 138 ± 5 µM and its action was more readily reversed by BIC (concentration ratio of 14 ± 3). Comparable concentration ratios for GABA and 3-APS of 1.6 ± 0.5 and 13 ± 4, respectively, were obtained using the Schaffer collateral-commissural (CA1) population spike as the test response. Raising the concentration of BIC from 100 µM to 500 µM did not further reverse the action of GABA. Baclofen, even at a concentration of 100 µM, did not reduce the perforant path population spike by more than 50%, although 20 µM baclofen abolished the Schaffer collateral-commissural population spike. When BIC (500 µM) and baclofen (100 µM) were superfused continuously, a 5 ml pulse of GABA (4 mM) still depressed the residual population spike. GABA also depressed the lateral perforant path EPSP with an EC_{50} of 4.5 mM and 100 µM BIC only weakly reversed this action (concentration ratio of 1.5). In contrast, baclofen little affected this response at concentrations as great as 100 µM.

Thus baclofen failed to reproduce some BIC-insensitive actions of GABA in the fascia dentata, although it reproduced all such actions in the CA1 area. These observations suggest that GABA exerts two types of BIC-insensitive action, only one of which is shared by baclofen. (Supported by NIH grant NS 16064.)

122.8 GABA-MEDIATED INHIBITION AT SYNAPSES FORMED BY CULTURED RETINAL NEURONS. E. Agardh*, H.H. Yeh and D.G. Puro (Spon: P.A. Dudley). Laboratory of Vision Research, National Eye Institute, NIH, Bethesda, Maryland 20205

A cell culture system was used to assess the effects of GABA on the release of acetylcholine at synapses formed in culture by embryonic chick retinal neurons. In our experimental system, striated muscle cells served as postsynaptic targets for cholinergic retinal neurons. Myotubes are useful as postsynaptic targets since they have a high density of cholinergic receptors and their large size permits prolonged intracellular monitoring of postsynaptic responses to acetylcholine.

We report here that microiontophoretically applied GABA could inhibit transmission at synapses between cholinergic retinal neurons and muscle cells. The GABA agonists, isoguvacine and muscimol, also inhibited cholinergic transmission. These inhibitory effects were blocked reversibly by the GABA antagonist, bicuculline. A benzodiazepine, flurazepam, could augment the GABA mediated inhibition of synaptic transmission.

It was possible, by plating a low density of retinal neurons, to study the effects of GABA on acetylcholine release from isolated, visually identified, cholinergic retinal neurons. Our results indicate that most of the cholinergic neurons examined in this culture system are responsive to GABA.

122.9 EFFECTS OF BENZODIAZEPINES AND RO 15-1788 ON INHIBITION IN THE HIPPOCAMPAL SLICE. Gregory L. King, Julie Johnson Knox*, and Raymond Dingledine, Dept. of Pharmacology and Neurobiology Curriculum, Univ. North Carolina, Chapel Hill, NC 27514.

Numerous studies have emphasized the complexity of the benzodiazepine-GABA receptor complex in the mammalian CNS. We have examined the effect of benzodiazepines (diazepam and chlordiazepoxide), and of Ro 15-1788, a benzodiazepine antagonist, on several indices of inhibition in the CA1 region of the in vitro hippocampal slice preparation: 1) orthodromic paired-pulse inhibition (PPI), 2) intracellular IPSPs evoked by either orthodromic or antidromic stimulation, and 3) the Ca^{++}-activated K^{+} conductance. All agents were applied either by continuous perfusion or as a small droplet (<1 nl) onto the surface of the slice. When the agents were perfused during the PPI studies, a range of interstimulus intervals was chosen such that drug effects could be examined on both strong (10-30 msec intervals) and weak (100-200 msec intervals) inhibition. In some slices, inhibition was observed only at the shorter interstimulus intervals. Both chlordiazepoxide and diazepam (0.1-1.0 μM) enhanced PPI by as much as 40%, with a greater effect on the peak inhibition than the decay phase. When the agonists, and Ro 15-1788, were applied by the nanodrop technique, a single interstimulus interval (10-30 msec) was used. Chlordiazepoxide and diazepam (0.1-1.0 μM) enhanced inhibition by as much as 90%, an effect which lasted 40-60 minutes. Ro 15-1788 (0.1-1.0 μM) decreased inhibition by as much as 100%, and in some cases facilitated the test response, with a timecourse similar to that of the agonists. In preliminary intracellular studies, application of diazepam (1.0 μM) by the nanodrop technique prolonged or enhanced the IPSP produced by orthodromic stimulation (n=3) with little or no effect on either the antidromic IPSP (n=2) or the afterhyperpolarization following a Ca^{++} spike (n=1). By contrast, Ro 15-1788 (1.0 μM) applied in a similar manner depressed the amplitudes of both the orthodromically activated IPSP (n=4 of 5) and the Ca^{++}-spike afterhyperpolarization (n=2), with no effect on the antidromic IPSP (n=2). Some micropipettes contained 50-100 mM QX-314, a quaternary lidocaine derivative which eliminates regenerative sodium conductances. These results support the hypothesis that benzodiazepines act to enhance synaptic inhibition. They also suggest that the enhanced inhibition is not achieved solely by an action on the GABA receptor complex. In addition, if Ro 15-1788 acts as a benzodiazepine antagonist, the presence in the hippocampus of an endogenous ligand for benzodiazepine receptors is implied. QX-314 was generously supplied by Dr. Bertil Takman of Astra Pharmaceuticals and Ro 15-1788 by Dr. J. O. McNamara. Supported by NS-06953, NS-07166 and NS-17771.

122.10 THE EFFECT OF FLURAZEPAM AND SEVERAL β-CARBOLINES ON SINGLE DOPAMINE CELL ACTIVITY. J. A. D'Amico* and D. A. MacNeil. Dept. of Pharmacology I, Hoffmann-La Roche Inc. Nutley, N.J. 07110

The activity of dopamine (DA) containing neurons of the substantia nigra pars compacta (SNC) can be modified by compounds that act at GABA receptors and at benzodiazepine (BZD) receptors (1,2,3). The present study describes the effects of flurazepam and muscimol on DA neuronal activity in the presence or absence of GABA. Additionally, several β-carbolines, which are known to interact at BZD sites, were also studied.

Sprague-Dawley rats were anesthetized (chloral hydrate, 400 mg/kg i.p.) and prepared for extracellular recordings from single DA cells in the SNC. Experimental compounds were administered intravenously. Flurazepam was dissolved in saline, while muscimol and the β-carbolines were suspended in a 0.2% carboxymethylcellulose/0.1% Tween 80 solution. In one set of experiments, the GABA synthesis inhibitor, 3-mercaptopropionic acid (3-MPA), was administered (90.0 mg/kg i.p.) 8 mins. before (4) flurazepam or muscimol.

Flurazepam produces a dose-dependent (0.1-1.2 mg/kg, i.v.) increase in DA neuronal activity. At 1.0 mg/kg the change in activity was greater than 200%. In rats that had been pretreated with 3-MPA, the effect of flurazepam was blocked, while the increase in firing rate produced by muscimol, a direct GABA agonist, was not influenced by 3-MPA. Thus the excitatory effect of flurazepam is dependent on the presence of GABA.

The β-carbolines (β-CCE, β-CCM, FG-7142) depressed the activity of DA cells to a limited degree (20-40%). In addition, the β-carbolines attenuated the facilitatory effect of flurazepam by approximately 10 fold. The data demonstrate that in a dopaminergic system the β-carbolines act as BZD antagonists as well as inverse agonists.

References
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122.11 INFLUENCES OF 2 ATYPICAL BENZODIAZEPINES, RO 5-3663 and RO 5-4864, ON GABA ERGIC MECHANISMS. D. A. MacNeil, J. A. D'Amico*, W. D. Horst, R. A. O'Brien and N. Spirt* Dept. of Pharmacology I, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

Ro 5-3663 is a convulsant benzodiazepine (BZ) which is known to interact with the BZ, GABA receptor-chloride ionophore complex, but has a very low affinity for either the "peripheral" or the "central" BZ binding sites. Ro 5-3663 has previously been shown to inhibit the GABA stimulation of diazepam binding (1). Ro 5-4864 is a BZ well known to have a high affinity for the "peripheral" BZ binding site (2). The present studies describe the influences of these 2 BZ's on GABA stimulated ^{3}H-diazepam binding and on the firing rates of dopamine (DA) neurons in the substantia nigra pars compacta. Experimental procedures were conducted as described elsewhere (1,3).

Ro 5-3663 and Ro 5-4864, at concentrations of 10^{-6}M, inhibit the GABA enhancement of ^{3}H-diazepam binding to rat brain (cortex) tissue by 32 and 43% respectively. Although neither drug influenced the firing rates of DA neurons when given alone, both drugs blocked the GABA mediated excitatory effects of flurazepam on DA neuron firing rates, Ro 5-3663 at cumulative doses of 0.3 mg/kg (i.v.) and Ro 5-4864 at cumulative doses of 2.0 mg/kg (i.v.). Neither drug influenced the excitatory effects of the direct GABA agonist, muscimol, on DA neuron firing rates.

The observations here that Ro 5-3663 and Ro 5-4864 have similar properties in blocking GABA mediated mechanisms along with the previous demonstration that Ro 5-3663 has no affinity for the "peripheral" BZ site (2) suggests that Ro 5-4864 is not specific for the "peripheral" BZ site but may also interact at a site on the BZ, GABA receptor-chloride ionophore complex. This may account for the proconvulsant and convulsant properties of Ro 5-4864 recently described by others (4,5,6).

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122.12 ANTICONVULSANT AND SEDATIVE PROPERTIES OF SOME BENZODIAZEPINES. M.W. Kalichman. Naval Health Research Center, San Diego, Calif. 92134.

If the anticonvulsant and sedative properties of the benzodiazepines are produced by the same mechanism, then one would predict that benzodiazepine relative potencies should be the same regardless of the property being studied. In order to test this hypothesis, the anticonvulsant & sedative properties of the benzodiazepines were studied in rats.

In Experiment I, the relative potencies of 6 benzodiazepines were compared as anticonvulsants against convulsions produced by 3-mercaptopropionic acid. Rats were each tested with three doses of each of 6 benzodiazepines: Chlordiazepoxide (CHLOR), Clonazepam (CLON), Diazepam (DIAZ), Flurazepam (FLUR), Lorazepam (LOR), Triazolam (TRI). Thirty minutes after i.p. benzodiazepine injections, subjects received 3-mercaptopropionic acid (40 mg/kg, i.p.). Based on the incidence of convulsive behaviors, the six benzodiazepines were ranked in order of decreasing potency as follows: TRI, CLON=LOR, FLUR, CHLOR, DIAZ. In estimates of relative potency for three of these drugs, it was estimated that TRI was twice as potent as CLON and 60 times more potent than FLUR.

In Experiment II, three benzodiazepines were tested as "sedative-hypnotics" in rats. Rats were tested with three doses of each of three drugs: CLON, FLUR, & TRI. Thirty minutes after benzodiazepine injections, subjects were scored for movement, position, and ptosis every five minutes for a period of 60 minutes. "Sedation" was defined as immobility, prostrate position, and at least 50% eye closure. By this criterion, the three benzodiazepines were ranked in order of decreasing potency as follows: TRI, CLON, FLUR. In a comparison of these three drugs at doses which would have produced sedation in 50% of the subjects tested, it was estimated that TRI was 10 times as potent as CLON and 400 times more potent than FLUR.

Although the orders of potency were the same in the anticonvulsant and sedative experiments, the relative potencies of the benzodiazepines were markedly different. If the mechanisms of the sedative and anticonvulsant properties of the benzodiazepines were the same, then the relative potencies of CLON, TRI, and FLUR should have been comparable in the two test procedures. Thus, the marked differences in relative potency are inconsistent with the hypothesis that these two properties are produced by the same mechanism.

122.13 THE ACTION OF FOOTSHOCK AND β-CARBOLINES ON GABA RECEPTORS IN THE BRAIN OF STRESSED AND UNSTRESSED RATS. G. Biggio, A. Concas*, M. Serra*, and M.G. Corda*. Institute of Biology, Chair of Pharmacology, University of Cagliari, Italy.

Cerebral cortex membranes from rats habituated to the manipulations that precede sacrifice (unstressed rats) have a higher total number of ^{3}H-GABA binding sites than membranes from naive rats (stressed rats) (1). Habituated and naive rats were used as a model to study the effect of β-carbolines, footshock and diazepam on ^{3}H-GABA binding sites. Diazepam (5×10^{-7} M), added to cortical membranes from naive rats, increased ^{3}H-GABA binding to the level of habituated rats, but failed to induce any further increase in membranes from the latter animals. Viceversa, foot shock delivered to habituated rats and β-carbolines (FG7142, β-CCE, DMCM) added to cortical membranes from habituated rats lowered ^{3}H-GABA binding to the level of naive animals, but caused no further decrease in the membranes from this last group. Diazepam removed the effect of both foot shock and β-carbolines in cortical membranes from habituated rats. Ro15-1788 (10^{-7} M) (a concentration which fails to affect ^{3}H-GABA binding both in habituated and naive rats) reversed both β-carbolines-induced decrease and diazepam-induced increase of ^{3}H-GABA binding. The increased capability of ^{3}H-GABA to bind to diazepam-treated membranes from naive rats returned to control level up on washing with buffer prior to binding assay. On the contrary, washings with the same buffer failed to abolish the decrease in ^{3}H-GABA binding elicited by the in vitro addition of β-carbolines to membranes from habituated rats. The results suggest: a) that β-carbolines elicit effects opposite to benzodiazepines by decreasing ^{3}H-GABA binding sites b) handling represents a stressful stimulus for naive animals c) stress (handling - foot shock) lowers GABA binding by releasing an endogenous ligand for benzodiazepine receptors possessing similar properties to β-carbolines.

(1) Biggio et al., Brain Res. 229, 363, 1981

122.14 INVOLVEMENT OF GABA IN THE FACILITATION OF PUNISHMENT-SUPPRESSED BEHAVIOR INDUCED BY BETA-CARBOLINES. M.G. Corda, A. Guidotti, E. Costa, Lab. Preclinical Pharmacology, Saint Elizabeths Hospital, Washington, D.C. 20032

The Vogel's conflict-punishment test for anxiolytic drugs was modified to study drugs increasing the shock-induced suppression of drinking. For this paradigm, 72 h. water-deprived rats were electrically shocked after every 3 sec. of continuous licking (= 1 licking period) with a low current intensity (0.2-0.3 mA). Due to the low intensity of the current delivered through the drinking tube, the animal behavior was not significantly suppressed during the conflict session (25±3 and 22±2 licking periods/3 min. for non-conflict and conflict sessions respectively)(Corda et al., Proc. Natl. Acad. Sci. USA 80:2072, 1983).

It has been recently reported that the β-carboline derivatives, FG-7142 (β-carboline-3-carboxylic acid ethyl ester methyl amide) and β-CCE (β-carboline-3-carboxylic acid ethyl ester) are able to induce anxiety in man, while DMCM (6,7 dimethoxy-4-ethyl- β-carboline-3-carboxylic acid methyl ester) and β-CCM (β-carboline-3-carboxylic acid methyl ester) are potent convulsants in animals (Braestrup et al., Science 216: 1241, 1982). In our experiments all these four β-carbolines enhanced shock-induced suppression of drinking, without modifying unpunished drinking. These results indicate a proconflict effect of β-carbolines, which is opposite to the anticonflict effect induced by benzodiazepines (BZ). Among the β-carbolines tested, the most potent in eliciting a proconflict action was β-CCM (IC_{30} 0.1 mg/kg i.v.). The effect of β-carbolines was antagonized by 1 mg/kg i.v. RO 15-1788 (ethyl-8-fluoro-5-6 dihydro-5-methyl-6-oxo-4-H-imidazo (1,5a)(1,4) benzodiazepine-3-carboxylate) and by 1 mg/kg i.v. CGS 8216 (2-phenylpyrazolo (4,3c) quinolin-3(5H) one) which were ineffective by themselves. Pretreatment of rats with subconvulsive doses (150 mg/kg s.c.) of Isoniazid, an inhibitor of the glutamic amino acid decarboxylase, resulted in 5 fold increase of the proconflict effect of FG-7142. In contrast strychnine, a glycine receptor antagonist, at subconvulsant doses (0.6 mg/kg s.c.) did not potentiate the effect of FG-7142. Pentylenetetrazol (PTZ), which increases anxiety in man (Lal and Shearman, Ann. Rep. Med. Chem. 15:51, 1980) and antagonizes the GABA-mediated postsynaptic inhibition by acting on the Cl^- channel, at doses of 15 mg/kg i.p. mimicked the proconflict effect of β-carbolines in rats. However this effect was not antagonized by RO 15-1788 in doses that prevented comparable effect of β-carbolines. These results suggest that a proconflict action can be elicited by drugs acting at different sites which are operative in controlling GABA receptor function. β-carboline derivatives alter the GABAergic transmission by acting on the BZ recognition site. This effect is potentiated by drugs that decrease GABA content in brain and is antagonized by the specific BZ antagonist, RO 15-1788. PTZ acts at a site on the Cl^- channel and thus its effect is not modified by RO 15-1788.

122.15 EFFECTS OF DIAZEPAM AND GABA-MODULATING DRUGS ON SELF-STIMULATION IN PREFRONTAL CORTEX OF RATS. Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine and Georgia Mental Health Institute, Atlanta, GA 30306.

The acquisition of intracranial self-stimulation (ICSS) behavior in medial prefrontal cortex (mPFC) resembles in several ways the phenomenon of seizure kindling in limbic structures. Furthermore, overt seizures may occur in animals during ICSS in mPFC. Epileptiform activity is known to be modulated by GABA neurotransmission; and diazepam, which has potent antiepileptic actions, can retard the acquisition of ICSS in mPFC as well as the development of kindling in other areas. In this study, male rats trained for ICSS with stimulating electrodes in mPFC were tested with diazepam and GABAergic drugs for effects on lever pressing rate during 15 min sessions. Stimuli consisted of 200 msec trains of square wave pulses 0.2 msec in duration at 100 Hz, and current values were chosen to give stable baseline ICSS rates but below the threshold for overt seizures. A GABA antagonist, picrotoxin (0.125 - 1.0 mg/kg), caused a dose-dependent decrease in the rate of ICSS that reached statistical significance at the 0.5 mg/kg (42 ± 10% vehicle) and 1.0 mg/kg (5 ± 2%) dose levels. A GABA agonist, muscimol (0.15 - 1.2 mg/kg), also caused a decrease in ICSS rate. However, unlike picrotoxin, the effect was not dose-dependent since 0.3 mg/kg (65 ± 13%) and 0.6 mg/kg (68 ± 9%) reduced ICSS more than 1.2 mg/kg (75 ± 5%), although all of these decreases were significant. In addition to the changes in ICSS observed 15 min after drug administration, 1.2 mg/kg muscimol produced a significant increase in ICSS rate (117 ± 4%) one day after its administration. In contrast to the GABAergic drugs, diazepam (0.1 - 3.0 mg/kg) produced an increase in ICSS rate at the lower 3 doses, statistically significant for the 0.3 mg/kg (129 ± 6%) dose level. At the highest dose of diazepam, animals appeared sedated with a resultant decrease in response rates (55 ± 22%) for most animals. These results suggest that drugs that affect seizure activity can have potent effects on ICSS in mPFC. Whether cortical discharges have facilatory or only rate-limiting effects on ICSS in mPFC is unknown, but epileptiform activity should be considered in investigations of the reinforcing processes occurring in mPFC. (Supported by Georgia Department of Human Resources.)

122.16 RELATIONSHIP BETWEEN BENZODIAZEPINE RECEPTORS AND THE ATTENUATION OF STRESS-INDUCED CORTICOSTERONE ELEVATIONS IN RATS. J.F. McElroy and J.S. Meyer. Division of Neuroscience & Behavior, Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Stress is accompanied by increased blood corticosterone (CS) levels and prior administration of benzodiazepines (BDZs) in relatively low doses can inhibit this response. Somewhat higher doses of BDZs however, can elevate CS concentrations in unstressed animals. Saturable, high-affinity binding sites for BDZs have been found in several mammalian tissues. Recent research has demonstrated the existence of two biochemically and pharmacologically distinct "central-type" BDZ receptors, as well as a peripheral-type BDZ receptor. Central Type I BDZ receptors are GABA-independent, display a high affinity for both BDZs and triazolopyridazines (TPZs), and are thought to mediate the anxiolytic actions of these drugs. Central Type II receptors are GABA-dependent, show a high affinity for BDZs but a low affinity for TPZs, and presumably mediate the depressant side effects associated with BDZ administration. The peripheral-type BDZ receptor is characterized by the ability of the clinically inactive BDZ Ro5-4864 but not the clinically active BDZ clonazepam to inhibit ^{3}H-diazepam binding to this site.

To establish whether the actions of BDZs on the pituitary-adrenocortical system can be related to a particular receptor type, the prototype triazolopyridazine CL218,872 was compared to chlordiazepoxide (CDP) in terms of its effects on serum CS levels in stressed and unstressed rats. Our results show that a relatively low dose of CDP (5 mg/kg i.p.) significantly attenuated the elevation of serum CS produced by a 15 min. exposure to sound stimulation (21.3 vs. 46.1 µg/100 ml in the saline-treated controls). A somewhat higher dose of CDP (15 mg/kg) when given to unstressed rats itself significantly elevated CS levels (from 11.8 to 37.3 µg/100 ml). With chronic administration (6 daily pretreatments), tolerance developed to only the latter effect of CDP. In contrast, CL218,872 (2.5 to 20 mg/kg) did not inhibit the CS elevation produced by sound stimulation and also failed to alter baseline CS levels in unstressed animals. Chronic CL218,872 pretreatment (10 mg/kg for 6 days) produced a cross-tolerance to the CDP-induced elevation of CS in unstressed rats, but apparently not to its attenuation of CS secretion in sound stimulated rats.

These results suggest that BDZs are not exerting their pituitary-adrenocortical actions via stimulation of central Type I receptors. Further experiments in progress are aimed at determining whether BDZs may be acting through central Type II or via peripheral BDZ receptors.

122.17 BENZODIAZEPINE RECEPTOR MEDIATION OF GENETICALLY-DETERMINED STEREOTYPE ABNORMAL BEHAVIOR OF TOTTERING MICE, P.J. Syapin, J.H. Schneider*, P. Larschied*, and J.M. Cook*. Dept. of Neurology, University of Southern California School of Medicine, Los Angeles, CA 90033 (PJS & JHS) and Dept. Chemistry, University of Wisconsin, Milwaukee, WI 53201 (PL & JMC).

The neurologic mutation tottering (gene designation tg) is expressed phenotypically as hindlimb ataxia and a "spontaneoulsy" recurring stereotypic pattern of abnormal movements. The reason for the occurrence of this behavior in (tg/tg) mice is unknown. We have shown that the stereotypic abnormal movements can be induced by a low, subconvulsant, dose of pentylenetetrazol, an effect blocked by diazepam (Syapin, Pharmacol. Biochem. Behav., 18:389, 1983). These results suggested an involvement of benzodiazepine receptors in this behavior. We now report on the effects of the benzodiazepine receptor ligand β-carboline-3-carboxylic acid ethyl ester (BCCE) and the specific benzodiazepine receptor blocker Ro 15-1788 on this behavior of (tg/tg) mice.

The effect of vehicle or 15 mg/kg BCCE IP on (tg/tg) mice was studied with or without 10 mg/kg Ro 15-1788 pretreatment. Mice were observed for 20 min following the last injection and scored blind for the presence of absence of the stereotypic abnormal behavior. The results are presented in the Table.

Treatment Group	Mice Showing Behavior / Mice Tested
vehicle, BCCE	8 / 9
Ro 15-1788, BCCE	1 / 10
vehicle, vehicle	1 / 11
Ro 15-1788, vehicle	2 / 12

Statistical analysis using the Cochran Q test for related nonparametric measures revealed a highly significant ($p < 0.001$) difference between treatment groups. Comparisons using the Fisher exact probability test showed a significant effect of Ro 15-1788 pretreatment on the ability of BCCE to elicit abnormal behavior ($p = 0.00975$). Treatment with Ro 15-1788 alone did not cause stereotypic abnormal behavior in (tg/tg) mice.

BCCE is known to cause experimental "anxiety" in monkeys, a benzodiazepine receptor effect that is blocked by Ro 15-1788 (Ninan et al., Science, 218:1332, 1982). Our results show that treatment with BCCE can cause a specific behavioral response in (tg/tg) mice and strongly suggest that this behavior occurs in response to "anxiety"-provoking stimuli. (Supported by a research grant from the Epilepsy Foundation of America to PJS).

122.18 GABA RECEPTORS MEDIATE CEREBRAL VASODILATION IN THE UNANESTHETIZED GOAT. C. Estrada*, G. Torregrosa* and E. Alborch* (SPON: C. Avendaño). Centro de Investigación, Hospital La Fe, Valencia, and Dpto. Fisiología, Facultad de Medicina, Universidad Autónoma, Madrid, Spain.

Recent evidence indicates that GABA may be involved in the regulation of cerebrovascular function. Several GABA agonists have been reported to dilate isolated pial vessels from different species (Edvinsson and Krause, Brain Res. 173:89, 1979), and gabaergic receptors have been biochemically characterized in bovine pial vessels (Krause et al., Brain Res. 185:51, 1980). However, the role of these GABA receptors in the overall regulation of cerebral circulation is not yet clear.

We studied the effects of GABA and muscimol on cerebral blood flow (CBF) of the unanesthetized goat. CBF was continuously measured by means of an electromagnetic flow probe chronically implanted on the internal maxillary artery after occlusion and thrombosis of the distal extracerebral vessels. Administration of GABA (1-100 μg) directly into the cerebral circulation produced dose-dependent increases in CBF, without accompanying systemic effects. Muscimol mimicked the effects of GABA at doses ten times lower. Administration of picrotoxin (1-5 mg) into the internal maxillary artery did not significantly change CBF, but inhibited in a dose-dependent manner the vasodilation induced by GABA or muscimol. Selective blockade of beta adrenergic or muscarinic cholinergic receptors by propranolol or atropine, respectively, did not modify the cerebrovascular response to the gabaergic agonists.

These results indicate that GABA increases total CBF, acting on specific receptor sites in the cerebral blood vessels. The absence of influence of picrotoxin on resting CBF suggests that the gabaergic receptors are not tonically activated under physiological conditions.

122.19 EFFECTS OF VALPROIC ACID IN TARDIVE DYSKINESIA: H.A. Nasrallah, M.K. McCalley-Whitters*, and F.J. Dunner*. VA Medical Center and Dept. of Psychiatry, University of Iowa College of Medicine, Iowa City, IA.

Introduction: Sodium Valproate is an anticonvulsant drug that increases brain GABA degradative enzymes. It has been used in Tardive Dyskinesia (TD) because of the neuro-chemical evidence that GABA may be involved in down-regulating dopaminergic activity, and thus GABAergic drugs may benefit TD. A total of four studies in the literature report good, mild, minimal or no therapeutic effects respectively. The dose was not high enough and blood levels were not monitored in two of the four studies. We present here a double-blind placebo-controlled trial of sodium valproate in TD, with blood level monitoring of the drug.

Method: Ten chronic schizophrenic or schizoaffective patients with persistent TD movements of two years duration and no neurological disorders that may simulate TD, consented to participate in a controlled trial of sodium valproate to test its effects on TD involuntary movements. The patients were kept on the maintenance medication that they were receiving (if any) at the time of referral to the study. Placebo capsules were given for 3 weeks followed by sodium valproate capsules (1000-2500 mg daily in four divided doses), and finally matching placebo capsules for 4 weeks. The patients were videotaped weekly and two raters blind to the drug or study design rated the patients' movements using the AIMS ratings on tape segments that were scrambled for sequence and edited for any cues or information. Blood for valproate serum concentrations were drawn at the end of each phase (placebo, active drug, placebo) to determine the maximum steady state achieved during the study.

Results: The global AIMS ratings for the 10 patients showed no significant chage during treatment with valproate (Wilcoxon Rank Sum Test). The mean serum concentration of valproate at the end of the active drug period was 83.8 mcg/ml, which is the highest of any TD study using valproate (therapeutic levels=50-100 mcg/ml for seizures). Side effects such as nausea with or without vomiting was noted in 55% of the patients.

Discussion: The study suggests that there are no therapeutic effect of valproate in a population of TD patients. However, we observed patients who showed improvement, and others who showed worsening or no change. Thus, although there may be no overall benefit from valproate in TD, some patients may derive some benefit from it. Implications for the role of GABA agonists in TD are discussed.

122.20 RESPONSE OF RAT HIPPOCAMPAL NEURONS TO TAURINE. KATHERINE H. TABER AND ROBERT H. THALMANN. Dept. of Cell Biology and Program in Neuroscience, Baylor College of Medicine, Houston Texas 77030

The response of CA1 neurons to taurine was examined as part of an evaluation of taurine as a possible neurotransmitter in mammalian brain. Slices of hippocampus were maintained at 37°C by standard methods. The response to taurine (20 mM in artificial CSF) applied by pressure from a glass pipette positioned on the surface of the slice was monitored by intracellular microelectrodes filled with KAc. Membrane resistance was measured with 0.5nA hyperpolarizing pulses injected through the recording electrode via a bridge circuit. Orthodromic postsynaptic potentials were elicited via stimulation of Schaffer collaterals. Within 1-5sec after application of taurine to the slice surface, there was a decrease in input resistance of up to 50% which returned to baseline within 5-60 seconds, depending on dose. When spontaneous action potentials were present, they were inhibited during the response. The taurine-induced change in membrane potential, typically a small hyperpolarization relative to the resting potential, had a reversal potential that ranged between -65 and -70mV. When Cl was injected into the neurons via recording electrodes filled with KCl rather than KAc, the estimated reversal potential of both the orthodromically elicited IPSP and the response to taurine usually shifted to a more positive value. Similar responses to taurine were obtained after action potentials and transmitter release in response to orthodromic stimulation had been blocked by incubation in TTX, reduced Ca(0.5mM), and 2mM Mn.

The reversal potential, increased conductance, and sensitivity to transmembrane Cl gradient suggest that an increased conductance to Cl constitutes a significant component of this response to taurine. In addition, the persistance of the response during TTX and blockade of Ca influx not only indicates that taurine can act directly upon the postsynaptic membrane of these cells, but also suggests that the response does not require postsynaptic Ca-activated conductances. We do not yet know whether the high concentration of taurine in the droplet was required because of factors such as uptake that might restrict the access of taurine to the postsynaptic membrane, or rather whether taurine might produce its response by binding to the receptor for a different molecule, such as GABA. Notwithstanding, these results indicate that taurine can produce a direct inhibitory action on hippocampal neurons, probably via an increased Cl conductance. Supported by National Institutes of Health, Grant NS11535.

123.1 Neurons Projecting to the Pituitary Gland and Median Eminence of the Hamster Determined by Horseradish Peroxidase (HRP) Histochemistry. Daniel B. Michael and Jerald A. Mitchell. Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

Previous studies have demonstrated the presence of a supraependymal complex of neurons (SEN) in the 3rd ventricle of the hamster which sends a fascicle of processes into the infundibular recess (Card & Mitchell; JCN 180: 43, '78). Retrograde transport of HRP injected into the pituitary was employed to determine what aspect of the gland is innervated by the SEN. Anesthetized female golden hamsters (58), age 8-24 weeks, body wt. 75-212g, were stereotaxically injected in the pituitary gland with 0.2 or 0.05 ul of 30% HRP (Worthington) or 0.05 ul of 1% lectin bound HRP (Sigma). After 24 hrs animals were sacrificed, brains were removed, post-fixed, and sectioned on a freezing microtome. Sections were reacted for HRP using benzidine dihydrochloride. Brain sections were examined for HRP labeled neurons from just caudal to the inferior colliculus to the level of the optic chiasm. Labeled cells were plotted using a camera lucida and photographed.

HRP was successfully injected into the pituitary gland in 35 of 57 animals. Injections were restricted to the anterior lobe (AL) or included the neural lobe (NL) with diffusion into the intermediate lobe (IL), AL, or the external layer of the median eminence (ME). Injections which included the NL and IL resulted in labeling of neurons in the SEN. HRP positive cells were also noted in the subependymal layer of the arcuate N, and the internal, middle, and external layers of ME. Readily apparent, heavily labeled neurons in the supraoptic (SON) and paraventricular (PVN) nuclei were similar in size and shape to magnocellular neurons identified in neutral red counterstained and Nissl preparations. AL injections yielded cellular labeling in connective tissue and the external layer of ME adjacent to the injection site, but not in the SEN nor any other region of the brain examined. No labeled cells were seen in controls (uninjected or injected brains in which the pituitary was missed).

HRP retrograde labeling clearly demonstrated the classic magnocellular projections from SON and PVN to NL. This data also provides evidence for projections from neurons of the SEN, subependymal layer of the arcuate, and ME to the NL and/or IL. Such cells may give rise to a fiber system innervating the IL in the hamster, similar to that recently described in the rat (Westlund and Childs; Endocr. 111: 1761, '82). No conclusive evidence for direct neural projections to the AL was provided unless such cells are located in the external layer of ME. Labeling of these cells, however may be nonspecific due to injection site proximity.

123.2 FURTHER EVIDENCE FOR HYPOTHALAMIC ASYMMETRY IN NEUROENDOCRINE CONTROL. D.M. Nance, M. Bhargava* and G.A. Myatt*. Dept. of Anatomy, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, B3H 4H7.

Previously we have shown that the facilitory effects of hemigonadectomy (Hemi-x) on FSH release of prepubertal male rats is blocked by unilateral deafferentation of the hypothalamus on the ipsi-, but not the contralateral side (Brain Res. Bull., 8: 299, 1982). This potentially neurally mediated phenomenon was specific to surgeries on the right side of the brain. Subsequently, we found that ovarian compensatory hypertrophy (OCH) in prepubertal female rats could be blocked by unilateral hypothalamic deafferentation on the ipsi-, but not the contralateral side with respect to the Hemi-x (Nance, et al., Brain Res. Bull., in press). This phenomenon was specific to surgeries on the left side of the brain. We report here the effects of unilateral kainic acid (KA) lesions (1.0 µg injected in a 1.0 µl volume) in the retrochiasmatic area on OCH in adult female rats that were Hemi-x on the ipsi- or the contralateral side. Briefly, KA or saline was infused unilaterally into the hypothalamus and the animals Hemi-x or given sham surgery on the ipsi- or contralateral side following brain surgery and percent OCH was assessed 10 days later. Brain histologies revealed a modest amount of gliosis associated with the unilateral KA lesions; however, a morphometric analysis of the hypothalamus indicated that the KA injections, relative to saline, significantly reduced the distance between the fornix and 3rd ventricle on the injected side of the brain when compared to the uninjected side. Mean reductions in the fornix -3rd ventricle distance on the injected side of the brain were 20.5 $\pm$ 2.1 and 7.5 $\pm$ 2.5 percent for the KA and saline injected animals, respectively. Similar to the effects of hypothalamic knife cuts, unilateral KA infusions blocked OCH if the lesions were located on the ipsi-, but not the contralateral side of the brain with respect to Hemi-x. Likewise, this phenomenon was clearly more apparent if the lesions were located on the right side of the brain. Morphometric analysis of the brain histologies indicated comparable lesions on the two sides of the brains. Additional observations indicated that whereas Hemi-x or KA lesions alone had no effect on vaginal cycles, the combination significantly reduced the incidence of estrus. However, these effects on estrous cycles were not specifically associated with lesions or Hemi-x on either side, thus excluding alterations in cyclicity as an explanation of the differences between the endocrine effects of KA on the two sides of the brain. In general, these results support the previous evidence for a direct neural contribution to endocrine control and further suggest a functional difference in the two-halves of the hypothalamus in neuroendocrine regulation.
Supported by MRC.

123.3 SPECIFICITY OF METHYLTRIENOLONE (R1881) BINDING IN BRAIN AND PITUITARY. K.L. Olsen and A.M. Etgen. Department of Psychiatry and Behavioral Science, SUNY @ Stony Brook, NY 11794 and Department of Biology, Rutgers University, Livingston Campus, New Brunswick, NJ 08903.

Methyltrienolone (R1881=17β-hydroxy-17α-methyl-estra-4,9,11-trien-3-one) is a synthetic steroid with potent androgenic activity in both peripheral and central target tissues. Unlike naturally occurring androgens, R1881 is not readily metabolized. It also binds with a higher affinity than DHT to androgen receptors. Hence, R1881 may prove useful in evaluating the role of receptors in mediating androgen-regulated behaviors. A potential problem is that R1881 binds to cytosol progestin receptors and has progestin-like behavioral effects; as little as 1ug of R1881 synergizes with estrogen to activate female sexual responses. Therefore we sought to evaluate the degree to which (^{3}H)R1881 could be displaced by androgens and progestins in preoptic-hypothalamic (HPOA) and pituitary (PIT) cytosols.

Initial competition experiments used HPOA and PIT cytosols from gonadectomized (gonadx) male and female King-Holtzman rats. In HPOA, 100-fold excess of unlabeled progestins (progesterone, R5020) was 60-70% as effective as unlabeled DHT and R1881, while in PIT, the progestins were 77-87% as effective in displacing (^{3}H)R1881 binding. These data indicate that a significant component of (^{3}H)R1881 binding in the CNS can be attributed to progestin receptors.

Since it is well known that estrogen treatment elevates progestin receptor levels, competition experiments were carried out with estrogen primed gonadx male and female rats. Results of the one point assay with 2nM (^{3}H)R1881, indicated that estrogen priming increased total R1881 binding, but did not alter the pattern of competition by the unlabeled progestins and androgens. Scatchard analysis of (^{3}H)R1881 binding suggests the presence of two binding components.

Similar competition studies were performed using tfm rats that are deficient in the number of neural androgen receptors. R5020 and progesterone were more effective in displacing (^{3}H)R1881 binding in androgen resistant rats than in wild-type males and females. Moreover, unlabeled progestins were better inhibitors than DHT in HPOA and PIT cytosols from estrogen primed tfm rats and in PIT of nonprimed tfm rats. In HPOA, R5020 and progesterone displaced binding 98% when compared with unlabeled R1881 itself.

These studies indicate that progestins, as well as androgens, contribute significantly to R1881 binding in brain and Pit. Thus, caution should be taken when interpreting results from studies using this synthetic steroid.

123.4 IN VIVO MEASUREMENT OF LUTEINIZING HORMONE RELEASING HORMONE IN UNANESTHETIZED, OVARIECTOMIZED RHESUS MONKEYS USING PUSH-PULL CANNULAE. N.J. Schultz*, M.D. Loose*, and E. Terasawa (SPON: W.T. McKinney). Wis. Reg. Primate Research Ctr., Neurosciences Training Prog. and University of WI, Madison, WI 53715-1299.

In vivo measurements of luteinizing hormone releasing hormone (LHRH) had been limited to hypophyseal portal plasma collections until Levine and Ramirez (Endocrinol. 107: 1782, 1980) described the use of push-pull cannulae to measure LHRH release in the mediobasal hypothalamus (MBH) of conscious rats. The present study reports a modified method for use of push-pull cannulae applied to rhesus monkeys that allows repeated measurements of in vivo release of LHRH in various locations of the median eminence. Several months prior to the experiment 4 ovariectomized monkeys were implanted with a cranial pedestal under halothane anesthesia. Using X-ray ventriculography the center of the pedestal was placed above the infundibular recess at the midline level and was used for a reference point. Two days before the perfusion, the animal was placed in a primate chair under ketamine anesthesia. Then a push-pull cannula composed of an outer (pull) cannula (20 ga) and inner stylet (28 ga) was inserted into the brain with a hydraulic microdrive unit, originally designed for recording experiments. This unit allowed positioning of the cannula along the X,Y,Z cordinates. Cannula placement was verified by X-ray. The cannula was left in place for 2 days to allow clearance of cellular debris to avoid potential clogs, so that collection could continue for more than 4 hrs. Although the animals were restrained, they were fully conscious and able to take food and water throughout the experiment. On the 3rd day, the stylet was replaced with an inner (push) cannula (28 ga), and artificial CSF was perfused at 20 µl/min by two identically calibrated peristaltic pumps. A U-tube was connected to the pull side allowing detection of a change in fluid level indicating a clog. When observed, the pumps could be stopped avoiding imbalancedflow and subsequent tissue damage. Ten-minute fractions were collected continuously on ice and acidified to 0.1N HCl and stored at -20°C. Perfusate samples were neutralized and assayed for LHRH by RIA using antibody supplied by Dr. Nett. LHRH release was detected in the perfusates of all monkeys. LHRH release was pulsatile with mean interpeak intervals of 30-80 min. This is consistent with reports of LHRH release patterns measured in hypophyseal portal plasma as well as data on pulsatile serum LH in monkeys. Peak LHRH release was 1.8 to 2 pg/10 min, while nadir values ranged from 0.3 pg/10 min to undetectable levels (assay sensitivity was 0.2 pg). With this method perfusates can be obtained repeatedly from conscious animals to analyze the correlates of various biochemical and neuroendocrine functions in addition to LHRH release. (Supported by NIH grants RR00167, HD15433 and HD11355.)

123.5 REGIONAL DIFFERENCES IN NEURONAL MODULATION IN RODENT HIPPOCAMPUS.

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Previously we have observed a gender specific modulation of hippocampal pyramidal cell excitability in rodent as measured by an increased amplitude of the monosynaptic population spike. Slices taken from male rats were facilitated following exposure to 100pm 17 beta estradiol (E2) and 10pm delta 9 tetra hydrocannabinol (THC) (Foy et al, Brain Res. Bull., 1982, B(4):341-345). Slices taken from females were facilitated following 100pm testosterone application (Teyler et al, Science, 1980, 209: 1017-19). The effect reached peak levels in 5-7 minutes. Classical cytosol receptor kinetics do not allow for such rapid effects.

Recent E2 receptor blocker experiments suggest a receptor mediated mechanism (Foy & Teyler, Brain Res. Bull., 1983, in press). If such is the case, the strength of the steroid effect should bear some relationship to receptor concentration. The present investigation attempts to examine this contention. No measurable cytosol E2 receptors have been observed in CA1, but significant levels were observed in the dentate gyrus (Rainbow et al., J. Neuroscience, 1982, 2:1439-45).

In the present investigation, no measurable effects of E2 and THC were observed in the dentate gyrus of slices taken from male rats, while CA1 showed the usual pattern of facilitation. In light of the evidence supporting a receptor mediated mechanism, these results are not consistent with the known distribution of cytoplasmic receptors. A recently described class of cell surface receptors (Towle and Sze, J. steroid Biochem., 1983,10(2):135-43) may provide an alternative mechanism for the rapid modulating effects of these compounds.

(NINCDS Grant NS 16507)

123.6 THE AMYGDALA IS NOT INVOLVED IN THE NEURAL CONTROL OF PRECOCIOUS PUBERTY IN CHICKS. W. J. Kuenzel* and R. R. Gabel* (SPON: W. Hodos). Poultry Sci. Dept., Univ. of Maryland, College Park, MD 20742.

Precocious puberty has been shown to be induced by surgical manipulation of the hypothalamus in female rats (Donovan, B. T. and J. van der Werff ten Bosch, Nature, London 178:745, 1956; Gellert, R. J. and W. F. Ganong, Acta Endocrin. 33:569, 1960). Other studies have demonstrated that the medial portion of the amygdaloid complex and stria terminalis are involved in the control of sexual maturation since bilateral destruction of these structures resulted in precocious ovarian stimulation (Elwers, M. and V. Critchlow, Amer. J. Physiol. 198:381, 1960; Amer. J. Physiol. 201:281, 1961).

Recently, precocious puberty has been demonstrated in male chicks following parasagittal knife cuts of the lateral hypothalamic area, extending from the preoptic to the mamillary region. Viable sperm, capable of fertilizing eggs, was obtained when chicks were 9 weeks of age (Mass, J. H. and W. J. Kuenzel, Devel. Brain Res., in press, 1983). Luteinizing hormone (LH) and androgen (A) were assayed in the plasma of chicks at weekly intervals from time of hypothalamic knife cuts. Maximum levels of LH and A were observed 3 and 4 weeks after surgery, respectively (Kuenzel, W. J. and P. J. Sharp, Neuroendocrin., submitted, 1983). Since extrahypothalamic structures have been implicated (Elwers, M. and V. Critchlow, Amer. J. Physiol. 198:381, 1960) to control the onset of puberty by inhibiting gonadotropin secretion, a study was conducted to isolate the nucleus taeniae and archistriatum (portions thought to be homologous to the mammalian amygdala) from medially situated brain structures.

Two groups of chicks received bilateral knife cuts. The first group (n=24) had cuts isolating both the nucleus taeniae and archistriatum from known connections to the hypothalamus (stereotaxic coordinates: Anterior (A) = 8.6-4.1 mm; Depth (D) = 3.0 mm; Lateral (L) = + 3.5 mm). The second group (n=12) received bilateral knife cuts located between cuts used to isolate the amygdala (first group) and bilateral hypothalamic cuts known to induce precocious puberty (Mass, J. H. and W. J. Kuenzel, Devel. Brain Res., in press, 1983). Stereotaxic coordinates of the second group were: A = 8.2-4.2 mm; D = 3.0, 2.5, 2.0 mm; L = + 2.0 mm).

No advancement of sexual maturation occurred in either group. Since both experimental groups received bilateral cuts designed to sever all known connections between the amygdala and hypothalamus, it is concluded that unlike mammals, the amygdala does not appear to have fibers which inhibit the onset of gonadal development in chicks. It is hypothesized that more medially placed structures, perhaps in the preoptic area or nearby medial telencephalic region contain neurons which function to effect maturation of the gonads.

123.7 **TESTOSTERONE 5α-REDUCTASE AND 3α-HYDROXYSTEROID OXIDOREDUCTASE IN LAMINAE OF THE RAT OLFACTORY TUBERCLE.**

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The enzymes that catalyze the conversion of testosterone to active and inactive metabolites in brain play key roles in determining the effects of testosterone on behavior. Yet little is known about the properties and localizations of these enzymes in brain. Here we describe the localizations of testosterone 5α-reductase and 3α-hydroxysteroid oxidoreductase (3α-OHSOR) within the laminae of the rat olfactory tubercle. Testosterone 5α-reductase reduces testosterone to dihydrotestosterone, and 3α-OHSOR inactivates dihydrotestosterone by converting it to 3α-androstandiol.

Tangential sections (16u) were cut from the frozen tubercle and pooled into three groups of 16 sections each. These were homogenized and assayed for 5α-reductase and 3α-OHSOR. Incubations were carried out with radiolabelled substrate in 35 ul volumes by methods similar to those described previously. (J. Neurochem. (1983) 40, 1460). Products were separated from substrates by TLC and quantitated by scintillation counting. 5α-reductase activity was highest in the deepest layers of the tubercle and lowest in the most superficial layers. 3α-OHSOR was then studied with higher resolution by homogenizing and assaying each 16u section. Virtually all of the activity was confined to the outermost sections. The dominant anatomical feature visible in these sections is the pia. Perhaps 3α-OHSOR in the pial layer opposes the movement of dihydrotestosterone across this boundary by inactivating it. The distribution of testosterone 5α-reductase that we observed is consistent with a localization to the granule neurons of the islands of Calleja in the olfactory tubercle.

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123.8 SEX AND DRUGS: CAN PRIMING IN ADULTHOOD AFFECT SEXUALLY DIFFERENTIATED RESPONSE PROFILES IN RODENTS?

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In 1978, we demonstrated the modulating effects of 100 pM concentrations of 17-beta-estradiol (E2) and testosterone (T) on in vitro hippocampal slices of normal adult rats (Vardaris and Teyler, Neurosci. Abs., 1978, 4, A1145). In 1979, we reported that the modulation of hippocampal excitability in adult rats gonadectomized two days prior to testing was similar to that seen in normal rats: E2 enhanced the amplitude of the CA1 population spike in the male and T enhanced the same response in the female (Teyler, Foy and Vardaris, Neurosci. Abs., 1979, 5, A1568). The observed differential responsitivity to gonadal steroid may reflect: 1) sexual differentiation in the hippocampus with respect to androgen and estrogen receptors, or 2) the priming effect of plasma gonadal steroids acting upon receptor populations.

Our present investigation features a paradigm whereby adult male rats were castrated, allowed a two week recovery period, and then primed (100 ug, i.p.) with estradiol benzoate (EB), testosterone propionate (TP), or vehicle for 7 days before testing in vitro with E2 or T (100 pM). The following trends were observed following addition of steroid to the bathing Ringer solution: Animals primed with EB exhibited response enhancement only when challenged with T; animals primed with TP exhibited an enhancement when challenged only with E2.

These data indicate that the sexually differentiated response profiles we have previously observed can be abolished or reversed by manipulation of adult circulating steroid levels, at least in the male. Since the sexually differentiated hippocampal response can be blocked in males with 17-alpha-estradiol (Foy and Teyler, Brain Res. Bull., 1983, in press) and tamoxifen, we suspect that circulating steroid levels can induce heterotypic receptor elaboration that are perhaps cell surface receptors (Towle and Sze, J. steroid Biochem., 1983, 10(2)). In this respect, it is of interest that only the TP primed castrated males showed a response enhancement to delta-9-tetrahydrocannabinol (THC) comparable to that reported earlier (Foy, Teyler and Vardaris, Brain Res. Bull., 1982, 8(4)), suggesting that THC is acting at the same inducible estrogen receptor. (NINCDS Grant NS 16507)

PRIMING DRUG	VEHICLE PRIMED			EB PRIMED			TP PRIMED		
TEST DRUG	T	E2	THC	T	E2	THC	T	E2	THC
% PRE DRUG CONTROL	106	97	130	169	94	111	106	153	165

123.9 REGIONAL VARIATION IN THE UP-REGULATION OF GLUCOCORTICOID BINDING PROTEINS IN RAT BRAIN. Barbara B. Turner. Dept. of Physiol., College of Med., East Tenn. State Univ., Johnson City, TN 37614.

Increases in 3H-corticosterone binding occur in the hippocampus and other brain structures of the rat following adrenalectomy (ADX). With the exception of the hippocampus, it is not clear whether receptor binding capacity or affinity has been increased. Post-ADX changes in 3H-corticosterone binding may also reflect alterations in tissue levels of membrane associated, "transcortin-like" binding proteins. Differential responsiveness of neural tissues to ADX is of importance since the ADX rat is the source of much of our knowledge concerning the pattern of glucocorticoid receptor distribution in the brain.

Glucocorticoid binding in four brain regions (hippocampus, hypothalamus, amygdala and cortex), pituitary, and liver was examined at 10h and 96h post-ADX. Tissues from perfused male rats, were homogenized (1:10 w/v) in a Tris-EDTA buffer containing glycerol and molybdate. Cytosol was incubated for 3-5h with steroid at 4C before separation of bound steroid by passage through LH-20 columns. 3H-dexamethasone (3H-dex) was used to determine the binding capcity and affinity of cytosol receptors. "Transcortin-like" binding in tissue was estimated from the amount of 3H-corticosterone specifically bound at 20 mM in the presence of excess dexamethasone.

Differences were found in the percent increase in maximal binding capacity (Bmax) among brain tissues and pituitary between 10h and 96h post-ADX. A 40% increase in Bmax was observed in both the hypothalamus (150+3 vs 213+25 fm/mg P) and the cortex (178+16 vs 250+39 fm/mg P). In the hippocampus and amygdala, a near doubling of Bmax occurred between 10h and 96h post-ADX (169+16 vs 330+24 fm/mg P, and 124+20 vs 235+47 fm/mg P, respectively). The pituitary showed only a 30% increase in 3H-dex binding (146+11 vs 194+4 fm/mg P).

In several brain tissues the percent increase in transcortin-like binding exceeded by many fold the increase observed in plasma transcortin (24%), but was less than that seen in liver (450%). The change in transcortin-like binding appeared to be tissue specific, being unrelated to changes in cytosol receptor number. In both the hypothalamus and cortex the increase in transcortin-like binding (191% and 171%) was far greater than the increase in 3H-dex binding. In the hippocampus, the increase in transcortin binding was 83%, less than the increase in 3H-dex binding. No increase in transcortin-like binding occurred in the amygdala.

A portion of the transcortin produced by the liver may be intended for tissue utilization by plamsa membranes where it may act to regulate corticoid entry into cells, thus controlling the "set-point" for receptor occupancy in the cell. If this is the case, then large increases in transcortin-like binding in a tissue following ADX, may reduce the uptake of steroid by the cell and render it relatively "blank" on autoradiographs. This might be the case with the hypothalamus and the cortex in the ADX rat. Supported by VA Grant 1A(74) 111-430108.

123.10 RESERPINE INDUCES DEPLETION OF CRF-41 IMMUNOREACTIVITY FROM THE EXTERNAL LAYER OF THE MEDIAN EMINENCE. S. Cummings, R. Elde and V. Seybold. Dept. of Anatomy, Univ. of Minnesota, Mpls. MN 55455

Passive immunization studies demonstrate that corticotropin releasing factor (CRF) secreted from hypothalamic neurons terminating in the median eminence regulates ACTH release. Brain catecholamines have been implicated in inhibition of CRF release, while vasopressin has been demonstrated to potentiate the activity of this peptide. We have previously demonstrated tonic catecholamine inhibition of vasopressin/neurophysin nerve terminals in the external layer of the median eminence using reserpine and other agents known to perturb monoaminergic function (Seybold, Elde and Hökfelt, Endocrinol. 108:1803, 1981.)

To determine if similar mechanisms regulate CRF release, 4 rats were injected with reserpine (10 mg/kg, ip, Serpasil, Ciba), and sacrificed by vascular perfusion with buffered 4% paraformaldehyde 24 hrs. later. Four rats were injected with reserpine vehicle to serve as controls. The median eminences of 2 reserpine-treated animals and 2 vehicle-treated animals were composed in a single block of mounting media and cryostat sectioned at 10 μm. Alternate sections of the tissue block were processed for indirect immunofluorescence using primary antibody directed against CRF-41 (Immuno Nuclear) or arginine vasopressin (VP)(Immuno Nuclear). Specificity of staining was determined by absorption controls.

Evaluation of the sections indicated a decrease in the level of immunoreactive CRF and vasopressin from the external layer of the median eminence of reserpine-treated animals as compared to vehicle-treated controls. Qualitative changes were not apparent in the internal layer of the median eminence.

To quantify these findings in the external layer of the median eminence, 9 observers used a blind protocol to rate, on a scale of 0-4, the intensity of fluorescence immunoreactivity of tissue sections prepared as described above. Analyses (t-tests) of the mean scores revealed decreases of 57% ($p<.005$) and 69% ($p<.005$) in CRF and VP staining intensity, respectively, compared to controls.

While these results do not determine the relationship between CRF and vasopressin, they are consistent with a model for brain monoamines in inhibition of CRF and vasopressin. Further experiments to examine the pharmacology associated with reserpine-induced alterations in CRF in the external layer of the median eminence and the morphological relationship of central monoamine systems to hypothalamic CRF are currently in progress.

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123.11 PLASMA CORTISOL LEVELS IN OPIATE ADDICTS BEFORE AND AFTER DETOXIFICATION, A. D. Pheterson*, C. A. Dackis*, M. S. Gold, D. R. Sweeney, Fair Oaks Hospital, Summit, N.J. 07901.

Anti-endorphin effects of exogenous opiates have been suggested and demonstrated in a preliminary study.[1] It has also been demonstrated that corticotropin (ACTH) and beta endorphin are secreted, stored, and released in similar sites under similar conditions.[2] Multiple sites for the production of pro opiomelanocortin in the pituitary and hypothalamus have recently been determined[3], with suggestion of differential, varying secretion presumably in an attempt to maintain toward organismic homeostasis. To study the effect of exogenous opiates upon ACTH-Adrenal activity we determined cortisol levels in individuals with prolonged and current opiate intake. The effect of subsequent opiate abstinence was then examined.

Midnight plasma cortisol levels were measured in 21 chronic opiate addicts within hours of their hospital admission and last drug intake. Ages ranged from 19 to 34 years with a mean of 27 years. Within 72 hours of detoxification with clonidine, a second midnight sample was drawn. Mean admission plasma cortisol was 3.85 + 2.71 μgms while mean post detoxification cortisol was 5.87 + 2.62 μgms. The rise from admission level was 52.48%, which was statistically significant ($t=2.86$, $df=20$, $p<.01$). These data are consistent with previous reports of anti-adrenal and possibly antihypothalamic-pituitary-adrenal effects of opiates.

The clinical significance of depressed cortisol production in addicts has been previously described in a pilot study.[4] Effects include fatiguability, anorexia, weakness, depression, and perhaps represent a strong need to self-medicate with continued opiate use. The 'rebound' of cortisol after detoxification suggests an ACTH-adrenal resilience not previously described. The possible role of clonidine is discussed.

1. Gold, M.S., et al Drug Alcohol Depend, 8:257-262, 1981.
2. Guillemin, R., et al Science, 1367-1369, 1977.
3. Berger, P.A., et al Ann Rev Med, 33:397-415, 1982.
4. Dackis, C.A., et al Lancet, 2:1167, 1982.

123.12 NEUROENDOCRINE AND PHYSIOLOGICAL RESPONSES TO ENDOTOXIN IN THE RAT. M.F. Mazurek,* J.B. Martin and N.W. Kasting. Dept. of Neurol., Massachusetts General Hospital, Boston, MA 02114.

We have previously shown that endotoxin administered intravenously to the rat results in a prompt suppression of GH & TSH, with reversal of these effects by anti-somatostatin antiserum. We now report the dynamics of plasma vasopressin (AVP), β-endorphin (β-End) and prolactin (PRL) secretion in relation to endotoxin-induced changes in plasma volume, blood pressure, body temperature, and plasma osmolality.

Experiments were performed on freely-behaving 300-350 gm Charles River rats, housed in isolation boxes. All parameters were measured every 30 minutes from 10:00 to 17:00 on both day 1 (control) and day 2 (endotoxin given via intravenous catheter at 10:30). In 3 separate groups of rats, blood was sampled (N=5) via right atrial cannula; blood pressure was measured (N=5) via right carotid catheter; and body temperature was followed (N=4) via probe in the carotid sheath.

Intravenous endotoxin produced a prompt increase in plasma AVP in all animals (mean baseline 2.3 ± .4 pg/ml; 30 min. 7.8 ± 3.3 pg/ml; 60 min 29.4 ± 4.7 pg/ml), with return to baseline by 180 mins. Plasma β-End also rose sharply after endotoxin in all rats (mean baseline 51 ± 12 pg/ml; 30 min 279 ± 111 pg/ml; 60 min 1650 ± 120 pg/ml), then slowly declined (390 min 102 ± 14 pg/ml). Plasma PRL increased significantly after endotoxin, but not as soon as AVP and β-END (mean baseline 0.7 ± 0.1 ng/ml; 30 min 1.1 ± 0.2 ng/ml; 60 min 25.1 ± 9 ng/ml), then returned to baseline by 210 mins. Plasma volume abruptly decreased about 20% following endotoxin (baseline hematocrit 37.6 ± 0.3%; 30 min 41.8 ± 0.6%; 60 min 42.8 ± 0.8%), then slowly returned towards baseline. Plasma osmolality was unchanged. Blood pressure response was triphasic, with a sharp initial rise, followed by an abrupt fall (mean baseline 121 ± 5 mmHg; 30 min 133 ± 3mmHg; 60 min 112 ± 2 mmHg), and later by prolonged hypertension (mean BP>129). Body temperature change was biphasic, with hypothermia after 1 hour (mean baseline 36.6 ± .6°C; 30 min 36.8 ± .1°; 60 min 35.5 ± .6°) followed by a later hyperthermia (mean temp > 38.2).

These results show that endotoxin administered intravenously to the rat produces prompt and marked increases of plasma AVP, β-End and PRL. These coincide with alterations of plasma volume, blood pressure and body temperature. The relative importance of the latter physiological changes in mediating the observed hormonal responses, and the interactions between the hormones themselves, are being investigated.

123.13 NEURONAL, NEUROVASCULAR AND NEUROPEPTIDE ORGANIZATION OF EMBRYONIC HYPOTHALAMIC TRANSPLANTS IN THE THIRD CEREBRAL VENTRICLE OF ADULT BRATTLEBORO RATS. D. E. Scott, W. K. Paull*, D. Sherman*, and D. M. Gash. Dept. of Anatomy, Univ. of Missouri Sch. of Medicine, Columbia, MO.; and Univ. of Rochester Medical Center, Rochester, N.Y.

Female homozygous rats with autosomal diabetes insipidus were utilized in this investigation. Circadian water consumption and urine output was recorded. Rats were anesthetized and prepared for stereotaxic surgery. Fragments of anterior hypothalami from 17 day old fetal rats were stereotaxically introduced into the third ventricle, following the technique of Gash and Sladek, 1980. Host rats were killed 60 days later and prepared for correlative scanning-transmission electron microscopy (SEM-TEM), microangiography, and correlative microangiography-immunocytochemistry. SEM analysis revealed the presence of numerous large grafts on the ventral thalamic and dorsal hypothalamic walls of the third cerebral ventricle. Numerous neuron-like cells were evident on the surface of transplants and upon the adjacent hypothalamic wall, coupled with a profusion of cell processes which formed thick feltworks underlying these cells. Subsequent TEM analysis confirmed these elements to be bonafide neurons and axons. TEM coupled with microangiography demonstrated that the grafts were routinely invaded by parenchymal vessels from the adjacent paraventricular bed as well as by fenestrated vessels from the underlying mantel plexus of portal vessels of the median eminence. These vessels were routinely surrounded by extensive perivascular spaces. Correlative angiography and ICC with antineurophysin and antivasopressin revealed the presence of numerous immunopositive cell bodies and processes in the grafts. Neurophysin positive fibers appeared to arise from the adjacent periventricular stratum. These observations suggest an intimate neurovascular and neuroanatomical relationship between host and transplant and establishes a potential substrate for the central delivery of neuropeptide hormones between explant and host.

123.14 CHANGED VASOPRESSIN IMMUNOREACTIVITY OF SUPRAOPTIC MAGNOCELLULAR NEURONS FOLLOWING INTERRUPTION OF CATECHOLAMINE NEURONAL INPUT L.D. Wilkin, R.L. Zerbe*, J.Z. Kiss*, T.H. Williams, and M. Palkovits*, Dept. of Anatomy, University of Iowa, Iowa City, IA 52242; and Neuroendocrine Unit Lab. Clin. Sci., NIH, Bethesda, MD 20205.

Recent studies have demonstrated increased vasopressin (AVP) secretion after brainstem lesions or pharmacological treatments that disrupt afferent catecholamine (CA) inputs to the hypothalamic supraoptic nucleus (SON), suggesting that this neuronal input exerts a tonic inhibitory action. In the present studies, changes in hypothalamic AVP neurons were examined by immunocytochemistry and radioimmunoassay (RIA) following disruption of CA pathways.

In the first series of experiments, rats were allowed to survive for 2, 6, or 14 days after intraventricular injections of 250 µg 6-hydroxydopamine. Following perfusion and processing for AVP immunocytochemistry, 6-OHDA-treated brains were compared with controls. Clear changes in AVP staining intensity were observed in all 6-OHDA treated brains, as summarized in the following table:

	2 day	6 day	14 day
supraoptic nucleus (SON)	↓	↑	↑
fibers of lateral retrochiasmatic area	↓	↑	↑
median eminence	?	↑	-

Since the A1 and A2 neurons of the medulla are the main source of CA projections to the AVP neurosecretory cells of the SON, groups of rats received knife cuts that transected the ascending medullary CA fibers. Following bilateral transection, water intake decreased from 51 ml/d to 11 ml/d, and urinary output dropped sharply. After an 8-day survival period, the brains were removed and frozen, and micropunches of SON and the pituitary were assayed by RIA for AVP content. AVP content (in ng/mg protein) of the pituitary decreased from 12151 ng to 8474 ng while SON AVP increased from 95.5 ng to 153.8 ng. Following unilateral transection, water intake was also reduced and pituitary AVP dropped to 11085 ng. No changes were seen in the ipsilateral SON, but the contralateral SON showed a marked increase of AVP to 152.2 ng. This unilateral increase indicates that the observed changes were due to interruption of neural input and not to a disturbance of body fluid homeostasis.

These studies demonstrate that interruptions of catecholaminergic neuronal input to SON change the hormonal content of AVP neurons. Our results provide new evidence that brain stem CA neurons may tonically inhibit the activity of AVP-secreting neuroendocrine cells.

123.15 CONTRASTING EFFECTS OF STIMULATION OF A1 AND A2 CATECHOLAMINE CELL GROUP AREAS OF THE MEDULLA ON ACTIVITY OF PARAVENTRICULAR NEUROSECRETORY NEURONS. T.A.Day*, A.V.Ferguson and L.P.Renaud. Montreal General Hospital Research Institute and McGill University Montreal, Quebec, Canada H3G 1A4.

The neurohypophysial hormones oxytocin (OXY) and vasopressin (VP) are synthesized in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei. Although both nuclei receive noradrenergic (NA) inputs from the A1 cell group of the ventrolateral medulla, the PVN also receives substantial NA inputs from the A2 group of the dorsomedial medulla and the A6 group of the pons. Following the completion of studies which indicated that NA afferents arising from the A1 group selectively activate VP neurons in the SON, we have now begun to examine the role of NA projections from A1 and A2 in regulating the activity of PVN neurosecretory neurons.

Extracellular recordings of PVN neurosecretory neurons identified by antidromic invasion from the neurohypophysis were obtained in rats anaesthetized with nembutal or urethane. The effects of cathodal pulses (1-3 pulses, 50-400µA) delivered to either the A1 or A2 cell group areas via a monopolar electrode (tip exposure 50-100µM) were examined by means of post-stimulus histograms. Following each experiment the location of the stimulating electrode relative to NA cell groups was determined using a catecholamine histofluorescence technique.

Stimulation of sites corresponding to the A1 NA cell group enhanced the activity of 42% of PVN units tested (n=24), the remaining 58% being unaffected. On the basis of firing patterns and responsivity to baroreceptor activation 8 of the cells tested were classified as VP-secreting and 7 as OXY-secreting neurons. A1 stimulation excited 7 of the 8 putative VP neurons, but had no affect on any of the putative OXY neurons.

Stimulation of the A2 cell group region increased cell firing in 25% of the neurons tested (n=20) and was without effect on the remaining 75%. Eleven cells were classified as OXY-secreting, 4 of which were excited following A2 stimulation.

Pharmacological verification of the identity of the pathways mediating these effects remains to be obtained. Nevertheless, these preliminary data suggest that, as in the SON, NA afferents arising from the A1 cell group of the ventrolateral medulla selectively activate PVN VP neurons which project to the neurohypophysis. The influence of NA afferents from the A2 group appears to differ from that of afferents originating in A1, given the observed activation of OXY-secreting neurons following stimulation in the A2 region.

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123.16 ACUTE ADRENALECTOMY INCREASES METABOLIC ACTIVITY IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. M. Kadekaro, M. Ito, P.M. Gross, L. Sokoloff, Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Perikarya for corticotropin-releasing factor (CRF) have recently been identified by immunocytochemical studies to be located in the hypothalamic paraventricular nuclear complex. Additionally, a heavy density of CRF-immunoreactive fibers is present in the median eminence. It has been suggested that CRF secretion is influenced by noradrenergic input from the locus coeruleus. We studied the energy metabolism of the locus coeruleus, paraventricular nucleus, median eminence and pituitary anterior lobe of acutely adrenalectomized conscious rats with the autoradiographic [^{14}C]deoxyglucose method. Rats were bilaterally adrenalectomized or sham-operated under light halothane anesthesia and allowed 5 hours for recovery. Dexamethasone (0.25 mg/kg i.m.) was injected during the surgical procedure in sham-operated or adrenalectomized animals. The table shows rates of glucose utilization expressed as µmoles/100 g/min (means ± SEM).

	Sham-operated (n=7)	Adrenalectomy (n=8)	Sham + dexamethasone (n=6)	Adrenalectomy +dexamethasone (n=6)
Locus coeruleus	65±1	93±4 *	68±2	72±4 †
Paraventricular n.	60±2	75±4 *	66±3	65±4 †
Median eminence	62±6	96±8 *	51±6	57±2 †
Pituitary anter. lobe	20±2	26±2 *	14±2	16±2 †

* $p < 0.05$ compared to sham-operated group.
† $p < 0.05$ compared to adrenalectomy group.

The results demonstrate that, in the absence of steroid hormones, metabolic activity is high in the axis of structures regulating corticotropin secretion and in the locus coeruleus, which probably provides important afferent input to this regulation. Glucocorticoid replacement in adrenalectomized rats reduces the rate of glucose metabolism to normal levels in these structures.

123.17 INTERACTIONS OF SOMATOSTATINERGIC AND SOMATOCRININERGIC SYSTEMS IN THE GENERATION OF EPISODIC GROWTH HORMONE SECRETION: STUDIES IN THE FREELY-BEHAVING MALE RAT. L.C. Terry, M. Zorza* and N. Petersen*. Neuroendocrine Lab., Univ. of Michigan and VA Medical Center, Ann Arbor, MI 48105.

Growth hormone (GH) secretion in freely-behaving male rats is characterized by an ultradian rhythm composed of secretory episodes that occur every 3-4h. The zenith and nadir of this rhythm are believed to be regulated by two hypothalamic peptides, somatostatin (SRIF), which inhibits GH secretion, and somatocrinin (growth hormone-releasing factor, GRF). The purpose of the present experiments was to determine the contribution of SRIF and GRF in the generation of this rhythm. Experiment 1: Thermal lesions were placed in hypothalamic ventromedial-arcuate nuclei bilaterally to destroy GRF-producing neurons. Animals were subsequently implanted with chronic indwelling intra-atrial cannulae. Two weeks later, blood samples were removed serially every 15 min for 5h and assayed for immunoreactive GH. One group received cysteamine (150 mg/kg sc), an agent known to deplete CNS and peripheral SRIF, and the other group, vehicle. Prolactin was also assayed to determine if the A12 (arcuate) dopaminergic system was disrupted. Such lesions suppressed or eliminated GH secretory episodes, but did not alter nadir levels, and caused a 12 fold increase in prolactin. Cysteamine caused a significant rise in nadir GH levels, but did not reinstitute or enhance episodic release. Experiment 2: Cannulated rats were administered hpGRF (10 ug iv) or vehicle at 1005 or 1205h. hpGRF prolonged GH secretory episodes when given during a zenith (1000h), and stimulated GH to zenith levels if administered during a nadir (1200h). Administration of hpGRF during a nadir did not alter the frequency nor synchronization of the GH rhythm. Experiment 3: The SRIF antagonist analogue cyclo [Ahep-Phe-D-Trp-Lys-Thr(Bzl)] (CPP), reported to stimulate GH secretion in nembutal-anesthetized rats (J.L. Fries et al, Peptides 3:811, 1982), or vehicle was administered (10 ug/kg sc) to rats at 1005 or 1205h. Other groups were pretreated with either pentobarbital (50 mg/kg ip) or vehicle at 1005h and received the CPP at 1030h. The pentapeptide did not stimulate GH secretion in unanesthetized animals, but did cause GH release (above pentobarbital-stimulated levels) in the anesthetized group.

These studies indicate the following: 1) episodic GRF release generates the rhythmic pattern of GH secretion, 2) local tonic control of GH secretion by SRIF plays a minor role in the regulation of this rhythm under nonstressful conditions, and 3) the somatostatin analog CPP does not have a significant antagonistic action on pituitary SRIF receptors under physiologic conditions. (Supported by AM 28443 and a VA Merit Review Grant.)

123.18 LOCAL RATES OF CEREBRAL PROTEIN SYNTHESIS IN CHRONIC HYPOTHYROIDISM IN THE ADULT RAT. D. Dow-Edwards, C. Smith*, and L. Sokoloff, Department of Neurosurgery, SUNY Downstate Medical Center, Brooklyn, NY, and Laboratory of Cerebral Metabolism, NIMH, Bethesda, MD.

Although thyroid hormones are known to regulate protein synthesis in the developing brain, no effects of thyroid hormones on the average rate of protein synthesis in brain taken as a whole have been observed in mature animals. Altered thyroid function has, however, been shown to influence the activity of several brain enzymes, to alter neurotransmitter levels, and to modify behavior. In order to determine if altered thyroid function might influence rates of protein synthesis in specific regions of the brain in vivo, we employed the recently developed autoradiographic method for measuring local rates of L-leucine incorporation into protein (Smith et al., 1980, Trans. Amer.Soc. Neurochem. 11, 94) in chronic hypothyroid rats.

Male Sprague-Dawley rats (250 g) were surgically thyroidectomized under pentobarbital anesthesia. Body weights were recorded during the following 3 months to confirm the effectiveness of the thyroidectomy. The measurement of protein synthesis was carried out in 5 hypothyroid animals and 4 sham-operated controls. The animals were administered an intravenous pulse (100 µCi/kg of body weight) of L-[1-^{14}C]leucine (59 mCi/mmol). Timed arterial samples were then taken for determination of the time courses of the arterial plasma concentrations of [^{14}C]leucine and leucine. At 60 minutes the animals were killed, and the brains were removed, frozen, and later sectioned for autoradiography as described by Sokoloff et al. (1977, J.Neurochem. 28, 897-916). The sections were fixed to remove the unincorporated [^{14}C]leucine and autoradiographed on Kodak MR film. Areas of interest were analyzed densitometrically to determine the local concentrations of ^{14}C in the tissues. From the local tissue ^{14}C concentrations and the time courses of the plasma [^{14}C]leucine and leucine concentrations local rates of leucine incorporation into protein were calculated by the operational equation of the method. Of the 51 brain regions examined there were significant decreases in the rates of protein synthesis in 13 structures of the hypothyroid animals. These structures included mainly components of the extrapyramidal motor system, nuclei of cranial nerves, and hypothalamic nuclei. There were no significant changes in visual or auditory pathways or in any region of the cortex. Chronic hypothyroidism, therefore, appears to decrease rates of protein synthesis in a few selected areas of brain.

123.19 A HYPOTHALAMIC PITUITARY SYSTEM STIMULATES PLASMINOGEN ACTIVATOR IN PLASMA. G. Fink, C.V. Prowse* and R.C. Dow*. MRC Brain Metabolism Unit and Blood Transfusion Service, Edinburgh, Scotland.

Plasminogen activator (PA) cleaves the proenzyme plasminogen allowing subsequent lysis of blood clots by degradation of fibrin. In man PA is released into plasma by a variety of stimuli including systemic infusion of adrenaline or 1-desamino-8-D-arginine vasopressin (DDAVP). In man, adrenaline acts directly on peripheral (endothelial) stores to cause PA release, whereas DDAVP acts by way of a central receptor (Cash et al, Clin. Sci. Mol. Med., 54:403, 1978). Regional infusion of DDAVP to the head in dogs released PA suggesting that DDAVP stimulates the release of a PA-releasing factor (PARF) which releases PA from a peripheral site. We have investigated the site of production of the proposed PARF and its central nervous control by means of electrical stimulation of the hypothalamus and infusion of extracts of anterior pituitary tissue.

All experiments were carried out on adult male rats anaesthetised with urethane. In preliminary experiments the ability of intravenous adrenaline or DDAVP to raise PA levels (measured by effect on euglobulin clot lysis times; Cash & Allan, Brit. J. Haematol., 13:376, 1967) was demonstrated. Electrical stimulation of the median eminence (ME) of the hypothalamus (Fink & Jamieson, J. Endocrinol., 68:71, 1976) in intact Wistar rats had a similar effect. The mechanism of PA release by such electrical stimulation did not depend upon either vasopressin or adrenaline since ME stimulation also caused PA release in homozygous Brattelboro rats (totally deficient in vasopressin) and in Wistar rats from which the adrenal glands had been removed. The PA response to ME stimulation was, however, abolished by section of the pituitary stalk. The release of PA was also induced, in a dose-dependent manner, by intravenous infusion into normal Wistar rats of a saline extract of anterior pituitary tissue obtained from either normal Wistar or Brattelboro rats. The activity of the pituitary extracts was destroyed by boiling, but not by treatment with di-isopropyl fluorophosphate which inhibits PA. Infusion of the forty-one amino acid residue corticotropin releasing factor was without effect.

These results show that the anterior pituitary gland contains a heat-labile factor which acts on peripheral stores to release PA into blood, and that the release of PARF is stimulated by a hypothalamic factor. The mechanism of action of PARF and its release does not require the presence of vasopressin, adrenaline, glucocorticoids or adrenal enkephalin.

123.PO ACTH INFLUENCE ON TYROSINE HYDROXYLASE ACTIVITY IN THE LOCUS COERULEUS. K.A. Markey and P.Y. Sze. Dept. of Biobehavioral Sciences, The University of Connecticut, Storrs, CT 06268.

Our previous study shows that chronic administration of corticosterone to mice during a sensitive period of early postnatal development elicits an increase of tyrosine hydroxylase activity in the locus coeruleus (Endocrinology 111: 1519, 1982). However, in adult mice, administration of glucocorticoids does not have any effect on the enzyme activity in the noradrenergic neurons. In the present study, we found that bilateral adrenalectomy resulted in an increase rather than the expected reduction of tyrosine hydroxylase activity in the locus coeruleus, suggesting that the response of the enzyme to pituitary-adrenal influences may change as ontogenesis proceeds. The enzyme activity in the locus coeruleus rose slowly in adrenalectomized adult mice, with a maximum increase of the enzyme activity (52% above control) occurring seven days after the surgery. No such increase of tyrosine hydroxylase activity was found in dopaminergic neurons of the substantia nigra. The increase of enzyme activity in the locus coeruleus following adrenalectomy was totally prevented by corticosterone replacement. Moreover, the adrenalectomy effect was abolished by hypophysectomy, indicating the involvement of the pituitary rather than the adrenocortical function. Chronic administration of ACTH (20 I.U./kg, i.p., daily) resulted in an increase of tyrosine hydroxylase activity in the locus coeruleus, with a time course and magnitude similar to those found after adrenalectomy. Additionally, the effects of four ACTH analogs were determined. ACTH(1-24) and ACTH(4-10) were as effective as the whole ACTH molecule, whereas ACTH(4-10,7-D-Phe) and ACTH(11-24) were ineffective. The effect of ACTH (4-10), a peptide fragment with no adrenocorticotrophic activity, further indicates that glucocorticoids are not involved. From these data, it appears that tyrosine hydroxylase in adult locus coeruleus is under the regulatory influence of pituitary ACTH. It remains to be determined whether the hormone can be transported from its pituitary origin to the locus coeruleus and exerts a direct action on the noradrenergic neurons. Regardless of the mechanism, the response of the noradrenergic neurons to pituitary activity may be an important component in physiological adaptation of the central nervous system to chronic stress. [Supported by USPHS Grant MH-29237.]

124.1 THE DEVELOPMENT AND EXTINCTION OF CONDITIONED FEAR FOLLOWING ACUTE EXPOSURE TO TRIMETHYLTIN. H.S. Swartzwelder, C.T. Johnson, C. Robinson*, E. Kestler*, and C. Steinbach*. Institute of Animal Behavior, Towson State Univ., Towson, Md.21204. Trimethyltin (TMT) is a known neurotoxin which produces deficits in learning and memory as well as a distinct limbic neuropathology (Brown et. al.,1979; Dyer et. al., 1982). Although the effects of TMT upon locomotor activity (Swartzwelder et. al., 1981), avoidance learning (Walsh et. al., 1982) and problem solving (Swartzwelder et. al., 1982) have been well documented, little is known about the effects of this compound upon classical conditioning phenomena. Conditioned suppression is recognised as both a measure of classical conditioning and neurobehavioral toxicity (Rescorla, 1968; Nation et.al., 1982), and animals with lesions in the hippocampus show impaired development of this response (Brady, 1958; Molino, 1975). In our study, conditioned suppression was measured in the control and TMT-treated rat. TMT-treated animals were given either 5.0 or 7.0 mg/kg/ml TMT via intragastric intubation and controls received the saline vehicle in equal volumes. All rats were trained to leverpress for food reinforcers on an FR-20 schedule. Once this baseline was established, the operant sessions were divided into three eight-minute blocks each. For three days a 2900-Hz tone was presented during the eighth minute of each block and a 0.5-s footshock was presented at the end of each block. Although the rate of suppression did not vary between groups, the TMT-treated rats showed less suppression than controls when the tone was presented alone during the first extinction test (i.e., without shock). These results suggest that the TMT-treated animal is less responsive to the normal suppressive effects of an aversive conditioned stimulus, and will be discussed in light of the effect of exposure to inorganic lead upon conditioned suppression.

124.2 TRIMETHYL TIN: BEHAVIORAL, NEUROCHEMICAL AND NEUROANATOMICAL EFFECTS. C.C. Loullis, R.L. Dean, D.I. Benson*, A.S. Lippa*, R.T. Bartus and J. Coupet. Dept. of CNS Research, Medical Research Div. of American Cyanamid, Lederle Labs, Pearl River, NY 10965.

The effects of acute administration of trimethyl tin (TMT) are distinguished from other organotin compounds by selective and persistent neuropathological changes in hippocampal pyramidal cells. This relatively selective cell destruction by TMT may provide a neurobiological probe for investigating neurochemical connections in the hippocampus. Cognitive deficits also persist following TMT administration and therefore it may also be a useful model in assessing hippocampal involvement in these deficits. The purpose of the present experiment was to investigate the behavioral neurochemical and cytological effects of TMT. Rats were intubated once with either 0.9% Saline or TMT (3.5 mg/kg). Two weeks later, following the disappearance of the acute toxic behavioral effects, animals were trained on a single trial passive avoidance task. Twenty four hours later they were tested for retention. Following this, animals were sacrificed, the hippocampus was quickly dissected on ice and stored at -20° C for neurochemical analysis and histological examination. Animals which received TMT exhibited a marked deficit in retention. Scatchard analysis using ^{3}H(-)QNB revealed a 21% decrease in muscarinic receptor density. Histological examination of the hippocampus from treated animals revealed a loss of pyramidal cells. These results indicate that muscarinic receptors reside on the hippocampal pyramidal cells and that these cells and receptors are critical in mediating the passive avoidance behavior.

124.3 A NEW PROCEDURE FOR SPECIATION OF NEUROTOXIC TRIMETHYLTIN IN IN-VIVO-EXPOSED MAMMALIAN TISSUES. K.L. Hulebak, J.C. Means*. Div. Toxicology, Dept. Environ. Hlth. Sci., The Johns Hopkins Univ., Baltimore, MD 21205, and Chesapeake Biological Lab. and Dept. of Chemistry, Univ. of Maryland, Solomons, MD 20688.

Exposure to trimethyltin results in morphologically detectable damage to the CNS and possibly to the kidney. Dose-response relationships have been described, but a fast method for quantitating levels of various methyltin species in target organs has been unavailable. It is possible to measure methyltins as total tin using atomic absorption spectrometry, but speciation of methyltins has proven difficult. We present a new procedure for the quantitative analysis and speciation of methyltins, directly from exposed tissues.

Trimethyltin chloride is purged from in vitro- or in vivo-exposed, freshly homogenized mouse liver, kidney, and brain using $NaBH_4$. The volatile organotin hydrides produced are cryogenically trapped on the head of a gas chromatographic column (at -40° C). The compounds are detected using selected ion monitoring in a Hewlett Packard 5985 B mass spectrometer in either the electron impact or the chemical ionization mode. Quantitation is achieved by integration of the areas of the chromatographic peaks. Linear response is obtained over the range of 1 ng to 30 µg. Recovery of trimethyltin spiked into tissues is greater than 85%. Nanogram quantities of trimethyltin can be recovered from in-vivo-exposed tissues. Trimethyltin is the only methyltin detected in organs from trimethyltin-exposed mice.

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124.3 ALTERATIONS IN SENSORIMOTOR FUNCTION FOLLOWING ACUTE TRIETHYL LEAD EXPOSURE: TIME AND DOSE RESPONSE ANALYSIS. T. J. Walsh * and H. A. Tilson * (SPON: K. Yoshikawa), Lab. Behav. Neurol. Toxicol., NIEHS Research Triangle Park, NC 27709.

Triethyl lead (TEL) is a neurotoxic organometal reported to produce a distinct pattern of limbic system pathology, involving the hippocampus, amygdala and pyriform cortex (Seawright et al., in Methods in Toxicology and Hazard Evaluation, 1980). Since the limbic forebrain is believed to modulate the sensitivity and/or behavioral reactivity to environmentally relevant stimuli one effect of TEL might be to disrupt sensorimotor processes. Therefore, the studies reported here examined the effects of TEL on various aspects of sensorimotor function using a neurobehavioral test battery that assesses motor activity, fore- and hindlimb grip strength, spectrally analyzed whole body movement, acoustic startle and reactivity to a noxious stimulus using the hot-plate test.

Adult male Fischer-344 rats were injected (s.c.) with either distilled water, or 1/4 (2.63 mg/kg), 1/2 (5.25 mg/kg) or 3/4 (7.88 mg/kg) the acute LD50 of TEL Cl and tested on days 1-2, 7-8, 14-15, 21-22 and 28-29 following dosing. TEL was found to affect sensorimotor function in a time and dose-related manner. The 1/4 LD50 dose of TEL failed to affect hot-plate latencies, motor activity or forelimb grip strength at any time after dosing but did increase hindlimb grip strength (10-20 %) on post-treatment days 1, 14 and 21. The intermediate dose of TEL (1/2 LD50) increased ambulation and rearing (60-140 %) on days 7, 14 and 21 and hot-plate latencies (40-60 %) up to 15 days following dosing. The highest dose of TEL (3/4 LD50) increased hot-plate latencies (26-176 %) 2, 8, 15 and 29 days after dosing. Hindlimb grip strength was increased (9 %) 1 day following dosing and there was a biphasic effect on rearing and ambulation. Both components of motor activity were decreased approximately 50 % 1 day after dosing; however, animals subsequently developed a persistent hyperactivity (ie. both measures increased 55-239 %) which was evident throughout the course of testing. Spectral analysis of movement revealed dose-related increases in power (amplitude), particularly in the 2.5 -10 Hz region. Changes in the spectral profile of activity were observed one week after dosing and persisted throughout the period of study.

Acute administration of TEL affected several indices of sensorimotor function. The most sensitive endpoints for detecting TEL toxicity were reactivity to a noxious stimulus, motor activity and spectrally analyzed whole body movements. Studies are now in progress to correlate these behavioral changes with alterations in central and peripheral nervous system pathology and brain neurotransmitter and peptide systems.

124.5 SOME ASPECTS OF FEEDING AND LOCOMOTOR ACTIVITY IN RATS EXPOSED TO TETRAETHYL LEAD. D. A. Czech, E. Hoium* and T. C. Trusk. Depts. of Psychology and Biology, Marquette Univ., Milwaukee, WI 53233

Previous research has shown that organolead compounds can lead to changes in locomotor and consumatory behavior. In addition to observing that exposure to tetraethyl lead (TEL) is followed in a dose related way by increased locomotor activity and reduced food and water intake in rats, we found that when food intake of rats in a control group was limited to that of yoked counterparts in a TEL exposed group, activity increased in both. Further, food intake and activity were significantly correlated in both groups, suggesting a possible link between reduced food intake and behavioral arousal in leaded rats. To pursue this issue, we yoked rats injected i.p. with 7 mg/kg TEL, a dose found effective for the aforementioned behaviors, with controls (peanut oil vehicle), supplementing food intake of TEL rats by stomach loading them with the difference between their voluntary daily intake and that of a yoked control. A third group, also given 7mg/kg TEL, was not food yoked, and served as control for noting lead effects without intervention. Observations were made over 3 pre- and 18 post-TEL days. Food loads were delivered to the yoked-TEL group over the initial 9 post-TEL days; other groups received sham loads over this period and all rats were sham intubated over days 10-18. Both TEL groups showed significantly higher activity(relative to pre-TEL levels) over all but the first few days of the post-TEL period. Food loads to leaded groups did not effectively prevent or reduce activity shifts. Leaded animals exhibited slower weight gains over the first 9 post days; a less effective nutritional state may therefore have contributed in part to increased activity. However, over the last 9 days, neither body weights nor food intakes were significantly different across groups, while activity remained elevated in TEL groups. Consequently, it is unlikely that nutritional state was the sole factor for all of the variance. Periodic tests over succeeding weeks revealed continued high activity in TEL groups. Significance levels were set at $p < .05$ for all analyses. These data suggest a more direct lead contribution, but are somewhat equivocal. Procedural limitations and possible mechanisms are discussed.

Related work in our lab also showed that TEL exposed rats will eat palatable foods, while rejecting lab chow, and may return to normal feeding levels sooner if maintained on such a diet, prompting two further studies. In the first study, a group of "recovered" TEL rats (7 mg/kg) and a control group matched for body weight were challenged with 10 U/kg insulin, 600 mg/kg 2-deoxy-D-glucose and 0.9% saline. No significant food intake differences were noted between groups to substances after both 1 and 6 hour tests. In the second, TEL and control rats were similarly challenged 3-7 days after TEL dosing. These results were complex.

124.6 DEFICITS IN SPATIAL MEMORY PERFORMANCE IN EIGHT ARM MAZE FOLLOWING EARLY LEAD EXPOSURE. L.L. Garten*, C. Winder* and P.D. Lewis* (SPON: A.J. Patel). Department of Histopathology, Royal Postgraduate Medical School, London W12, U.K.

Behavioral alterations have been reported in clinical and experimental studies of lead neurotoxicity. Lead induced changes in development and structure of the hippocampal region have also been indicated.

Maternal rats were dosed with 300 and 1000ppm lead acetate in drinking water throughout gestation and lactation; neonates were weaned to tapwater at 21 days of age. This model has been shown to produce blood lead levels in weaned offspring equivalent to childhood clinical and subclinical levels. No body weight differences between control and lead treated animals were seen at any stage. At 100 days of age, blood lead levels of dosed rats were similar to those of controls. Adult female rats (130-160 days) perinatally exposed according to this regime were used for behavioral testing.

Performance in the elevated radial 8 arm maze has been shown to be selectively affected by hippocampal damage. Radial maze tasks involve a flexible, rather than consistent response, using working memory and cognitive mapping strategies.

Performance in this maze was consistently and significantly impaired in lead dosed rats. Acquisition of the task was delayed and after attainment of a criterion of one error in three consecutive trials performance continued to be inferior to that of controls. The number of errors made by animals in both dose groups was significantly increased. However, latency, or time taken to make the required eight choices, was not consistently affected.

The deficits seen in performance following perinatal low level intake suggest that the hippocampus may be susceptible to lead exposure. That changes persist into adult life implies the possibility of a long-term or even irreversible disfunction.

124.7 EARLY ELECTROPHYSIOLOGIC CHANGES IN MAMMALIAN NERVE INDUCED BY ORGANOPHOSPHORUS AGENTS. R. J. Anderson, J. H. Carlson and C. B. Dunham* Dept. of Pharmacol., George Washington Univ. Med. Ctr., Washington, D.C. 20037.

Exposure to some organophosphorus (OP) compounds leads to the development of a delayed neuropathy, which in most cases correlates with inhibition of neurotoxic esterase (NTE). However, the steps between NTE inhibition, which occurs rapidly, and the onset of the histopathology, which usually takes more than a week, are not known. The purpose of this study was to examine the electrophysiologic changes which occur during chronic administration of several delayed neuropathy-inducing OPs. Male rats were dosed ip with trichlorfon (TCF) (200 mg/kg), DFP (1 mg/kg), or soman (0.05 mg/kg) in single daily doses for up to 45 days. At intervals rats were withdrawn from the study and tested for changes in sciatic nerve excitability. All three OPs induced a shift toward shorter relative refractory periods, reaching a maximum after 15 days of TCF dosing, 10 days of DFP and only 1 day of soman. Also, the nerve compound action potentials had shorter durations and faster conduction velocities than the controls. These effects correlated with a loss of plasma and acetyl cholinesterase activity. Despite continued dosing, the electrophysiologic effects returned to control in 45 days with TCF, about 20 days with DFP and 4 days with soman. Enzyme activities, though not completely recovered at these times, nevertheless increased in activity. Thus, these early electrophysiologic changes seem to correlate with cholinesterase inhibition.

The sciatic nerves of these animals were also challenged with potassium-induced depolarization using the sucrose gap technique. Chronically administered DFP lead to increased nerve sensitivity to potassium, inducing greater depolarizations than the controls. This effect was maximal after 20 days of DFP dosing, a time at which both enzyme activity and nerve conduction are almost fully recovered. The effect on potassium depolarization seems to correlate with the time course of delayed neuropathy induced by DFP and may be an index of the development of this damage. Soman produced variable changes in the sucrose gap measurements which may have been due to the small dose which was used. Prior to sacrifice none of the animals showed evidence of motor deficits using behavioral measures, and histologic examination revealed only mild evidence of neuropathic changes in some, but not all of the nerves.

These results show that electrophysiologic changes do occur in peripheral mammalian nerves after chronic exposure to OPs. Some features of nerve excitability seem to correlate better with acute toxicity (cholinesterase inhibition) while others appear to correlate with the development of delayed neuropathy.

This work was supported in part by USAMRICD.

124.8 BEHAVIORAL TOXICITY OF CARBON DISULFIDE IN THE RAT. J.T. Concannon and M.D. Schechter. Program in Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, Ohio 44272.

Adult rats were trained under the influence of carbon disulfide (CS_2) to perform two types of operant tasks: (a) a drug-discrimination task and (b) a nose-poke task to escape inhaled CS_2. In the first task, rats were trained to discriminate between the stimulus properties of intraperitoneally-administered CS_2 and its vehicle in a two-lever, food-motivated operant task, using either 12.5 or 25.0 mg/kg CS_2. The results of an extensive, 56-session training schedule showed that only one rat met the criterion of 9 agent-appropriate lever selections out of 10 consecutive trials, but its subsequent performance deteriorated to below criterion level. Post-training biochemical determinations, however, revealed that both doses of CS_2 reliably depleted cerebellar levels of norepinephrine (NE), without affecting "rest-of-brain" levels of dopamine (DA) or NE, when determinations were made using high-performance liquid chromatography (HPLC). These results suggest that biochemical measures of brain status may be even more sensitive indices of injected solvent-induced "toxicity" than is operant drug-discrimination.

The second task used a system which simultaneously delivered continuous, high levels of CS_2 to some ("chronic") animals, while rapidly decreasing CS_2 levels contingent upon an operant nose-poke escape response in other ("trained") animals. This computer-assisted system meets the following criteria: (a) gas concentrations are readily controlled and accurately monitored; (b) technicians are protected from exposure to the irritant gas; and (c) the subject orients toward the gas source and performs a simple response to terminate the gas. Preliminary results show that trained animals can learn this task compared to "yoked-control" animals who have the same CS_2 exposure, in terms of intensity and duration, but who cannot terminate CS_2 exposure. However, the learning is a very gradual process and required extensive training. Inter-trial interval (ITI) responses also indicates that CS_2 escape learning is not due to simple activity-increasing effects of CS_2. Manipulations which increased the level of CS_2 escape responding will be discussed. These results suggest that CS_2 is detectable and can serve as a warning stimulus for subsequent aversiveness.

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124.9 ALTERED RETROGRADE AXOPLASMIC TRANSPORT BY SINGLE DOSES OF ACRYLAMIDE. Matthew S. Miller and Peter S. Spencer, Institute of Neurotoxicology, Depts. of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, New York 10461.

Monomeric acrylamide (ACMD) induces central-peripheral distal axonopathy in laboratory animals and humans following repeated exposure. Little is known regarding the underlying biochemical mechanisms. Recent studies suggest that small changes in bidirectional fast axonal transport in distal portions of peripheral nerve are associated with ACMD-induced axonopathy. However, it is unclear whether these changes in axonal transport precede, or occur secondary to, axonal degeneration. This study determined the effects of single doses of ACMD on retrograde axonal transport. To assess retrograde axonal transport, purified iodinated tetanus toxin (^{125}I-TX) was unilaterally injected into the gastrocnemius muscle of male CD-1 mice (20-30 g). After ^{125}I-TX injection (2.5-12.5 hours), animals were killed, and accumulation of ^{125}I in ipsilateral and contralateral spinal-cord anterior horn (L_3-L_6) and dorsal root ganglia (L_3-L_6) was determined by gamma counting. The difference in ^{125}I content of ipsilateral and contralateral tissues was considered to be the result of axonal transport. In control mice, preferential accumulation of ^{125}I in ipsilateral anterior horn and dorsal root ganglia was detected within 5 hours and attained maximum levels approximately 7.5 hours after peripheral injection of ^{125}I-TX. The effect of single doses of ACMD and N,N'-methylene-bis-acrylamide (a non-neurotoxic analog of ACMD) on retrograde axonal transport of ^{125}I-TX was then investigated. One hour after peripheral injection of ^{125}I-TX, mice received a single intraperitoneal injection of ACMD (0-100 mg/kg) or equimolar doses of N,N'-methylene-bis-acrylamide. Animals were killed 5 hours later and accumulation of ^{125}I in anterior horn and dorsal root ganglia was assessed as described above. ACMD was administered after ^{125}I-TX to avoid potential effects of ACMD on the neuronal uptake of radiolabeled toxin. Single doses of ACMD produced a dose-dependent decrease in the transport of ^{125}I-TX to the perikarya of primary sensory neurons in dorsal root ganglia and lower motor neurons in anterior horn. The threshold dose for inhibition of transport by ACMD was approximately 25 mg/kg in both sensory and motor neurons. The dose of ACMD required to inhibit retrograde axonal transport maximally was 50 mg/kg in sensory neurons and 100 mg/kg in motor neurons. Substantially greater doses of N,N'-methylene-bis-acrylamide were required to alter the axonal transport of ^{125}I-TX by peripheral sensory and motor neurons. It is concluded that single doses of ACMD alter retrograde axoplasmic transport in peripheral sensory and motor neurons. Retrograde axoplasmic transport in primary sensory neurons is more susceptible to inhibition by single doses of ACMD than that in lower motor neurons. Alterations in axonal transport may reflect the primary biochemical lesion associated with ACMD-induced central-peripheral distal axonopathy. (Supported by NS07063 and OH00851).

124.10 BEHAVIORAL EFFECTS OF CHRONIC HALOTHANE EXPOSURE IN DEVELOPING RATS. Edward D. Levin*, Nellie K. Laughlin*, Robert E. Bowman, and Etsuro Uemura. Dept. of Psychology, Primate Laboratory, Univ. of Wisconsin-Madison, WI. 53715, and Dept. of Anatomy, University of Iowa, Ames, Iowa. (SPON: M. H. Weiler).

Halothane, a commonly used surgical anesthetic has been found to cause neural and behavioral deficits in rats exposed to low, chronic levels during perinatal development. The present study further examined the behavioral effects and will correlate these with neuropathological studies of synaptic density and dendritic branching. Rats were exposed to 100 parts per million halothane in the air during gestation and until 60 days after birth. The intermittant exposure group (N=6 litters) received halothane for 8 hours/day for 5 days/week. The continuous exposure group (N=6 litters) received halothane continually throughout the exposure period except for about one hour/day needed for animal husbandry and testing. The control group (N=4 litters) received no halothane but were kept in exposure chambers identical to those of the other two groups.

At 20, 40 and 60 days of age, the rats were tested for spontaneous alternation in an unbaited T-maze. This classic test of hippocampal development was chosen to complement the neuropathological studies of the hippocampus. Latency and direction of choice was measured for a series of six trials. Using ANOVA no significant halothane effects were seen with the percent alternation measure although the controls did show a significant increase in alternation with development ($p<.01$) while both halothane groups did not. ANOVA of the latency data revealed no main effect of halothane but there were significant effects of halothane x age ($p<.01$), halothane x trial ($p<.05$) and halothane x age x trial ($p<.001$). These significant effects reflected a lengthening of choice latency over trials of the two halothane groups at day 20 as compared with the controls who did not change their latency over trials. This effect was no longer apparent at days 40 and 60.

An additional female and male offspring from each litter were tested in an open field for 3 consequetive days beginning at weaning (Day 25). ANOVA of the number of squares entered indicated that halothane treated animals were less active than controls ($p<01$) and that the pattern of changes in activity within sessions was different across groups ($p<.025$). The decreased activity in the open field and longer response latencies in the alternation task both may suggest a similar effect of halothane on locomotor exploration. We will also report data on social behavior and on 8-arm exploration and learning. Supported by NIH grant NS 17107.

124.11 BEHAVIORAL TOXICITY IN RABBITS FOLLOWING PROLONGED ALUMINUM (Al) EXPOSURE DURING DIFFERENT DEVELOPMENTAL STAGES. R.A. Yokel and M.T. Willhite.* College of Pharmacy and Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536.

Al has been implicated as the causative agent in dialysis encephalopathy and osteomalacia and has been found to accumulate in Alzheimer diseased brains. A study (Neurobehav. Toxicol. Teratol. 5: 41-46, 1983) conducted in adult rabbits showed evidence of toxicity following repeated systemic Al exposure (5 sc inj/wk x 4wk of 0 to 400 µmole Al/kg/inj). Body weight loss was 3% in those receiving 400 µmole Al/kg/inj whereas controls gained 46% by wk 5 after Al inj. Acquisition of a classically conditioned reflex (nictitating membrane extension, NME) 2.5 wk after Al exposure showed lower terminal % conditioned responses (CR) (80 and 75% of control for the 200 and 400 µmole Al groups) and longer latencies to CR (327 and 310 vs. 261 msec for controls) indicating less well developed CR acquisition.

To assess Al toxicity in younger organisms, pregnant rabbits received 5 inj/wk x 4wk of 0, 25, 100 or 400 µmole Al/kg/inj during gestation. Weight gain was little effected in the surviving offspring (54%) of 400µmole Al exposed does. The 25 µmole Al exposed group showed facilitation of NME acquisition (higher % CR and shorter CR latency) whether conditioned at 6.5 or 10.5 wk of age. This effect may relate to the epileptogenic potential of Al. The acquisition of NME in the other Al exposed offspring was not different from controls.

Treatment of lactating does with 5 inj/wk x 4wk of 0 to 800 µmole Al/kg/inj resulted in weight loss, to a low of 90% of original weight at wk 5 in 400 µmole Al exposed does. Does exposed to 800 µmole Al died at 4wk. Suckling offspring of 25 µmole Al exposed does increased in body weight from control levels at wk 8 to 111% of controls by wk 12. Offspring of 400 µmole Al exposed does were 75% of control body weight from wk 1 to 6 then increased to 89% of controls by wk 12. NME acquisition was more impaired in 25 µmole Al exposed offspring (70% of controls) when conditioned at 6.5 or 10.5 wk of age, than offspring exposed to higher Al levels.

Five inj/wk x 4wk of 400 µmole Al, 50 µmole Pb, or 1 µmole Sn/kg/inj to rabbits beginning at 2 days of age produced significant inhibition of weight gain in Al treated rabbits but no consistent effects on NME acquisition in any metal exposed groups compared to controls.

Body weight changes did not consistently correlate with behavioral changes suggesting no cause-effect relationship. Although the mature rabbit seems to be more affected by Al than the immature rabbit, Al does not appear to have great potential to produce behavioral toxicity in the organism with normal renal function. (Supported by NIH Grant ES 2676.)

124.12 CONDITIONED TASTE AVERSION INDUCED BY THE ORGANOPHOSPHORUS INSECTICIDE DICHLORVOS IN RATS. L.G.Costa*, P.Roney* and S.D.Murphy* (sponsor I.J.Butler). Div. of Toxicology, Dept. of Pharmacology, University of Texas Medical School and School of Public Health, Houston, Texas 77025.

Conditioned taste aversion (CTA) is a behavioral paradigm that is rapidly acquired by animals when the ingestion of a novel substance is followed by certain drugs, toxic chemicals or X-irradiation. CTA has been proposed as a test for evaluating the toxic effects of chemicals on the CNS. In the present study we investigated the ability of the organophosphorus insecticide dichlorvos (2,2-dichloroethenyl phosphoric acid dimethyl ester; DDVP; analytical grade, 97%) to induce CTA. Male rats were caged individually and adapted to a daily restricted (30 min) water availability schedule. When intakes had stabilized (usually after 5-7 days), rats were given simultaneous access to water and to a 0.15% (w/v) saccharin solution. Immediately after the session rats were injected with saline, lithium chloride (6 meq/kg, as a positive control) or DDVP (1.5, 3.0, 4.5, 6.0, 9.0 mg/kg,ip). Moderate signs of cholinergic intoxication were observed only after administration of the 9.0 mg/kg dose. Three days later the animals were presented again with the water-saccharin choice. Saccharin consumption (as percent of total fluid intake) was 83.9 for control, 18.5 for LiCl-treated rats and 70.4, 65.5, 27.5, 23.4, 24.6 for rats given increasing doses of DDVP. Values were significantly different from control ($P < 0.05$, ANOVA) at 4.5, 6.0 and 9.0 mg/kg. Total fluid consumption did not differ among groups.

Cholinesterase activity was measured 30 min after the administration of DDVP and found to be inhibited 38.2 and 67.6% (plasma), 60.3 and 76.5% (whole brain) and 51.8 and 69.4% (diaphragm) after 4.5 and 9.0 mg/kg, respectively. The 4.5 mg/kg dose of DDVP did not have any effect on either spontaneous motor activity in an open field nor on motor coordination in an inclined plane test. This dose of DDVP also did not alter pain perception in the tail-immersion and hot-plate tests. At the dose of 9.0 mg/kg, DDVP had significant effects in all these tests.

These results indicate that DDVP causes CTA at doses at which no other apparent sign of toxicity is evident and suggest that CTA may be an useful test in evaluating neurobehavioral toxicity of organophosphorus insecticides. (Supported in part by grant ES-01831 from NIEHS).

124.13 PERSISTENT LOCOMOTOR HYPERACTIVITY IN OFFSPRING OF RHESUS MONKEYS EXPOSED TO POLYCHLORINATED OR POLYBROMINATED BIPHENYLS. Susan L. Schantz* and Robert E. Bowman, Dept. of Psychology Primate Laboratory, Univ. of Wisconsin, Madison, WI 53706.

Locomotor activity of rhesus monkeys born to polychlorinated or polybrominated biphenyl (PCB or PBB) exposed mothers was compared with that of controls. Experimental mothers were fed diets to which either the PCB mixture Aroclor 1248 or Aroclor 1016 or the PBB mixture Firemaster FF-1 were added. Experimental diets were begun prior to breeding and continued throughout gestation and nursing of offspring. Doses were as follows: (1) Aroclor 1248: 0.5 or 1.0 ppm 3 times weekly (2) Aroclor 1016: 0.25 or 1.0 ppm daily (3) Firemaster FF-1: 0.3 ppm daily. Following weaning of their offspring, Aroclor 1248 and Firemaster FF-1 exposed mothers were maintained on their experimental diets for a second breeding round from which offspring were also studied. The experiment also included the following recovery rounds of offspring born after experimental diets were discontinued (1) Three groups of offspring born in three successive years following termination of daily exposure to a diet containing 2.5 ppm Aroclor 1248 (2) One group of offspring born following termination of the 1.0 ppm Aroclor 1016 diet and (3) one group of offspring born following termination of the 0.3 ppm Firemaster FF-1 diet. All infants were weaned at 4 mo. of age. Locomotor activity was assessed in an activity chamber with crossed photobeams at approximately yearly intervals beginning when the animals were 12 mo. of age. For each activity test an animal was placed in the activity chamber for a 75 min. session 5 days per week for a total of 20 sessions. Photobeam breaks were converted to counts per hour and averaged over blocks of 4 sessions for analysis by ANOVA. Most PCB and PBB-exposed groups consistently displayed locomotor activity levels that were elevated with respect to controls. Locomotor activity levels in some groups remained elevated until 36 mo. of age. In a previous study we reported a transition from juvenile hyperactivity to adolescent hypoactivity in a single group of monkeys born while their mothers were being exposed to 2.5 ppm Aroclor 1248 (Bowman, R.E., & Heironimus, M.P., Neurobehavioral Toxicology and Teratology, 3:16-18, 1982). There were as yet no indications of such a trend in the current study. The hyperactivity seen in these offspring of PCB- and PBB-exposed mothers was more persistent than hyperactivity we have previously reported in offspring of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-exposed mothers (Schantz, S.L., & Bowman, R.E., The Toxicologist, 3:1(1),68,1983). However, the dose of TCDD was very low (5 ppt in the mother's diet) in the latter study. Supported by NIEHS grant ES0 1847.

124.14 ACUTE EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) ON NEUROMUSCULAR FUNCTION IN RATS. P. A. Bernard*, E. Toyoshima*, C. U. Eccles, R. F. Mayer, K. P. Johnson, and S. R. Max. (SPON:) R. A. Sjodin). Dept. of Neurol., Univ. of Md. Sch. of Med., Dept. of Pharmacol., Univ. of Md. Sch. of Pharm., and V.A. Medical Center, Baltimore, MD 21201.

The herbicide 2,4-D has been reported to cause abnormalities of neuromuscular function in man and animals. To assess this possibility we carried out a multidisciplinary study of the effects of 2,4-D on neuromuscular function in adult male rats (Fisher 344N). We investigated the effects of injections of 2,4-D (dissolved in Emulphor/ethanol/H_2O; 3/2/5, v/v/v) at 200 mg/kg body weight (i.p.) at 3, 15 and 24 h. Controls received vehicle. Spontaneous locomotor activity was monitored for 20-min periods by means of an electronic activity meter employing an 8 x 8 array of infrared photobeams arranged at 2-inch intervals. At 3 h after injection spontaneous locomotor activity was 9% of vehicle injected control values, and it recovered to 50% of control values at 24 h. Acetylcholinesterase (AChE) and choline acetyltransferase (CAT) activities were assessed in sciatic nerve as well as in white quadriceps, red quadriceps, extensor digitorum longus, soleus and diaphragm muscles. At 3 h post-injection AChE was decreased (20%, $p < .05$) in white quadriceps with no change in sciatic nerve or the other muscles. At 15 h AChE was decreased (20%, $p < .05$) in white and red quadriceps and diaphragm with no change in soleus, extensor digitorum longus or sciatic nerve. At 24 h AChE was decreased (20%, $p < .05$) in diaphragm. There was no change in CAT activity at any time point. Electromyographic recordings with a concentric needle electrode in awake animals showed prominent myotonic discharges (repetitive muscle action potentials with waxing and waning features) in leg and buttocks muscles 3 h after 2,4-D injection. Myotonic discharges persisted but were reduced in duration and had less waxing and waning 24 h after injection. These data suggest that 2,4-D causes an acute postjunctional deficit. The studies further suggest that the defect in muscle membranes resulting from 2,4-D may be related to loss of AChE. (Supported by the Research Service of the U.S. Veterans Administration.)

124.15 EVALUATING THE NEUROTOXICITY OF NEW INTRATHECAL CONTRAST MEDIA. L.A. Hayman*, L.C. Abbate*, E.T. Hedley-Whyte, L. Miller*, J. Taveras*. Contrast Media Rsh. Lab., Mass. General Hospital, Boston, MA 02114.

The rapid introduction of a number of new water-soluble intrathecal contrast agents necessitates the development of an effective way to compare the neurotoxicity of these agents. Available testing methods are inadequate. Clinical trials largely measure how successful the medical team is in preventing the medium from reaching the cranial cisterns rather than intrinsic neurotoxicity. Animal models usually measure only very crude indicators of neurotoxicity (i.e., seizures or death). In vitro studies fail to consider important in vivo differences in the CSF clearance and brain absorption of different media.

This report outlines a method for quantifying neurotoxicity by power spectral analysis of electroencephalograms (EEG) obtained after cisternal injection in awake Wistar rats. This method was used to evaluate a 100 µl bolus of ^{3}H-labelled Iopromide, which is a new water soluble myelographic contrast medium. These data were correlated with: 1) the CSF clearance, 2) the CNS concentration, and 3) the regional and cellular CNS location of the Iopromide. Measurements of the last two parameters were done from film and microscopic autoradiographs obtained 4, 12 and 20 hours after intrathecal injection. ^{3}H polyethylene glycol (PEG-900) was given intrathecally to provide control EEG and autoradiographic measurements. Positive and negative chemography controls were obtained for all microscopic autoradiograms.

The results of the film autoradiographs showed striking accumulation of ^{3}H Iopromide within grey matter regions of the CNS with relative sparing of myelinated white matter areas. The same distribution was noted after intrathecal injection of the control compound. Accumulation of the ^{3}H Iopromide within neurons was noted on microscopic autoradiographs done 4 hours after injection. The brain concentration diminished over time. The EEG showed periodic perturbation which began 4 hours after intrathecal injection of ^{3}H Iopromide. These bursts increased in sharpness and intensity between 4-8 hours after injection. They diminished but did not disappear in EEG recordings done 8-20 hours after injection. No EEG abnormalities occurred in the control rats.

124.16 LESIONS OF VENTRAL PALLIDUM ENHANCE BEHAVIORAL RESPONSE TO NICOTINE. C. Ksir and D.M. Benson*. Department of Psychology, University of Wyoming, Laramie, WY 82071.

Lesions made in the region of the ventral pallidum by injection of kainic acid (KA) have been shown to produce decreases in cortical acetylcholine-related enzyme activities and histopathological changes resembling those seen in Alzheimer's disease (Coyle, Price and DeLong, Science, 1983). Before beginning behavioral testing, pilot studies were performed on six rats in order to ensure that similar lesions made in this laboratory would result in decreased staining for acetylcholinesterase in the cerebral cortex.

Eighteen male albino rats were given injections of KA (0.75 ug in 1.0 ul artificial CSF) bilaterally in the region of the ventral pallidum and substantia innominata. Six other rats received similar injections of the artificial CSF vehicle alone (VEH). Eighteen unoperated control rats were also tested (UNOP). One week following surgery, and again three days later, each rat was tested in one of 16 photocell activity cages. After 2 hours of adaptation to the test cages, each rat was given a subcutaneous injection of saline or of 0.1, 0.2 or 0.4 mg/kg nicotine (base weights). Each rat received one injection of saline and one of nicotine, with half the rats receiving saline on the first test day. Six UNOP animals received each dose of nicotine, as did six of the KA animals. The six VEH rats all received 0.2 mg/kg nicotine. Activity was measured by breaks of the photocell beams in each cage for two hours after injection.

Activity levels after saline injections were similar among all groups. KA rats showed significantly greater behavioral activation in response to nicotine than either the UNOP or the VEH controls. The mean number of beam breaks during the first hour after injection of 0.2 mg/kg nicotine was 249.9 for the UNOP, 217.9 for the VEH, and 839.4 for the KA group.

These results suggest that decreased levels of ACh and related enzymes in the cerebral cortex, as is seen in Alzheimer's disease, may be accompanied by a hyperresponsiveness to the behavioral effects of nicotine or other compounds acting at nicotinic receptors.

125.1 EFFECT OF SYSTEMIC AMFONELIC ACID ON CHRONIC INTRACAUDATE INFUSION OF AMPHETAMINE ON ROTATIONAL BEHAVIOR IN THE RAT.
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We have studied the effects of sustained high doses of amphetamine (70 ug/hr) when unilaterally infused into the caudate for 7 days. This paradigm will induce ipsilateral rotational behavior to the side of the chronic treatment following systemic injection of amphetamine (2-5 mg/kg). Furthermore, these systemic amphetamine injections produce ipsilateral rotational behavior in a dose-dependent manner (Dougherty & Ellinwood, 1981). The behavioral changes that occur following chronic amphetamine infusion in the caudate appear to reflect a reduction in dopamine and/or number of terminals.

Recently Steranka (1982) has demonstrated that amfonelic acid (AA) can block the long-term dopamine depleting effects of systemically administered amphetamine with iprindole, which inhibit its metabolism. We tested whether systemic injections of AA could also block the changes in ipsilateral rotational behavior in our chronic intracaudate amphetamine paradigm.

Male Sprague-Dawley rats were implanted with bilateral 25 ga cannulae into the caudate nuclei. Following recovery baseline rotational behavior was measured in each rat, and a preferred direction for turning responses was determined. Rats were divided into three treatment groups: (1) amphetamine alone, (2) amphetamine + AA, and (3) artificial cerebrospinal fluid (CSF). Amphetamine and artificial CSF were continuously infused unilaterally into the caudate for 7 days at a rate of 1 ul/hr through a 32 ga cannula that was attached to an Alzet osmotic pump. On each day of infusion, the amfonelic acid group received a single daily systemic injection (2 mg/kg AA), and the other two groups received control injections. Following pump removal, rats were injected systemically with amphetamine (5 mg/kg) and rated for rotational behavior and for intoxication effects. Four weeks after the last test, rats were sacrificed for determination of striatal dopamine levels using HPLC coupled with electrochemical detection. Dopamine levels in the infused caudate were compared to the contralateral side.

Rats treated with AA showed significantly lower levels of amphetamine-induced rotational behavior than controls. These results are in agreement with Steranka (1982), and demonstrate that systemic AA is capable of blocking the behavioral effects of chronic intracaudate amphetamine infusion.

Dougherty & Ellinwood, Biol Psychiatry, 16, 479-488, 1981.
Steranka, Brain Res, 234, 123-136, 1982.

125.2 PROGRESSIVE ALTERATIONS IN BEHAVIOR DURING TWO DOSE LEVELS OF CONTINUOUS AMPHETAMINE.
G. Ellison, Fredricka Martin*, and Lynn Wilkinson*. Dept. of Psychology, UCLA, Los Angeles CA 90024

Amphetamines, when administered continuously, reliably elicit psychoses in humans. In animals, continuous amphetamines have unique neurotoxic effects in caudate and elicit a "late-stage" of abnormal social and other behaviors.

In order to clarify the stages of behavioral alterations during continuous amphetamines, and the role of motor stereotypies in the elicitation of these effects, rats were observed repeatedly throughout 14 days of very stable levels of drug administration. Two doses of continuous amphetamines were studied: a "high" dose which elicited appreciable motor stereotypies throughout the first 10 days after pellet implantation, and a "low" dose which resulted in minimal or no stereotypies. The animals, and controls, were observed while in their home cage using time-sample procedures. Closed-circuit TV images were scored by blind observers using 26 response categories which were directly fed into minicomputer for subsequent analysis.

The animals administered the high dose were rarely inactive throughout the first ten days following pellet implant. Their motor stereotypies progressed from sniffing the floor of the cage to biting at the floor, as has been reported with repeated injections of amphetamine. These animals showed a consistent suppression of most forms of grooming, such as body washing and scratching, but an increase in grooming of the paws which developed into mild self-mutilation in several rats. Tolerance to the activating and appetite-suppressing properties of amphetamine had developed by the 10th or 11th day after pellet implantation on several measures.

In contrast, the low-dose animals did not enter motor stereotypies, but did show a similar progression from initial sniffing at the ceiling of the cage, in brief but frequent bouts, to biting at the ceiling of the cage. Thus, motor stereotypies are not essential for this progression from sniffing to biting. The low dose animals also showed increased body shakes and limb-flicks during the later stages of continuous amphetamines, whereas these behaviors were actually suppressed in the high dose animals throughout pellet action.

Motor stereotypies thus may have an active role in suppressing or masking "late-stage" behaviors.

125.3 AGGREGATION INCREASES LONG-LASTING SEROTONIN DEPLETION INDUCED BY d-METHAMPHETAMINE.
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While there is considerable evidence that aggregation enhances the lethal as well as the short-term monoamine-depleting effects of amphetamines (Moore, K.E., J. Pharmacol. 142:6, 1963; Lokiec et al., Eur. J. Pharmacol. 44:391, 1977), there is a paucity of data on whether aggregation and other environmental variables influence the recently discovered long-term, apparently toxic, effects of amphetamines on brain monoamine-containing neurons. The purpose of this study was to determine whether aggregation increased the toxic effect of d-methamphetamine (METH) on brain serotonin (5HT) and dopamine (DA) neurons. Four groups of male albino rats weighing approximately 250 g were used: saline-isolates, saline-aggregates, METH-isolates and METH-aggregates. Isolated rats were housed in suspended wire-mesh cages measuring 7.5 x 9.5 x 7". Aggregate rats were housed 12 per cage in similar but wider cages measuring 25 x 9.5 x 7". Rats administered METH received the drug subcutaneously at a dose of 15 mg/kg every 6 hours for 24 hours. Control rats were injected with saline. Aggregation was begun just prior to the first injection and terminated 6 hours after the last injection. Aggregated rats that died during the drug regimen were replaced with untreated rats. At all other times during the experiment, all rats were housed singly with ad libitum access to food and water. Ambient temperature was 22 ± 1°C. Two weeks after the last injection, 8 rats were randomly selected from the surviving rats in each of the above-mentioned groups and killed for hippocampal 5HT assay. 5HT was measured by cation-exchange liquid chromatography coupled with electrochemical detection. Aggregation increased the lethal effect of METH. Mortality in the METH-isolate group was 10% (1/10) whereas in the METH-aggregate group it was 25% (6/24). Aggregation also increased the long-lasting 5HT depleting action of METH. Hippocampal 5HT was decreased by 51% in the METH-isolated group, whereas in the METH-aggregate group it was decreased by 78%. This difference was significant at the 0.01 level. Aggregation per se did not lower 5HT level. These results indicate that environmental factors such as aggregation can enhance the toxic effect of amphetamines on central 5HT neurons. Whether aggregation also enhances the DA neurotoxic effect of amphetamines is currently being examined. Finally, the mechanisms underlying this effect are also under investigation. G.A.R. supported by USPHS Training Grant (Pathobiology) GM-07190; L.S.S. and C.R.S. by DA-00085 and DA-00250; P. Malpas, MH-14274, Training Grant, L.S.S.: RSA MH-10562.

125.4 EEG STUDIES DURING TOXIC LEVELS OF AMPHETAMINE.
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These studies are carried out on 12 male and female cats using hardwire and telemetry. Chronic electrodes are placed in the basal amygdala, nucleus accumbens and anterior sigmoid gyrus. Repeated doses of d- and l-amphetamine showed symptoms of moderate intoxication. Symptoms associated with these doseslevels are: tachycardia, hypertension, hyperthermia, dilated pupils, muscular dyskinesia stereotypy, rapid shallow breathing, vomiting, diarrhea, and urinary retention. Hallucinatory-like behavior and confusion was apparent on the second and third days of amphetamine.

Analog data is collected on FM tape and recordings are made on a polygraph. The data is analog to digitally converted and processed on a V-72 Varian minicomputer. Power spectral, cross correlation and coherence spectral analyses are used. Changes in the electrical activity of the amygdala, nucleus accumbens and anterior sigmoid gyrus showed desynchronization at 1 to 44 Hz with mild toxic levels of amphetamine. At moderate toxic levels of amphetamine, there is increased synchronization above 40 Hz up to 100 Hz, with increased coherence at these frequencies. There is an increase in coherence above 250 Hz on the second to fifth day of amphetamine. In some cats this is related to confusion and hallucinatory-like behavior.

125.5 DOPAMINERGIC AND NEOSTRIATAL NEURONS: DOSE-DEPENDENT CHANGES IN SENSITIVITY TO AMPHETAMINE FOLLOWING LONG-TERM TREATMENT. Katsuo Kamata* and George V. Rebec (SPON: W.D. Neff). Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Certain components of the behavioral response to amphetamine are enhanced with repeated injections. This augmentation may reflect, in part, a subsensitivity of autoreceptors on dopaminergic (DA) neurons in the substantia nigra pars compacta (SNC). According to this view, a decrease in autoreceptor sensitivity would mean less inhibition of DA neurons and, thus, an enhanced effect on postsynaptic neurons in the neostriatum (Muller, P. and Seeman, P., Eur. J. Pharmacol., 55:149, 1979). Recordings of amphetamine-induced changes in SNC activity, however, have produced conflicting findings. Thus, whereas a reduction in the sensitivity of inhibitory autoreceptors has been reported following long-term treatment with 4.0 mg/kg (Antelman, S.M. & Chiodo, L.A., Biol. Psychiat., 16:717, 1981), other investigators (Staunton, D.A. et al., Brain Res., 188:107, 1980) found no differences in the responsiveness of DA neurons following pretreatment with either saline or with even higher doses of amphetamine. To resolve this issue, we recorded neuronal activity simultaneously from postsynaptic neurons in the neostriatum and from DA neurons in the SNC following long-term treatment with saline or with a wide range of amphetamine doses.

Adult, male rats were pretreated with saline, 1.0 or 5.0 mg/kg d-amphetamine twice daily for 6 consecutive days. Single-unit recording on the following day revealed a differential shift in the iv dose of the drug required to produce at least a 50% change in neuronal activity (ED50) depending on the pretreatment dose. In the SNC, pretreatment with the low dose significantly reduced the ED50 compared to saline controls, whereas in rats pretreated with the high dose the ED50 was significantly increased. Moreover, in the high-dose group an iv challenge injection of 0.25 mg/kg d-amphetamine accelerated SNC activity--an effect that was never observed in control animals. In the neostriatum, control rats responded to increasing incremental doses of d-amphetamine with either an inhibition followed by an excitation or an excitation. Following pretreatment with 1.0 mg/kg neurons responded with either a depression or an excitation, whereas only amphetamine-induced excitations were recorded in the neostriatum following pretreatment with 5.0 mg/kg. Thus, although the changes in firing rate produced by repeated exposure to amphetamine are dose-dependent in both sites, a decrease in autoreceptor sensitivity may explain our results in the SNC following long-term treatment with high amphetamine doses.

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125.6 AMPHETAMINE TOLERANCE AND BODY WEIGHT SET POINT: A DOSE RESPONSE ANALYSIS. D. L. Wolgin and J. Salisbury*. Psychology Dept., Florida Atlantic University, Boca Raton, FL 33431.

Rats given amphetamine and access to food initially eat very little. However, if the drug is given chronically, intake gradually recovers. Such recovery is generally thought to reflect tolerance to the drug. Recently, Stunkard (Life Sci., 1982, 30, 2043) has proposed that amphetamine lowers a set point for body weight regulation, and that tolerance does not develop to this effect. According to this view, the decrease and subsequent increase in food intake during chronic amphetamine administration are secondary to achieving, and then maintaining, a lower weight level. To further evaluate this hypothesis, we examined the effect of long-term exposure to different doses of amphetamine on food intake and body weight.

Rats were given 0, 2, or 4 mg/kg of d-amphetamine sulfate 20 min before daily access to sweetened milk, which was available for 30 min. Both drugged groups were initially anorexic and lost weight. While milk intakes recovered to control levels on Days 11 and 18, respectively, body weights remained below the level of controls throughout the 48 days of drug administration. Rats given 2 mg/kg maintained their weights at 94-96% of controls. Rats given 4 mg/kg maintained their weights at 86-88% of controls for 15 days, but then increased their intakes and gradually gained weight to 94% of controls by Day 48. Thus, at the 4 mg/kg dose, partial tolerance developed to the initial weight suppressant effect of amphetamine.

Drug doses were then increased to 4 and 6 mg/kg, respectively, for an additional 63-74 days. Intense stereotyped movements developed in both groups of rats during this period. Milk intake was suppressed in both groups during the first month, and then recovered during the second month, although control levels of intake were not consistently maintained. Body weights in both groups dropped to as low as 76%, but were generally maintained between 78-82% of control levels. However, for individual rats, milk intake during this period of maintained body weight was extremely variable from day to day, particularly in the group given 6 mg/kg. These large fluctuations in intake, along with the confounding effects of drug-induced stereotypy, challenge any simple interpretation of the data in terms of a lowered set point for body weight.

125.7 FOREBRAIN DOPAMINE BLOCKADE DISAPPEARS DURING 6 MONTHS OF NEUROLEPTIC ADMINISTRATION. K.J. Pittman*, A. Jakubovic, and H.C. Fibiger (SPON: E.G. McGeer). Div. of Neurological Sciences, Univ.of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Although the antipsychotic properties of neuroleptic drugs have been attributed to their capacity to block dopaminergic transmission, recent studies have shown that behavioral and biochemical indices of DA blockade within the striatum disappear after approximately 6 months of continuous neuroleptic administration, followed in some instances by a supersensitive condition. The present study sought to determine whether a similar situation applies to terminal regions of the mesocortical/mesolimbic dopamine projections; whether the rate and degree of tolerance is dependent upon the type of neuroleptic administered; and whether prolonged exposure to neuroleptics affect striatal choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) activities.

Male Long-Evans rats received cis-flupenthixol (FLU) (.8-1.0 mg/kg/day) or trifluperazine (TRI) (2.0-3.0 mg/kg/day) for 1 week, 1 month, 3 months, or 6 months and were compared to control animals on measures of spontaneous locomotion, amphetamine-induced locomotion, and apomorphine-induced stereotypies. The levels of DA, DOPAC, HVA, 5-HT, and HIAA in the striatum, nucleus accumbens, olfactory tubercles, and medial frontal cortex were measured by HPLC.

Although behavioral responses were attenuated to a similar degree following one week of either drug, complete tolerance appeared by 3 to 6 months on all measures. Furthermore, animals treated with TRI showed a supersensitive response to the locomotor-inducing effects of amphetamine as early as 1 month which persisted to the end of the study. The neuroleptics caused a significant elevation in the levels of DOPAC and HVA in all brain regions after one week but this disappeared by 3-6 months. Concentrations of DA, 5-HT, and HIAA were not consistently altered at any time in any area. Striatal GAD and CAT activities were not influenced by 1 week or 6 month administration of either neuroleptic.

These results suggest functional measures of dopamine receptor blockade in the rat forebrain disappear in the course of 6 months administration of neuroleptic drugs. As these findings contrast markedly with the long-term antipsychotic efficacy of these compounds, a reassessment of theories concerning their mode of action may be warranted. The augmented response to amphetamine of animals treated with the phenothiazine drug TRI suggests that this class of neuroleptic may more easily elicit dyskinetic symptoms than thioxanthene drugs such as FLU.

Supported by grants from MRC and AHFMR.

125.8 SENSITIZATION OF NEURONS IN THE AMYGDALOID COMPLEX TO CLOZAPINE, BUT NOT HALOPERIDOL, WITH LONG-TERM TREATMENT. Glenn D. Anderson* and George V. Rebec (SPON: T.R. Bashore). Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

We have previously shown that, unlike the neostriatum and the nucleus accumbens, the amygdaloid complex is differentially responsive to cataleptogenic and non-cataleptogenic antipsychotic drugs (Rebec, G.V. et al., Pharmacol. Biochem. Behav., 14:49, 1981). Haloperidol, for example, exerts little if any effect on amygdaloid activity even at doses that produce intense catalepsy, whereas clozapine, which fails to produce this response, typically accelerates firing rate in this site. Because these drugs exert their greatest clinical effects with long-term use, we extended our investigation of the amygdaloid complex to chronically treated rats.

After receiving 2 daily injections of saline, 1.0 mg/kg haloperidol, or 10.0 mg/kg clozapine for 6 consecutive days, rats were prepared for single-unit recording on the following day and challenged (ip) with either haloperidol or clozapine. Consistent with previous evidence, clozapine, but not haloperidol, accelerated amygdaloid activity in saline controls. Thus, whereas haloperidol failed to change firing rate in the amygdaloid complex, clozapine accelerated activity to approximately 200% of the preinjection baseline rate. Following chronic treatment, this difference became even more pronounced. Clozapine, for example, routinely increased firing rate to beyond 700% but haloperidol, as in saline controls, was largely without effect. In fact, haloperidol failed to alter amygdaloid activity in separate groups of rats pretreated with the drug for as long as 13 days. These results suggest that whereas amygdaloid neurons remain unresponsive to haloperidol even after long-term treatment, they become progressively more responsive to repeated injections of clozapine.

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125.9 APOMORPHINE-INDUCED INHIBITION OF NEOSTRIATAL ACTIVITY IS ENHANCED BY 6-HYDROXYDOPAMINE LESIONS BUT NOT BY LONG-TERM AMPHETAMINE ADMINISTRATION. Kevin D. Alloway and George V. Rebec. Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

It has been suggested that the behavioral augmentation produced by long-term amphetamine treatment is mediated, at least in part, by an increase in the sensitivity of postsynaptic dopamine (DA) receptors (Klawans, H. & Margolin, D., Arch. Gen. Psychiat., 32:725, 1975). We tested this hypothesis by recording the effects of increasing incremental doses of apomorphine, a DA receptor agonist, on single-unit activity in the neostriatum of locally anesthetized, immobilized rats that were pretreated twice daily with saline, 1.0, 5.0, or 10.0 mg/kg d-amphetamine for 6 consecutive days. In addition, because depletion of neostriatal DA increases DA receptor sensitivity (Creese, I. et al., Science, 197:596, 1977), we also included a group of rats that sustained unilateral 6-hydroxydopamine (6-OHDA) lesions 2 weeks prior to single-unit recording.

Examination of the dose-response curve for neurons inhibited by apomorphine (0.0025 to 0.32 mg/kg, iv) revealed that 6-OHDA, but not long-term amphetamine administration, increased the sensitivity of postsynaptic DA receptors. Thus, compared to saline controls, the median effective dose for producing a 50% inhibition of firing rate was significantly reduced in the neostriatum of 6-OHDA-lesioned rats but not in rats pretreated with amphetamine. In fact, whereas all the ipsilateral neurons sampled from rats with a 6-OHDA lesion were inhibited by apomorphine, this response was recorded in only 62% of control neurons and only in 44 to 62% of neurons from the 3 groups of rats pretreated with amphetamine. Moreover, neostriatal activity on the lesioned side was depressed to a greater extent than in control rats for apomorphine doses ranging from 0.01 to 0.08 mg/kg. Unexpectedly, neurons from the intact side of 6-OHDA rats also responded to apomorphine (0.005 to 0.02 mg/kg) with a greater decrease in firing rate than in saline controls. The response of amphetamine-pretreated rats, on the other hand, paralleled that of control animals. It appears, therefore, that long-term amphetamine administration produces an augmentation of behavior by some mechanism other than an increase in the sensitivity of postsynaptic DA receptors.

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125.10 BEHAVIORAL EFFECTS OF DRUG HOLIDAYS IN CHRONIC HALOPERIDOL ADMINISTRATION. W.W. Sant III, R. Cruz*, M. Morgan*, and G. Ellison. Dept. Psychology, UCLA, Los Angeles, Ca. 90024.

A number of clinicians have suggested that periodic drug-free intervals, drug holidays, in neuroleptic administration may decrease the incidence of tardive dyskinesia (TD) in general, or of persistent TD in particular. One retrospective clinical study found a positive relationship between drug holidays and persistent TD. However, this study also reported a positive relationship between number of drug holidays and length of neuroleptic administration. We undertook an examination of the effect of drug holidays on the behavioral effects of chronic haloperidol administration in rats while controlling for total exposure to haloperidol.

Two groups of female, Sprague-Dawley albino rats received haloperidol and lactic acid in their drinking water for 12 weeks. In one group haloperidol administration was uninterrupted, while in the other group three evenly spaced, one-week-long drug-free intervals were given. A control group of animals received lactic acid in their water throughout the experiment.

Animals receiving haloperidol had a mean daily intake of about 0.8 mg/kg of the drug and showed a gradual increase in body weight while control animals did not. Daily home-cage activity was dramatically depressed to about 25% of control levels on the first day of haloperidol administration and remained unchanged for the following three weeks of drug administration. Indeed, there was no attenuation of haloperidol's depression of home-cage activity after 9 weeks of either interrupted or uninterrupted haloperidol administration. During the first drug holiday, after three weeks of haloperidol administration, activity levels rose to control levels within two days and continued to increase, peaking on the fifth day of the holiday and then returning to control levels on days 6 and 7. Reinstitution of haloperidol administration caused an immediate depression of activity to previous haloperidol levels.

After final discontinuation of haloperidol administration an increase in oral movements and in home-cage activity was observed. The time-course of these increases differed between rats that had received drug holidays and those that had not, indicating that drug administration regimen can alter the time-course of behaviors seen after neuroleptic withdrawal.

(Haloperidol was generously supplied by McNeil Pharmaceuticals.)

125.11 ROLE OF ANXIETY IN MEDIATING CHLORDIAZEPOXIDE-INDUCED REDUCTION OF CORTICOSTERONE RESPONSE TO STRESS. G.B. Freeman and J.B. Thurmond. Neuropsychopharmacology Lab.,University of Louisville, Louisville, KY 40292.

Anti-anxiety drugs are effective in reducing stress-induced increases in corticosterone (CS) and emotional behavior in the mouse. It was proposed whether chlordiazepoxide (CDP) has a direct effect on the CS response or whether this drug indirectly affects CS levels by decreasing an animal's emotional responsiveness (anxiety) to acute noise stress. The role of the monoamines in CS response regulation and the mediation of emotional behavior in response to stress was investigated by combining agonist drug treatment (quipazine, apomorphine, clonidine) with acute CDP (5, 10, 20, 40 mg/kg).

The effects of stress did not produce a behavioral profile consistent with a model of anxiety. Exploration and locomotor activity were increased by stress. This activation enhanced the stimulatory effect of low doses of CDP and attenuated the depressant action of higher doses. Quipazine reduced the depressant effects of CDP in stressed animals. Its action failed to support an anti-serotonergic action of CDP. Clonidine antagonized the stimulatory action of low doses of CDP and provided support for the notion that the stimulatory effect of CDP may be due to enhanced catecholamine neurotransmission, especially NE.

Exposure to stress produced a two-fold increase in CS which was reduced by 5 mg/kg of CDP. An inverse relationship between behavior and CS was demonstrated for nonstressed mice. The depressant action of CDP in nonstressed mice may have stimulated the hypothalamic-pituitary system. The lack of a strong relationship between CS and behavior in stressed mice suggested that postulating anxiety as a theoretical intervening variable between the effect of the benzodiazepines and the modulation of the CS response may not be justified. Chlordiazepoxide may have affected the CS response in some other way than by reducing an animal's emotional responsiveness to stress. Alternatively, increases in CS levels, rather than behavioral performance, may be a more suitable index of the emotional responsiveness of an animal. Subsequent reduction of this response with low doses of CDP may be a more appropriate indication of the anxiolytic effect of the benzodiazepines.

Changes in CS resulting from agonist treatment indicated that the mechanisms controlling basal CS levels may be different and separate from those mediating the CS response to stress.

125.12 Ro15-1788 PARTIALLY BLOCKS DEVELOPMENT OF CHLORDIAZEPOXIDE PHYSICAL DEPENDENCE IN THE RAT. J.J. Guarino*, M.H. Gay*, N.R. Boisse, H.S. Kruger* and R.M. Periana*. Section of Pharmacology, Northeastern University, Boston, MA 02115.

Ro15-1788 (R/A) is a selective antagonist of the CNS effects of classical anxiolytic-sedative-hypnotic benzodiazepines (BNZs). R/A antagonizes BNZ actions by displacing BNZs from their receptors and is relatively devoid of other pharmacologic activity. Previously several laboratories including ours (Neurosci. Abstr., 396, 1982) have demonstrated that R/A can precipitate an acute withdrawal (W/D) reaction in several species of lab animals chronically treated with various BNZs. These studies support the concept that the displacement of active BNZs from the receptor triggers the expression of W/D after dependence has been induced. To further characterize the involvement of BNZ receptors in dependence, we have studied the effects of daily R/A administration during the induction of BNZ dependence. Dependence was induced by intragastrically administered chlordiazepoxide HCl (CDP), 75 mg/kg, bid for 5 weeks (chronic CDP group). The chronic CDP + R/A group received 25 mg/kg of R/A, p.o., 2 hours after each morning dose of CDP. Water controls (chronic H_2O) received equivalent volumes of H_2O, i.g., bid for 5 weeks. Chronic R/A controls (chronic H_2O + R/A) received 25 mg/kg R/A 2 hours after each morning dose of H_2O. On the last day of chronic treatment, all animals received R/A 25 mg/kg, i.p., 4 hours after the last a.m. CDP or H_2O dose. Precipitated W/D was measured by 4 or 5 independent experienced observers who rated the intensity of 20 W/D signs on a scale of 0 to 3. The WD score is the mean summed rating. The W/D syndrome was qualitatively similar but less severe for chronic CDP + R/A compared to chronic CDP. R/A precipitated 12.7 ($\pm$ 1.0 S.E.) and 14.8 ($\pm$ 0.6 S.E.) signs/rat for chronic CDP + R/A and chronic CDP, respectively. Peak WD scores were 16.6 ($\pm$ 1.7 S.E.) and 24.3 ($\pm$ 1.1 S.E.), respectively. The mean peak intensity of 9 individual graded signs was significantly less for CDP + R/A compared to the CDP group. Chronic H_2O and chronic H_2O + R/A controls showed negligible W/D manifestations. This study demonstrates that chronic co-administration of R/A and CDP attenuates the precipitated W/D and supports the involvement of BNZ receptors in both induction and expression of physical dependence. The incompleteness of dependence blockade in this study may reflect the extremely short half-life of R/A in the rat which provided only intermittent as opposed to continuous receptor blockade. Additional nonreceptor specific mechanisms may also contribute to dependence induction.

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125.13 EFFECT OF PROLYL-LEUCYL-GLYCINAMIDE (PLG) ON 17β-ESTRADIOL (E_2) SUPERSENSITIVITY OF DOPAMINE (DA) RECEPTORS. G. Rajakumar*, P. Chiu*, and R.K. Mishra*(SPON: C. R. Merril). Dept. Neurosciences, 4N52, Faculty of Health Sciences, McMaster Univ., Hamilton, Ontario Canada.

Estrogen administration to male rats has been shown to increase the density of striatal DA receptors without changing their affinity. This has been documented both from behavioural and direct binding studies. The effect of estrogen on DA receptor has potential clinical implications in view of the findings that chorea is associated with pregnancy or the use of oral contraceptives. It is also claimed that ovarectomy of guinea pigs decreased stereotypy and in rats decreased DA stimulated adenylate cyclase activity, but administration of estrogen reversed both these effects.

Earlier studies from this laboratory had shown that PLG suppressed the supersensitized DA receptors induced by neuroleptics. We now report the results of PLG administration on estrogen induced supersensitivity in male rats.

17β-Estradiol was administered(S.C.)20 µg/kg-5 days followed by 60 µg/kg-5 days to induce supersensitivity. PLG 20 mg/kg(S.C.) was administered prior to and concurrently with estradiol administration to separate groups of rats (6 in each group). Biochemical characterization (K_d & B_{max}) of striatal DA receptors was carried out by direct binding studies with ^{3}H-spiroperidol and 1 µm (d) butaclamol as the displacing agent.

E_2 administration produced 205% of control B_{max} of DA receptors suggesting the development of supersensitivy. While pre-treatment and concurrent treatment of PLG produced 69% and 92% of control B_{max} respectively.

	B_{max} f moles/mg protein^{-1}	%
Control	140	100
20 µg-5 days + Estrogen 60 µg-5 days	288	205
PLG pretreated (20 mg/kg-5 days)	97	69
PLG concurrent (20 mg/kg)	130	93

K_d values were identical in all cases.

Our study demonstrates that PLG could prevent and suppress the supersensitized DA receptors by estradiol.

(Supported by Ontario Mental Health Foundation and Parkinson Foundation of Canada)

PSYCHOTHERAPEUTIC DRUGS: ANTIDEPRESSANTS

126.1 THE AMYGDALA IS A SITE OF ACTION OF IMIPRAMINE IN A SCREEN FOR ANTIDEPRESSANT AGENTS. G.E.Duncan*, G.R.Breese and W.E.Stumpf. Depts. of Anatomy, Psychiatry and Pharmacology and the Biol.Sci. Res.Ctr., Univ. of North Carolina, Chapel Hill, NC 27514.

Many brain regions contain specific receptor-like binding sites for imipramine (IMP), (Palkovits et al. Br.Res., 210:493,1981). However, IMP's therapeutic actions and behavioral effects in animal models of depression may result from the interaction of the drug with specific neuroanatomical systems, rather than in all areas of the CNS that contain IMP receptors. In order to determine if the action of IMP can be localized to discrete areas of the CNS, IMP was infused into different brain regions of rats and tested using a behavioral model of depression (Porsolt et al., Eur.J.Pharm.47:379,1978). The Porsolt test is a "learned helplessness" paradigm in which rats are forced to swim in a cylinder containing water (25°C) on two days. The first day, rats are placed in the cylinder for 15 min. After approximately 10 min the rats become immobile and float in the water. On the second day, the rats are placed in the cylinder for a 5 min period. After one to two min of activity, saline-treated rats become immobile and float in the water. Antidepressant drugs selectively and markedly reduce the amount of time that rats are immobile during this 5 min period even though the drugs decrease open field activity (Porsolt,et al., 1978).

In the present study all rats were given 15 mg/kg IMP i.p.after the 15 min swim. On the second day of the test, IMP (0.1 to 10 ug) dissolved in 0.5 ul of 0.9% NaCl or 0.5 ul of 0.9% NaCl, was infused bilaterally into the central-lateral amygdala over a 5 min period. Three min after the termination of the infusion, the rats were placed in water and the amount of time that they were immobile was measured. The mean immobility (± SEM) for rats infused with saline was 216 ± 7 sec. Bilateral infusion of 1 ug of IMP into the amygdala produce a maximal reduction of immobility (106 ± 19 sec). The mean immobility for rats that received i.p. injections of saline and 15 mg/kg of IMP were, respectively, 194 ± 22 and 102 ± 10 sec. The infusion of 1 ug of IMP into the caudate nucleus (2 mm dorsal to the site of the amygdala infusions) produced no effect in the Porsolt test. To determine if the anticholinergic properties of IMP were responsible for the observed effects in the test, 1 ug of atropine was injected into the central-lateral amygdala. In contrast to IMP, atropine produced no significant reduction in the duration of immobility. Therefore, the present results suggest that the amygdala is involved in the action of IMP to suppress immobility in the Porsolt test.

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126.2 ANALGESIC PROPERTIES OF MJ 13754, A NONTRICYCLIC ANTIDEPRESSANT CANDIDATE. D. W. Smith*, M. S. Eison, L. A. Riblet, D. P. Taylor, J. P. Yevich, and D. L. Temple, Jr. CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville, IN 47721.

Recently we identified[1] MJ 13754, known chemically as 2-(3-[4(3-chlorophenyl)-1-piperazinyl]propyl)-5-ethyl-2, 4-dihydro-4-(2-phenoxyethyl)-3-1,2,4-triazol-3-one, as a nontricyclic antidepressant candidate exhibiting a selective serotonergic mechanism of action. MJ 13754 was found to protect against reserpine-induced ptosis in mice, thus suggesting antidepressant activity. MJ 13754 was further distinguished by in vitro receptor binding data. Adrenergic (α_1, α_2, and β), cholinergic (muscarinic), and histaminic receptor affinities were lacking. However, MJ 13754 was found to bind with high affinity in vitro to the serotonin S_2 receptor (IC_{50} = 39 nM).

Because many tricyclic antidepressants (TCA) enjoy analgesic properties, we have evaluated the antinociceptive properties of MJ 13754. Two models for analgesia were employed: phenylquinone induced writhing (PQW) in mice and shock-induced vocalization in the rat. The potency of MJ 13754 was compared with the following TCAs: amitriptyline, clomipramine, desimipramine, doxepin, and imipramine. Also included was the nontricyclic antidepressant trazodone; and the simple piperazines m-chlorophenylpiperazine (MCPP) and m-trifluomethylphenylpiperazine (MTFPP); and MJ 14858 (2-(3-[4-(3-trifluoromethylphenyl)-1-piperazinyl]propyl)-5-ethyl-2,4-dihydro-4-(2-phenoxyethyl)-3H-1,2,4-triazol-3-one). Of the TCAs examined, only amitriptyline was more potent than MJ 13754, MJ 14858, or trazodone (ED_{50}s = 57, 113, 53 µmole/kg, respectively, p.o.) in the PQW assay. In the vocalization assay using identical doses, MJ 13754, MJ 14858, and trazodone induced vocalization thresholds (VT_{50} = 0.42, 0.55, 0.38 mA, respectively) greater than all TCAs examined. Both piperazines, MCPP and MTFPP exhibited some antinociceptive activity. None of the aforementioned compounds were found to inhibit [^{3}H]diprenorphine binding in rat midbrain homogenate (IC_{50} values > 20 µM), suggesting a non-opiod mechanism. Rotarod failure and inhibition of spontaneous motor activity were used to assess drug-induced motor deficits. MJ 13754 and MJ 14858 were found to be less sedating than the above compounds which exhibited analgesia. These data strongly suggest that MJ 13754, in addition to its antidepressant profile, may possess an analgesic component. This analgesia may be attributed to centrally-enhanced serotonergic transmission.

[1] Taylor, D. P., Eison, M. S., Riblet, L. A., Smith, D. W., Temple, D. L., Yevich, J. P. Soc. Neurosci. Abst. 1982, 8, 465.

126.3 THE PREDICTION OF THERAPEUTIC NORTRIPTYLINE DOSAGE REGIMES AND RELATED PLASMA CONCENTRATIONS OF HYDROXYLATED METABOLITES IN GERIATRIC DEPRESSIVES. A. L. C. Pottash[1], D. M. Martin*[1], I. Extein[2], F. Mas[3], A. H. Jarvis*[3], R. G. Zirk*[1] and M. S. Gold[1]. Psychiatric Diagnostic Laboratories of America, Summit, N.J. 07901[1], Falkirk Hosptial, Central Valley, N.Y. 10917[2] and Gracie Square Hospital, New York, N.Y. 10021[3].

Nortriptyline (NOR) is one of the most widely used tricyclic antidepressants in the management of endogenous depression. This is due in part to the extensive documentation and utility of a plasma therapeutic window (50-140 ng/ml)[1] and single dose prediction test.[2] These measures allow physicians to rapidly achieve and maintain patients at therapeutic blood levels for 21 consecutive days which provides an objective, operationally defined antidepressant trial. Yet, during chronic NOR therapy plasma levels of NOR vary greatly between individuals on identical dosage regimes and a small group of patients within the therapeutic window have minimal clinical response. 10-hydroxynortriptyline (10-OH NOR), a biologically active metabolite of NOR, also varies as greatly if not more than NOR. 10-OH NOR metabolism can be responsible for interindividual variation of NOR levels as well as elevating active plasma levels above the therapeutic range. 10-OH-NOR is primarily cleared from the plasma compartment by renal excretion which can be greatly decreased in geriatric populations. This can result in accumulations of 10-OH-NOR and may be an important compound to therapeutically monitor and include in geriatric dose prediction studies.

An inpatient population of geriatric depressives (N=20) having a mean age of 63 years was studied over two months. They were maintained on varying doses of NOR as determined by a single 50 mg loading dose and subsequent plasma level obtained 24 hours later. Plasma levels[3] and clinical depression rating scales were obtained weekly. In a preliminary analysis it was noted that in all patients the presence of significant amounts of 10-OH-NOR 252 ± 131 ng/ml existed after steady state mean NOR concentrations 119 ± 38 ng/ml were attained. This observed concentration of 10-OH NOR was significantly higher ($p<.001$) than concentrations noted in an adult population (N=20) mean age 44, with therapeutic levels 128 ± 22 ng/ml of NOR and 110 ± 70 ng/ml of 10-OH-NOR. The incorporation of 10-OH-NOR into loading dose plasma analysis indicated patients who hypometabolized NOR 10-OH-NOR. It is this group of patients that yielded the highest 10-OH-NOR levels once therapeutic NOR plasma concentrations were attained. These and other clinical data will be presented by the authors.

1) Psychopharmacology 77:193-197, 1982.
2) Brit J Psych 139:413-417, 1981.
3) Neuroscience Abstract 7:644, 1981.

126.4 INTERRELATIONSHIPS BETWEEN ADAPTATION TO STRESS AND ANTIDEPRESSANT TREATMENT. J.E. Platt, R. Trullas*, A.V. Slucky*, and E.A. Stone. Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016

Repeated stress and chronic antidepressant treatment have been shown to have similar effects on noradrenergic receptor function in rat brain in that both lead to reduced beta adrenergic receptor density and a decreased cAMP response to norepinephrine. Repeated restraint stress has also been shown to induce a positive response on a behavioral screening test for antidepressants. The present studies examined further the interrelations between adaptation to stress and antidepressant treatment.

The first study was designed to test if pretreatment with antidepressants could mimic the effects of adaptation and thereby reduce the adverse effects of stress. Rats were treated with DMI according to a decending dose schedule (10mg/kg b.i.d. - 7.5mg/kg once daily). Restraint stress (5 h) was administered after 14 and 21 days of DMI. Adverse reactions to stress (anorexia, gastric ulcers and plasma corticosterone elevation) were assessed after the first and second stress sessions.

DMI-treated rats showed a significantly smaller decrease in food intake following acute stress (first stress session) than controls (14,47% ± 0.63% vs. 38.83% ± 1.3%, $p<.0001$). They also had significantly fewer ulcers (second stress session) (0.63 ± 0.42 vs. 2.00 ± 0.65, $p<.05$, one tailed) and were more likely to be ulcer-free (Fisher Exact Test, $p = .067$). Plasma corticosterone levels are currently being analyzed.

The second study was the converse of the first and was designed to determine if pretreatment with chronic stress (adaptation) could mimic the effects of antidepressants on a pharmacological screening test (attenuation of clonidine (100µg/kg) or reserpine (5mg/kg) hypothermia). Rats were treated for 10 days with restraint stress (2.5 h per day) or with DMI (10mg/kg i.p.). Tests were conducted 24 hours after the last stress or injection.

Stressed rats showed significantly less clonidine-hypothermia than controls at 60 ($p<.05$, one tailed), 90 and 120 min. ($p<.025$ one tailed) post-clonidine and were similar to DMI-treated animals ($p<.025$ vs. controls, one tailed, at all time points). The reserpine test is currently under investigation.

These results indicate further parallels between adaptation to stress and antidepressant therapy involving measures of resistance to stress and of preclinical antidepressant activity. They support the hypothesis that antidepressant therapy mimics the central neurochemical changes that occur during adaptation to chronic stress and that this common neurochemical alteration (noradrenergic or other) underlies the observed behavioral and physiological similarities. (Supported in part by grants MH 22768 and MH 08618 and CIRIT predoct. training grant to R.T.)

126.5 ENHANCEMENT OF SEROTONINERGIC NEUROTRANSMISSION BY SHORT-TERM LITHIUM TREATMENT: ELECTROPHYSIOLOGICAL STUDIES IN THE RAT. P. Blier and C. de Montigny, Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada H3C 3T8.

Long-term tricyclic antidepressant (TCA) drug treatment increases the response of forebrain neurons to microiontophoretically-applied serotonin (5-HT) in the rat. Lithium (Li) addition to the regimen of most TCA-refractory patients brings within 48 h a marked reduction of the depressive syndrome. It has been postulated that the rapid antidepressant effect of Li in TCA-refractory patients might be due to the enhancement of the activity of 5-HT neurons by lithium. The present studies were undertaken to assess electrophysiologically the effect of Li on 5-HT neurotransmission.

Male Sprague-Dawley rats (250-275 g) were fed with regular chow or chow containing 0.2% Li carbonate for 48 h producing plasma Li concentrations of 0.4#1.0 mEq/L. Three rats were pretreated with 5,7-dihydroxytryptamine (5,7-DHT) (200 µg, free base, intraventricularly) one h after desipramine (25 mg/kg, i.p.) and four weeks before Li treatment.

In a first series of experiments, five-barrelled micropipettes were used to record from CA_3 hippocampal pyramidal neurons under chloral hydrate anesthesia (400 mg/kg, i.p.). The response of these neurons to the electrical stimulation of the ventromedial ascending 5-HT pathway was determined from peristimulus time histograms and their responsiveness to microiontophoretic applications of 5-HT creatinine sulfate (0.5 mM in 0.2 M NaCl, pH: 4) and norepinephrine bitartrate (NE) (0.05 M, pH: 4) was estimated using the $I.T_{50}$ method (current x time required to obtain a 50% decrease of firing rate). The responsiveness to 5-HT and NE was not modified by the Li treatment whereas the effect of the electrical stimulation of the ascending 5-HT pathway was increased. The same stimulation failed to produce any suppression of the firing in the 5,7-DHT-pretreated animals.

In a second series, unitary recordings of 5-HT neurons were obtained from the mesencephalic dorsal raphe nucleus. Lithium pretreatment did not modify the firing rate of 5-HT neurons.

In conclusion, short-term Li modifies neither the responsiveness of postsynaptic neurons to 5-HT nor the electrical activity of 5-HT neurons but enhances the efficacy of the ascending 5-HT system. This effect of Li might be due to an increased release of 5-HT from forebrain terminals (Treiser et al., Science, 213: 1529, 1981). The present physiological demonstration of the presynaptic effect of Li on the 5-HT system is consistent with the hypothesis that its rapid antidepressant effect in patients treated with, but not responding to, a TCA drug might be due to an increased amount of 5-HT reaching postsynaptic neurons sensitized to 5-HT by the TCA pretreatment.

126.6 ACTIVITY OF NOREPINEPHRINE-CONTAINING NEURONS IN FREELY MOVING CATS: EFFECTS OF TRICYCLIC ANTIDEPRESSANTS. D.W. Preussler* and M.E. Trulson. Dept. of Pharmacol. Marshall Univ. Sch. of Med., Huntington, WV 25701.

Tricyclic antidepressant drugs have been hypothesized to exert their therapeutic effects via an inhibition of the uptake of norepinephrine (NE) and serotonin (5HT) into central nerve terminals. Antidepressants possessing a secondary amine side chain, such as desipramine and chlordesimipramine are much more potent in inhibiting the reuptake of NE than those with a tertiary amine side chain, such as imipramine and chlorimipramine. Since tertiary amine antidepressants are N-dealkylated in the liver to their secondary amine analogues, and the activity of the hepatic enzymes involved in these processes change with a number of factors (such as certain disease states, presence of additional drugs, etc.) we examined the effects of these antidepressants on the activity of NE-containing neurons in freely moving cats and in cats with altered hepatic metabolism. The activity of NE neurons was recorded in the locus coeruleus (LC) of adult male and female cats by means of movable 32 and 64 µ dia. insulated nichrome wires (see Brain Res., 1979, 263, 135-150 for complete methodology). Noradrenergic neurons showed a strong positive correlation with level of behavioral arousal, as previously described (Reiner and Morrison, Soc. Neurosci. Abstr. 8, 1982, p. 392). Mean discharge rates (spikes/sec) for the various behavioral states were as follows: active waking, 2.72 ± 0.27; quiet waking, 1.69 ± 0.20; slow wave sleep, 0.77 ± 0.13; and REM sleep, 0.09 ± 0.02. Impramine produced dose-dependent decreases in LC unit activity from no significant change at 0.1 mg/kg (i.p.) to a nearly total suppression of activity at 5 mg/kg, as compared to quiet waking. Similarly, chlorimipramine produced dose-dependent decreases in the activity of noradrenergic neurons, from no significant change at 0.5 mg/kg, (i.p.) to a complete suppression of activity at 25 mg/kg. When pretreated with a compound that inhibits drug metabolism (SKF-525A, 40 mg/kg, i.p.) 30 min prior to tricyclic antidepressant drug administration, the ID_{50} for imipramine was increased from 1.28 mg/kg in naive cats to 3.48 mg/kg in the pretreated subject, and the ID_{50} for chlorimipramine was increased from 11.1 mg/kg to 102.3 mg/kg. Conversely, when the animals were pretreated with a compound that increases drug metabolism (phenobarbital, 50 mg/kg/day, i.p., for 7 days) the ID_{50} for imipramine was decreased significantly to 0.54 mg/kg, and the ID_{50} for chlorimipramine was decreased to 2.4 mg/kg. These data demonstrate that altered hepatic metabolism dramatically influences the effects of tricyclic antidepressants on the activity of central noradrenergic neurons. Therefore, hepatic metabolism should be monitored in patients considered for tricyclic antidepressant medication.

126.7 NORADRENERGIC DENERVATION PREVENTS TRICYCLIC ANTIDEPRESSANT-INDUCED SENSITIZATION OF RAT FOREBRAIN NEURONS TO SEROTONIN. P. Gravel* and C. de Montigny. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, Canada H3C 3T8.

Green and Deakin have reported that brain norepinephrine (NE) depletion prevents ECS-induced enhancement of serotonin (5-HT)mediated behaviour (Nature, 285: 232, 1980). The present experiments were undertaken to determine if NE depletion also prevents tricyclic antidepressant (TCA)-induced sensitization to microiontophoretically-applied 5-HT.

Male Sprague-Dawley rats (200 g) were used. One group received an intraventricular injection of 6-hydroxydopamine (6-OHDA) (200 µg FB in 20 µl of a 0.9% NaCl and 0.1% ascorbic acid solution), one hour after zimelidine (25 mg/kg, i.p.) given to protect the 5-HT system from 6-OHDA neurotoxic action. Half of this group were subsequently treated for 14 days with 5 mg/kg q.d. of a TCA drug (imipramine or amitriptyline) administered either by an i.p. minipump (Alza) or by daily i.p. injections and the other half served as controls. Half of another group of intact animals received the same TCA treatments and the other half served as controls. Microiontophoretic experiments were carried out 15 days after the pump was installed or 24 h after the last injection under urethane anesthesia (1.25 g/kg, i.p.). Five-barrelled micropipettes were used for unitary extracellular recording of CA_3 dorsal hippocampus neurons. Side barrels were filled with 5-HT creatine sulfate (0.5 mM in 200 mM NaCl, pH: 4), NE bitartrate (50 mM in 50 mM NaCl, pH: 4) and acetylcholine chloride (ACh) (20 mM in 200 mM NaCl, pH: 4). Small currents of acetylcholine (1-3 nA) were used to activate silent or slowly discharging units. Neuronal responsiveness was estimated using the $I.T_{50}$ method (current x time required to obtain a 50% decrease of firing rate from baseline). The same micropipette was used for each pair of animals (one control and one TCAtreated rat) from intact and 6-OHDA groups in order to cancel any variation in micropipette efficacy.

The 6-OHDA pretreatment did not modify the $I.T_{50}$ values for NE and for 5-HT, but markedly prolonged the duration of the effect of NE, indicating adequate removal of NE terminals. The TCA treatments increased responsiveness to 5-HT in intact rats but failed to do so in 6-OHDA-denervated rats. The $I.T_{50}$ values for NE were modified by the TCA treatments in neither intact nor 6-OHDA rats.

These data indicate that the central NE system must be intact for the TCA-induced sensitization to 5-HT to occur. This suggests that sensitization of forebrain neurons to 5-HT produced by TCA drugs might result from their action on the NE system.

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127.8 LITHIUM MODIFIES NEUROPHYSIOLOGICAL RESPONSES IN THE CA1 REGION OF THE HIPPOCAMPAL SLICE PREPARATION. P.C. Rinaldi, M.D. Fairchild* & J.A. Kusske. Div. of Neurological Surgery, Univ. of Calif., Irvine, CA 92717 & V.A. Med. Ctr., Sepulveda, CA 91343.

Lithium has been employed clinically in the treatment of a number of psychiatric and neurological disorders and also appears to be helpful in the treatment of chronic alcoholism. However, the mechanisms through which lithium exerts its therapeutic effects and its actions on the CNS remain largely unexplained. The present study examines electrophysiological measures of CNS activity in tissue acutely exposed to lithium.

The hippocampus was dissected from brains of male Sprague-Dawley rats, prepared as in vitro slices, and maintained in a perfusion/recording chamber. Electrophysiological measurements were obtained while perfusing with normal media and with media in which LiCl was substituted for NaCl in 10, 20 and 30mM amounts.

Bipolar stimulation electrodes were positioned in the Schaffer/Commissural Projections (SCP) to the apical dendrites of CA1 pyramidal cells. Extracellular measures included assessment of the field potentials recorded from cell bodies in the s. pyramidale and from the dendritic area in the s. radiatum. Input/output (I/O) relations between the stimulus current applied to the SCP and the dendritic field potentials were determined. Field potentials from cell body and dendritic regions were monitored simultaneously so that stimulus intensities required to elicit detectable and maximal dendritic responses, in a range below threshold for elicitation of pyramidal cell discharge, could be measured. Evoked activity considered to represent the presynaptic fiber volley was measured in separate experiments in which conditions were optimized to record this response.

Slices perfused in 30mM lithium were found to be unstable and often exhibited seizure activity. With perfusion of a 10mM lithium media, many slices exhibited no change. However, slices perfused in 20mM lithium for periods of from six to twenty eight minutes exhibited reliable results. I/O relations were affected in such a way as to produce a shift in the curves describing current/response amplitude relations suggesting that lithium produced a decrease in the current required for a particular amplitude dendritic field potential. This occurred in 11 out of 12 slices tested. Varying degrees of recovery were obtained. The same concentration of lithium appears to reduce the amplitude of the presynaptic fiber volley (n=9). These results are in agreement with other studies that have pointed to lithium effects on neural fibers, possibly mediated through lithium interaction with the sodium-potassium pump. In addition, our results point to other lithium effects, possibly on synaptic transmission. (Supported by NIAAA grant # AA 03506).

127.9 **EFFECT OF PIRACETAM ON HIGH AFFINITY CHOLINE UPTAKE.** Vimala H. Sethy, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

A selective reduction in cholinergic markers such as choline acetyltransferase, high affinity cholie uptake (HACU) and acetylcholinesterase have been demonstrated in the cerebral cortex of patients with Alzheimer's Disease, suggesting that a specific cholinergic deficiency may be responsible for this disorder. Physostigmine, a cholinergic agent, seems to have beneficial effects in patients with Alzheimer's Disease. If a deficiency of the cholinergic system is the cause of cognitive impairment in patients with Alzheimer's Disease, an impairment of cholinergic functions may be observed in old rats. This hypothesis has been investigated by studying HACU in the hippocampus and cerebral cortex of young and old rats, and by investigating the effect of piracetam on this parameter. HACU was significantly ($p<0.05$) reduced in the hippocampus of both Sprague-Dawley and Fischer 344 old (24-month) female rats as compared to young (3-month) rats. A similar effect was not observed in the cerebral cortex. Acute administratin of piracetam at a dose of 100 mg/kg significantly ($p<0.05$) increased HACU in the hippocampus of both the young and old rats. Piracetam had no significant efffect on the HACU in the cerebral cortex. The increase in HACU in the hippocampus was found to be dose-dependent with piracetam, aniracetam (3, 10, 30 and 100 mg/kg) and 3,4-diaminopyrridine (1, 3 and 10 mg/kg). The results of the present investigations suggest that old rats may have reduced cholinergic activity, and piracetam improves it by increasing HACU.

127.10 TRICYCLIC ANTIDEPRESSANT DRUGS AS HISTAMINE H_2 RECEPTOR ANTAGONISTS IN BRAIN: SPECIES DIFFERENCES. Richard Straub*, Saul Maayani, Julia Ayala* and Jack P. Green. Department of Pharmacology, Mount Sinai School of Medicine of C.U.N.Y., N.Y., NY.

Similar to their blockade of the histamine H_2 receptor in guinea pig brain (Green, et al., Nature, 269:163, 1977), tricyclic antidepressant drugs (TA) are potent competitive antagonists of the H_2 receptor linked to adenylate cyclase in particulate preparations from rabbit brain. Classification of the histamine (HA) receptor in rabbit brain as the H_2 type was done by Schild analysis of the inhibition of HA response by antagonists (cimetidine, tiotidine, mepyramine) and by measuring stimulation of cAMP production by known HA agonists (HA, 4-CH_3-HA, 2-CH_3-HA) and partial agonists (dimaprit, impromidine). While the affinities of cimetidine and tiotidine do not differ between the two species, the TA exhibit between 2 and 25 times higher affinity for the H_2 receptor in the rabbit brain preparation. In contrast, the affinities of the TA for the muscarinic cholinergic binding site as measured by competition experiments with [^{3}H]-atropine is not species dependent.

	H_2 Cyclase		Muscarinic Binding	
	Kd±S.D.,nM, (n=2-8)			
DRUG	G.Pig Hipp.	Rabb. Hipp.	G.Pig Ctx.	Rabb. Ctx.
Nortriptyline	610±40	250±10	32±16	36±3
Amitriptyline(Ami)	310±170	12±6	9.7±1	7.2±2
Ami-CH_3I	23500±8400	4760±480	4.3±.5	6.8±1
Ami-C_4H_9I	--	3830±270	37±.5	34±8
Imipramine	410±65	34±12	66±3	89±23
(+)Trimipramine	83±27	5±3	21±7	27±11
(-)Trimipramine	83±30	13±7	22±6	36±9
(+)Trimipramine	92±40	9±4	22±4	26±.7
Iprindole	2344±257	350±190	--	--
Dibenzepin	3240±355	1040±180	724±150	710±40
Cimetidine	340±150	325±120	--	--
Tiotidine	7	4±1	--	--
(-)Atropine	--	--	.29±.04	.35±.02

The rank order for antimuscarinic potency is different from the rank order in inhibiting the HA H_2 response. The following structure activity relationships are evident for H_2 antagonism: (1)The rank order is the same in both species. (2)The quaternary analogs of amitriptyline are less potent than amitriptyline. (3) Branching of the side chain of imipramine(trimipramine) increases both H_2 and muscarinic affinity about three-fold, but the enantiomers of trimipramine are equipotent. It is suggested that the TA are interacting with the histamine H_2 receptor in a different and more complex manner than cimetidine or tiotidine. (Supported by MH31805 and Predoctoral Training Grant GM07163).

126.11 COMPARISON OF EFFECTS OF STRESS AND ANTIDEPRESSANTS ON NORADRENERGIC RECEPTOR FUNCTION IN RAT BRAIN. E.A. Stone, J.E. Platt, R. Trullas* and A.V. Slucky.* Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016.

Previous studies have shown that repeated stress reduces the function of noradrenergic receptors in the rat brain as evidenced by a decreased cAMP response to norepinephrine (NE) and a reduced density of beta adrenergic receptors (BARs). The present study was undertaken to investigate the generality of this effect and to compare it to the desensitization of receptors produced by tricyclic antidepressants (TADs). The stressors employed were restraint, food deprivation and hypercapnia. The former two represent common laboratory or natural stressors whereas the latter occurs in certain pathological states such as emphysema. Restraint was administered for 2.5 h per day for 10 days. Food deprivation consisted of six 48 h fasts given over a 3 week period. Fasts were separated by 1-2.5 days. For hypercapnia rats were exposed to a mixture of 10% CO_2, 20% O_2 and 70% N_2 for 1 h per day for 10 days. Desmethylimipramine (DMI), a TAD, was injected 10 mg/kg, i.p., b.i.d., for 10 days. Rats were killed immediately or 24 h after the last stress or injection.

DMI was found to significantly reduce the cAMP response to catecholamines in hypothalamic (35-40%) and cortical (35%) slices. The same effect was found after restraint but was smaller than that produced by DMI (hypothalamus, 9-22%; cortex, currently under investigation). Food deprivation and CO_2 have not yet been examined with regard to cAMP response. A similar pattern of results was obtained for BAR density: DMI produced significant decreases in the cortex (24%) and hypothalamus (16%) whereas the stressors food deprivation, restraint and CO_2 produced smaller decreases of 18, 8 and 2% respectively in the cortex and of about 5% each in the hypothalamus.

The present results indicate that many forms of repeated stress lower brain noradrenergic receptor function as evidenced by the effects of restraint and food deprivation on the above measures. The failure of CO_2 to reduce BAR density however suggests that this effect may not be general to all forms of stress. The reason that CO_2 was ineffective may be related to the fact that it represents a milder form of stimulation than the other conditions. The present results also indicate that the reduction in receptor function caused by DMI is much greater than that caused by these stressors. The significance of this finding is not yet known although it may mean that there is a physiological limit to the extent to which stress can desensitize brain noradrenergic receptors, and that antidepressants may exceed this limit. If this notion is correct it may have important implications for the mode of action of antidepressant agents. (Supported in part by grants MH 22768 and MH 08618 and CIRIT predoct. training grant to R. T.)

126.12 DEMONSTRATION OF A GROWTH REBOUND PHENOMENON FOLLOWING CESSATION OF CHRONIC METHYLPHENIDATE TREATMENT IN THE DEVELOPING RAT. W.J. Pizzi, J.E. Barnhart*, E.C. Rode* and V. Hirschenbein*. Northeastern Illinois University, Chicago, IL 60625.

Whether stimulants such as the amphetamines and methylphenidate lead to growth impairment and whether there is a rebound following the cessation of drug treatment has been hotly debated in the clinical literature. To date, only a few studies have been carried out on animals demonstrating a growth impairment. Furthermore, to our knowledge only research conducted in this lab has studied the phenomenon of growth rebound. In previous work we demonstrated that relatively high doses of methylphenidate can result in reduced femur lengths, along with decreased weights of testes, adrenal, thyroid, pituitary, and brain. In our original study all of these deficits were present in a random sample of animals immediately following drug cessation; however, these deficits were absent in a randomly selected group of animals at 500 days of age. The present study was carried out to determine the time course of the growth rebound.

In the present study rats were injected s.c. from days 5-24 of age according to varying injection schedules (35 mg/kg body wt. twice daily for males; 35 mg/kg body wt. once daily for females) with either methylphenidate or saline. Each housing unit contained one dam and a minimum of 6 and maximum of 8 pups. All animals received injections for 19 or 20 days. A group of randomly selected animals was necropsied within a 48-hour period following drug cessation. A second group of randomly selected animals was necropsied 30 days following drug cessation. Immediately following methylphenidate treatment both males and females showed decreased femur lengths, along with decreased pituitary and body weights. Further, the males showed a reduced brain weight, which was not present in the female animals treated once daily. The second necropsy carried out at 55 days of age revealed no significant differences in any of the above measures for either methylphenidate-treated males or females.

These data confirm our earlier finding of an acute growth suppression following chronic methylphenidate treatment in the developing rat. Further, it appears that a growth rebound occurs immediately upon cessation of drug treatment in the developing rat. A review of the literature indicates that growth impairments are seldom seen in children treated with less than 20 mg/day. These findings suggest that the stimulants should be used at the lowest effective therapeutic dose with frequent drug holidays when these are consistent with the treatment of the disorder.

(Supported by Committee on Organized Research, Northeastern Illinois University)

126.13 Effect of CI-844, a Cognition Performance Enhancer, on Rat Brain Biogenic Amine and Acetylcholine Turnover. T. Pugsley*, S. Myers*, Y. Shih*, L. Coughenour*, S. Stewart* and M. Gluckman. (SPON: R. F. BRUNS) Dept. Pharmacology Warner-Lambert/Parke-Davis Research, Ann Arbor, MI 48105.

CI-844 (3-phenoxypyridine) has been shown to enhance retention for passive avoidance learning in mice (Butler et al. J. Med. Chem 1981, 24: 346-350) and to increase avoidance in a rat aquisition procedure at 30 mg/kg i.p. (Boff et al. Soc. Neurosci., 1982, Abst 877 p. 320). Cognitive processes have been shown to be dependent on the function of brain biogenic amines and acetycholine neuronal systems. The purpose of the present study was to examine the effects of behaviorally active doses of CI-844 on dopamine (DA), norepinephrine (NE) and serotonin (5-HT) turnover, rat serum prolactin, and sodium-dependent high affinity choline uptake (HACU), and to determine its in vitro affinity for various rat brain receptors.

Without altering endogenous DA levels CI-844 caused a decrease in the DA metabolite 3,4-dihydroxyphenylacetic acid in rat striatum and hippocampus at 5 and 20 mg/kg i.p. but not at 1 and 0.1 mg/kg 2 hours after dosing; striatal homovanillic acid was decreased at 20 mg/kg. These findings suggested that CI-844 was decreasing DA turnover. CI-844 decreased rat serum prolactin at 25 mg/kg i.p. 1 and 2 hours after administration. This data would tend to suggest a DA agonist action of CI-844. However this effect is unlikely to be due to a direct interaction of CI-844 with DA receptors as it did not inhibit the in vitro binding of ^{3}H-haloperidol to rat striatum DA receptors at up to $1x10^{-5}$M. CI-844 (20 mg/kg i.p.) did not affect measures of NE (hippocampus and cerebral cortex) and 5-HT turnover (striatum and hippocampus) 2 hours after its administration. CI-844 (0.01 - 20 mg/kg i.p.) did not affect significantly in vitro HACU into rat brain hippocampal synaptosomes 30 minutes after being given suggesting that it was not altering hippocampal cholinergic neuronal activity. CI-844 exhibited no significant in vitro affinity for either rat brain adenosine A_1 or muscarinic receptors. The present neurochemical findings suggest that CI-844 may be exerting, its cognitive enhancing effects, at least in part, by increasing dopaminergic neuronal function.

126.14 BLOCKADE OF NOREPINEPHRINE (NE) AND DOPAMINE (DA) UPTAKE AND INDUCTION OF NE RECEPTOR SUBSENSITIVITY BY TAMETRALINE. B. Kenneth Koe, Susan W. Koch*, Katherine W. Minor* and Lorraine A. Lebel*. Central Research Division, Pfizer Inc., Groton, CT 06340

Tametraline, (+)-trans (1R,4S)-N-methyl-4-phenyl-1,2,3,4-tetrahydro-1-naphthylamine, was reported previously to be a potent blocker of NE uptake in rat heart in vivo (Sarges et al., 1974) and NE and DA uptake in synaptosomes of rat brain (Koe, 1976). The present study describes some neurochemical effects of tametraline and the antidepressant, nomifensine, also a NE and DA uptake blocker. The inhibition of synaptosomal uptake of NE, DA, and serotonin (5HT) by tametraline was found to be competitive; Ki values based on Dixon plots were: 0.018 µM (NE), 0.03 µM (DA), and 0.63 µM (5HT). Its effects on DA uptake in synaptosomes from naive and reserpinized rats suggested that tametraline does not belong to the amphetamine type of DA uptake blockers. In ex vivo studies, tametraline was one-fourth as active (ED_{50} 8 µmol/kg) as desipramine (DMI) in inhibiting NE uptake, twice as active (ED_{50} 47 µmol/kg) as nomifensine in blocking DA uptake, but less effective (ED_{50} >100 µmol/kg) than chlorimipramine against 5HT uptake. NE uptake ex vivo was maximally inhibited 1-2 hr after tametraline and returned to normal by 24 hr. In vivo, inhibition of NE uptake was demonstrated in rat heart (i.v. ^{3}H-NE; ED_{50} 5 µmol/kg, compared to 7 and 1 µmol/kg for nomifensine and DMI, respectively) and rat brain (intracisternal ^{3}H-NE). The depletion of NE and DA levels in rat brain induced by α-methyl-m-tyrosine was attenuated by tametraline, further indicating blockade of uptake of these amines in vivo. Tametraline by inhibiting DA reuptake, may increase DA concentrations at autoreceptors. Accordingly, tametraline was found to decrease striatal DA synthesis in naive rats as well as in rats treated with γ-butyrolactone to block impulse flow in DA neurons.

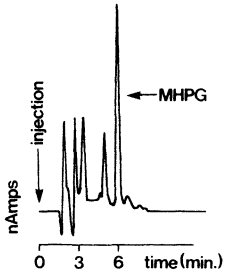
Tametraline and nomifensine were found to induce down-regulation of central NE neurons after subacute administration. After the injection of 75 µmol/kg i.p., b.i.d., for 8 days, these drugs decreased the NE response of the limbic forebrain adenylate cyclase in rats by 42% and 47%, respectively. Binding of ^{3}H-dihydroalprenolol to β-adrenoceptors was also decreased, but ^{3}H-spiperone binding to 5HT-2 receptors was not. With the same dosing regimen, tametraline was found to elicit subsensitivity of the adenylate cyclase after 4 days. Thus, both drugs elicited down-regulation, which may be predictive of clinical antidepressant activity. For nomifensine, DA uptake blockade may also contribute to the latter, since the major neurochemical property of bupropion and amineptine, both active against depression in man, appears to be inhibition of DA uptake.

126.15 BINDING SITES FOR IMIPRAMINE AND COCAINE ON A MODEL LIPID MEMBRANE: COMPARISON WITH CNS RECEPTORS. M.E.A. Reith*, H. Sershen* and A. Lajtha. Center for Neurochemistry, Rockand Research Institute, Ward's Island, N.Y. 10035.

Many antidepressant and stimulant drugs have been shown to bind with high affinity to specific sites in the CNS. The remarkable lipophilicity of these drugs raises the question of whether lipid constituents of brain membranes play a role in the observed high-affinity binding. In experiments with integral membranes it is difficult to disentangle interactions with proteins from those with surrounding lipids. We therefore used a pure lipid system of liposomes consisting of spherical membranes of phosphatidylcholine and cholesterol, and investigated its interaction with the antidepressant [^{3}H]imipramine and the stimulant [^{3}H]cocaine. The assays with liposomes contained concentrations of phosphatidylcholine and cholesterol similar to those calculated to be present in binding assays with brain membranes. Liposomes bound [^{3}H]imipramine and [^{3}H]cocaine with a dissociation constant in the micromolar range and a density close to 2 pmol/µg of phosphatidylcholine. There was a highly significant correlation ($r=0.9-1.0$, $n=13$, $p < 0.001$) between the potencies of various drugs in inhibiting the binding to liposomes and their lipophilicities as measured by octanol-water partition coefficients. In contrast, there was a much weaker correlation ($r=0.5-0.6$, $n=13$, $0.05 < p < 0.10$) between the potencies of drugs in inhibiting the binding of [^{3}H]imipramine and [^{3}H]cocaine to liposomes and their potencies in inhibiting the high-affinity binding to brain membranes. The potencies of drugs in inhibiting the binding to brain membranes were somewhat dependent ($r=0.6-0.7$, $n=13$, $p < 0.05$) upon their lipophilicities. These results indicate that model lipid membranes, devoid of proteins, can actually "bind" lipophilic substances, but that such lipid sites are not likely to be the primary sites of high-affinity binding of [^{3}H]imipramine and [^{3}H]cocaine to brain membranes. However, it is likely that lipophilicity is one of the components in the complex binding interactions between lipophilic substances and receptors attached to the lipid backbone of integral membranes.

126.16 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ELECTROCHEMICAL DETECTION AND GAS LIQUID CHROMATOGRAPHIC ANALYSIS OF 3-METHOXY 4-HYDROXY-PHENYLGLYCOL IN HUMAN URINE. D. M. Martin*, R. E. Petroski*, J. Alrazi*, R. Hahn*, A. L. C. Pottash, M. S. Gold, Psychiatric Diagnostic Laboratories of America, Summit, N.J. 07901.

3-methoxy 4-hydroxyphenylglycol (MHPG) has been reported to be the major urinary metabolite of norepinephrine in man and associated with treatment response to various antidepressant medications for over 10 years. The principal methodology for the analysis of urinary MHPG has been a Gas Liquid Chromatographic (GLC) separation of a trifluroacetic anhydride derivative and quantitation by electron capture detection. GLC techniques require complex extraction, derivatization and stabilization of precarious detector response all of which may add to analytical variability of the data. This in part may explain why there exists mixed reports over the clinical application of MHPG as a predictor of medication response in depressed patients. We report a High Performance Liquid Chromatographic Electrochemical Detection technique (HPLC-ECD) which eliminates many of the sources of analytical variability and methodological error inherent in GLC procedures. Congugated urinary MHPG was enzymatically hydrolyzed to the free form by incubating 250 uL of the 24 hour collection with 20 uL of glusalase for 20 hours. This hydrolysate was then basified with 50 uL of 2N NaOH and extracted into 3.6 mL of ethyl acetate. The MHPG extract was washed with 0.1M acetic acid to clean the organic phase. MHPG was then extracted from the organic into 450 uL of 8M urea/0.1M citrate and washed twice with hexane. 100 ul of the urea/citrate was injected into a reverse phase isocratic HPLC system using a mobile phase of 0.05M NaH_2PO_4, 10% MEOH, 0.1mM Na_2EDTA adjusted to pH=3.0 flowing over a 10 micron C-18 column. Detection was amperometric using a BAS glassy carbon electrochemical detector V=+0.70 volts, range 2-20 nA. Over 100 urine samples have been analyzed using both this technique and GLC yielded an excellent and significant linear relationship ($r=0.97$; $p<.001$) between the two groups of data and the day-to-day coefficient of variation of the assay was less than 12%. As little as 10 ng of MHPG could be detected which may allow quantitation of MHPG in plasma as well as urine. The precision reproducibility, ease of sample preparation and simplicity of instrumentation make this technique exceptionally well suited for routine clinical measurement of MHPG.



PSYCHOTHERAPEUTIC DRUGS: NEUROLEPTICS

127.1 CHOLINE IN RED BLOOD CELLS AND PLASMA IN CHRONIC SCHIZOPHRENIC INPATIENTS. W.B. Lawson, D.J. Jeste,* B.H. Phelps,* U. Kopp,* L.B. Bigelow, I. Hannin. Adult Psychiatry Branch, NIMH, Saint Elizabeths Hospital, Washington, D.C., 20032 and Pittsburgh, Pennsylvania

INTRODUCTION: Various measures of blood choline have been proposed as possible biological markers of central cholinergic activity. For example, elevated RBC choline concentrations have been reported in a proportion of depressed patients, associated with Tourette's syndrome, and with lithium treatment. Some indirect evidence also implicates cholinergic pathology in schizophrenia. However, very little work has been done with blood levels of choline in schizophrenia.

METHODS: All twenty-seven patients with RDC positive schizophrenia on our Research Wards at the Adult Psychiatry Branch of NIMH were examined. Two nurses on the ward rated psychopathology with the Brief Psychiatric Rating Scale. Two other nurses independently rated movement disorders with the Abnormal Involuntary Movement Scale and Abbreviated Rockland Dyskinesia Rating Scale. Blood was drawn on the same day. Plasma and RBC samples were sent to Pittsburgh where choline estimations were done "blind". In 17 patients this process was repeated two months later. Every patient had been either neuroleptic-free or on stable doses of neuroleptics for at least a month before each test-day.

RESULTS: Both RBC and plasma choline concentrations had intra-subject stability over the two month period, especially in patients whose medication status remained unchanged. No significant correlation was seen between RBC and plasma choline levels. RBC choline (mean 39 + S.D. 50 nmol/ml) had a much greater inter-subject variability than did plasma choline (8 + 3 nmol/ml). Neither measure correlated significantly with most psychopathology scores on the BPRS, including the depression subscale. A significant negative correlation was seen between neuroleptic dosage (converted to chlorpromazine equivalents) and RBC choline (Pearson's $r = -.41$; $p < 0.04$) that was independent of concomitant treatment with anticholinergics. No significant difference was seen between RBC choline levels in seven patients with tardive dyskinesia (46 + 50 nmol/ml) and nondyskinetic patients (42 + 54 nmol/ml). However, one patient with neuroleptic-induced Tourette's syndrome had a markedly elevated RBC choline concentration (130 nmol/ml).

DISCUSSION: RBC choline is a possible biological marker of central cholinergic activity that is relatively stable over time. We will discuss possible implications (and limitations) of our findings with reference to neuroleptic treatment, tardive dyskinesia, iatrogenic Tourette's syndrome, depression and cognitive functioning in schizophrenia.

127.2 INTERACTION OF PYRROLOISOQUINOLINE ANTIPSYCHOTICS WITH A D-2 DOPAMINE RECEPTOR SUBTYPE - EVALUATION BY ^{3}H-RO 22-1319 BINDING. T. Nakajima*, I. Kuruma*, and K. Nakamura*, Dept. of Pharmacology, Nippon Roche Research Center, Kamakura, Japan, and G. Olson*, H. Cheung*, E. Chiang*, L. Berger*, G. Bautz*, R.A. O'Brien, C. Burghardt*, T. Mowles*, E. Gamzu, A.B. Davidson and E. Boff*, Depts. of Chemistry and Pharmacology, Roche Research Center, Nutley, New Jersey 07110.

Ro 22-1319 is a novel, conformationally rigid pyrroloisoquinoline derivative which exhibits a potent antipsychotic-like profil in animal tests. It has weak cataleptogenic activity and does nc induce receptor supersensitivity upon chronic dosing (Davidson, et al., Psychopharmacol., 79:32-39, 1983).

^{3}H-Ro 22-1319 has been found to bind in a sodium-dependent manner to a single component of rat striatal homogenates with hic (nM) affinity. The binding is saturable and highly stereo-specific for the pharmacologically active 4aR,8aR-(-)-enantiomer, (-)-Ro 22-1319. The binding of Ro 22-1319 and a series of pyrroloisoquinoline analogs to the ^{3}H-spiroperidol-labelled receptor also exhibited a sulpiride-like dependence on sodium ion, suggesting that the pyrroloisoquinoline series may be selective for a D-2 receptor subtype. Other D-2 dopamine antagonists, sulpiride, metoclopramide, and molindone are similarly more potent inhibitor of ^{3}H-Ro 22-1319 binding than of ^{3}H-spiroperidol binding.

Structure-activity relationships indicate that changes in the lipophilicity of the basic nitrogen substituent of compounds of the pyrroloisoquinoline class can modulate their binding to the D-1 (adenylate cyclase) and D-2 (spiroperidol) receptors (Bautz, et al., Soc. Neurosci. Abstr., 7:866, 1981) via their interaction with an auxiliary binding site identified through receptor modeling (Olson, et al., J. Med. Chem., 24:1026-1034, 1981; Olson, et al., in "Dopamine Receptors", in press). The affinity of pyrroloisoquinoline analogs for the ^{3}H-Ro 22-1319 receptor is less dependent on the size and lipophilicity of the group on the basic nitrogen. These observations suggest that Ro 22-1319 and analogs bind to a distinct D-2 receptor subtype which differs at the molecular level from the ^{3}H-spiroperidol binding site by having a greatly diminished hydrophobic character in its auxiliary binding site.

127.3 SELECTIVE ACTIVITY OF MDL 17,214EF IN TESTS PREDICTIVE OF "ANTIPSYCHOTIC" ACTIVITY. Francis P. Miller and Donald L. Braun,* Merrell Dow Research Center, Cincinnati, Ohio 45215.

Initial results with MDL 17,214EF (4-(4-(6-chlor-2-naphthalenyl)carbonyl-1-piperidinyl)-1-(4-fluorophenyl)-1-butanone methane sulfonate (1:1)) indicated that it might possess antipsychotic-like properties. The present experiments were carried out to determine the profile of activity of MDL 17,214EF in several tests where known antipsychotic agents are active. The results of these studies are shown in the table below, accompanied by comparative results with haloperidol (H) and thioridazine (T).

	ED50 (mg/kg i.p.)		
	MDL 17,214EF	H	T
A. Amphet. Aggreg. Toxicity	1.7	0.17	0.86
B. Pernicious Preening - Mice	2.16	0.51	2.65
C. Fighting Mice	9.0	2.7	8.7
D. Apomorph. Climbing Mice	75.8	0.15	3.2
E. Apomorph. Stereotypy-Rats	> 320	0.50	30.3
F. Amphet. Stereotypy-Rats	> 320	0.33	53.6

Although H and T are equally efficacious antipsychotic agents, it is believed that T has a much lower propensity to cause extrapyramidal symptoms (EPS). Since both antipsychotic activity and EPS are thought to be mediated via blockade of dopamine receptors and certain animal tests used to evaluate antipsychotic agents are sensitive to dopaminergic blockers, the clinical differences between H and T should be reflected in these animals tests. Observation of the data in the table above shows that, although T is 1/3-1/5 as potent as H in tests A-C, it is only 1/20-1/160 as potent in tests D-F. The relatively weak activity of T vs H in Tests D-F suggests that these tests are more reflective of liability to cause EPS, whereas the relatively more potent activity of T vs H in Tests A-C suggests that these tests are more reflective of antipsychotic activity. This interpretation is supported by data with several other antipsychotic agents with known liability to cause EPS.

For MDL 17,214EF, the selectivity of blockade in these tests is very impressive. In Tests A-C, thought to be related to antipsychotic activity, MDL 17,214EF is 1/3-1/10 as potent as H. However, in Tests D-F, thought to be related to EPS, MDL 17,214EF is <1/500 as potent as H. We suggest that these highly selective behavioral effects of MDL 17,214EF are indicative of a highly selective dopamine blocking agent, and presumably an antipsychotic agent with minimal, if any, liability to cause EPS.

127.4 HETEROGENEOUS ROTATIONAL BEHAVIOR RESPONSES TO APOMORPHINE, SKF 38393, 3-PPP AND PERGOLIDE. H.M. Fenton* and J.M. Liebman. Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

After unilateral destruction of the ascending nigro-neostriatal pathway by 6-hydroxydopamine microinjections, rats show rotational behavior (RB) when injected systemically with drugs that enhance dopaminergic neurotransmission. Postsynaptic dopamine (DA) agonism is inferred from contralateral RB away from the lesioned side, presumably due to the stimulation of hypersensitive striatal DA receptors. This preparation has been used to evaluate the postsynaptic DA agonist properties of the putative DA autoreceptor agonist, TL-99 (Goodale et al., Science, 210:1141, 1980) and 3-PPP (Hjorth et al., Life Sci., 28:1225, 1981) as well as the proposed D-1 agonist, SKF 38393 (Stoof and Kebabian, Nature, 294:366, 1981). Two inconsistencies have emerged. First, some investigations showed that TL-99 and 3-PPP are capable of inducing RB in lesioned rats (Martin et al., Eur. J. Pharmacol., 76:15, 1981) but others reported little or no RB (Goodale et al., 1980; Fallon et al., Fed. Proc., 41:1065, 1982). Secondly, the reported ability of TL-99, 3-PPP and SKF 38393 to induce RB was inconsistent with the absence of postsynaptic DA receptor activation in behavioral models using normal, unlesioned animals (Martin et al., 1981; Setler et al., Eur. J. Pharmacol., 50:419, 1978). To address this controversy, we systematically compared the RB induced by various doses of apomorphine, SKF 38393, 3-PPP and pergolide in a large colony of lesioned rats.

One week after unilateral nigral microinjection of 6-hydroxydopamine (10.8 µg in 4 µl), rats were placed (without a restraining harness) inside clear Plexiglas cylinders (13.5 cm radius, 29.5 cm high) centered in Omnitech Digiscan activity monitors. Each complete rotation was automatically recorded by commercially available software. Rats were tested in 30 min sessions that began 5 min after s.c. injection of the DA agonist.

Forty-eight rats each completed at least 35 contralateral turns per session in response to initial testing with 0.5 mg/kg apomorphine, and were selected for further testing. Two subgroups emerged: one subgroup (n=28) averaged 58 contralateral rotations after 0.03 mg/kg apomorphine and showed comparable contralateral RB after 1-10 mg/kg 3-PPP, 3-10 mg/kg SKF 38393 and 0.3 mg/kg pergolide. The other subgroup (n=20) failed to rotate in response to 0.03 mg/kg apomorphine or at doses as high as 30-100 mg/kg SKF 38393, 30 mg/kg 3-PPP and 0.3 mg/kg pergolide. A higher dose of apomorphine (0.25 mg/kg), however, induced RB in this subgroup that was comparable in magnitude to that in the more sensitive subgroup. Thus, careful preselection of animals is necessary to yield readily interpretable results when DA agonists are assessed in the RB model. It will be of interest to determine the basis for the differential sensitivity noted with these subgroups.

127.5 THE AVERAGE DAILY DOSE OF ANTIPSYCHOTIC DRUGS AS CLINICALLY PRESCRIBED IN AN INPATIENT SETTING. D. Miller*, A. C. Andorn*, S. Stern*, E. Seideman* (SPON: L. Hershey). Dept. of Psychiatry, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106.

Discharge medication was tabulated on a µmole/kg patient weight/day basis on sequential admissions to the psychiatric service in 1979. The only patients included (N=200) were those demonstrating psychotic symptoms on admission and discharged on only one antipsychotic medication. The preliminary findings indicate that the frequency with which these drugs are prescribed is: haloperidol > trifluoperazine > thioridazine = cis-thiothixene > chlorpromazine>fluphenazine>perphenazine>mezoridazine. The range of doseages used is extensive. The doses used for each drug do not appear to distribute normally which probably accounts for the large standard deviations observed in the mean doses below.

DRUG	AVERAGE DAILY DOSE umole/kg	N
Haloperidol	0.93 +/- 0.77	51
Trifluoperazine	0.85 +/- 0.52	45
Cis-thiothixene	0.88 +/- 0.83	27
Thioridazine	15.20 +/-11.60	27
Chlorpromazine	19.94 +/-17.16	17
Fluphenazine	0.57 +/- 0.59	13

If we could use the average daily dose to compute a rank order of these drugs we would note it is different from previous literature reports, and the actual doses used are higher. Further studies will be included in an effort to determine the type of distribution of dosages. It appears the the average daily dose of antipsychotic drugs has yet to be established. Ideally, both retrospective and prospective, multi-institutional studies should be done on large numbers of patients in order to determine the actual daily dose of antipsychotic medication.

127.6 THIORIDAZINE PHARMACODYNAMICS: IN VITRO CORRELATIONS AND DEPENDENCE ON DRUG METABOLISM. Mark H. Lewis, Laura Staples*, Donna McCorkle and Richard B. Mailman. Biological Sciences Research Center and the Departments of Psychiatry and Pharmacology, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514.

At least two metabolites of the clinically useful antipsychotic drug thioridazine (THD) (Mellaril®) are known to have dopamine receptor blocking properties and also cause clinical antipsychotic effects. Earlier in vivo and in vitro work has suggested that THD-2-sulfoxide (MES) is approximately equipotent to THD whereas THD-2-sulfone is approximately 5 times more potent than the parent drug. We have gathered evidence demonstrating that the conversion of THD to these two active metabolites is catalyzed in large measure by the hepatic flavin containing monooxygenase (EC 1.14.13.8), an enzyme high in hamsters and humans but not in rats. Therefore, we sought to compare the in vivo and in vitro activity of THD and its metabolites in the rat vs. the hamster. THD has sometimes been termed an "atypical" neuroleptic, in part, because of its inability to antagonize apomorphine-induced stereotypies in the rat. A computer supported observational scoring method was used that measured multiple behavioral topographies following apomorphine administration. In these studies, we found that although THD or MES was not a very potent antagonist of apomorphine-induced stereotypies in the rat, both were quite potent in inhibiting apomorphine stereotypies in the hamster. Moreover, THD and MES both antagonized amphetamine-induced locomotion in the rat, at much lower doses than required for inhibition of stereotypies. Despite this result, little difference was noted in the ability of THD or its metabolites to displace [^{3}H]-spiperone from striatal versus mesolimbic receptors. Despite clinical data to the contrary, we found no major difference in the potency of MES vs THD in either behavioral test. Further, we have found that in the rat both THD and MES at low doses (5-10 mg/kg) potentiated both amphetamine-induced locomotion and, under certain conditions, apomorphine-induced stereotypies. Whether such potentiation is a pharmacokinetic phenomenon, or represents ligand-receptor interactions, awaits further study. The present results suggest that the pharmacodynamic effects of THD appear to be determined, at least in part, by drug metabolism. Further, clinical hypotheses concerning the effects of THD might better be based on experiments conducted with species that metabolize THD in a manner similar to humans. (Supported in part by PHS grants HD/MH16834 and HD03110).

127.7 GAMMA-VINYL GABA: GABA AGONIST TREATMENT IN TARDIVE DYSKINESIA. G.K. Thaker,* C.A. Tamminga, T.N. Ferraro, T.A. Hare. Maryland Psychiatric Research Center, U. of Maryland, Baltimore, Md. 21228; Thomas Jefferson University, Philadelphia, Pa. 19107.

Enhancement of central GABA neuronal function has been attempted in individuals with hyperkinetic motor disorders to diminish dyskinetic symptoms. Although some studies in tardive dyskinesia (TD) have been positive (Arch. Gen. Psychiat. 36:595, 1979), others have reported equivocal antidyskinetic action or prominent drug-induced side effects in response to GABA agonist treatment. Since most clinical studies have been carried out in schizophrenic individuals receiving concomitant neuroleptic treatment, the action of GABA agonists to exacerbate neuroleptic-induced Parkinsonism may obscure the primary ameliorative effect of GABA stimulation in TD. We have administered the indirect acting GABA agonist gamma vinyl GABA (GVG) to seven individuals with TD who were otherwise free from centrally active drugs. All participants were withdrawn from neuroleptic treatment at least four weeks prior to testing. The double-blind, crossover design began with a fixed two-week placebo period and was followed by three weeks of GVG or placebo, then crossed with three weeks of placebo or GVG. Motor symptoms and mental status were assessed by blind live ratings twice weekly, and videotapes were done weekly and rated at the end of the protocol. Each participant received 3000 mg GVG daily during the drug period. All seven patients demonstrated a clear antidyskinetic response to GVG. The mean baseline TD score ($\pm$ SEM) was 16.1 $\pm$ 3.2; mean TD score during drug treatment was 9.5 $\pm$ 2.3 compared with 15.8 $\pm$ 3.6 during placebo treatment ($p<.01$). The average decrease in dyskinetic symptomatology was 43.9%. No clinically significant motor or cognitive side effects occurred. CSF GABA levels increased by 148% during GVG treatment (99 $\pm$ 24 pM/ml, placebo; 213 $\pm$ 23 pM/ml, GVG). Analysis of amino acid levels in CSF during placebo and drug periods demonstrated a selective increase in GABA and homocarnisine without concomitant increases in other amino acids. Thus, GVG may be both an effective and practical antidyskinetic agent. Substantia nigra, pars reticulata GABA-containing efferent neurons may mediate this GABA agonist effect in the described terminal areas: thalamus (VM/VL), superior colliculus, or reticular system.

127.8 NEUROLEPTIC EFFECTS ON METHIONINE ADENOSYLTRANSFERASE KINETICS. L. C. Tolbert, J. A. Monti, W. Walter-Ryan* and J. R. Smythies. Neurosciences Program and Dept. of Psychiatry, Univ. of Alabama in Birmingham, Birmingham, Alabama 35294.

Previous reports from this lab have documented decreased activity of the enzyme methionine adenosyltransferase (MAT) in erythrocytes obtained from either Feighner or DSM-III diagnosed schizophrenics as compared to normal subjects. Other reports in the literature such as decreased rates of oxidation of methionine to CO_2, lower plasma levels of S-adenosylmethionine, and exacerbation of symptoms following methionine loading are also consistent with the general concept of abnormalities in methionine metabolism in some schizophrenic subjects. Nonetheless, one of the concerns was that the patients in both MAT studies were medicated with a variety of neuroleptics and this was possibly the source of the apparent differences in enzyme activity. For this reason studies were initiated to evaluate the effects of neuroleptics on MAT kinetics. Two approaches have been taken. One approach has been to collect a blood sample from a drug-free (at least 1 month) schizophrenic upon admission and a second blood sample after initiation of neuroleptic therapy. Due to the short-term nature of the inpatient facilities this second sample is usually obtained after two weeks of neuroleptics. Both erythrocyte samples are assayed for MAT and comparisons made between these paired samples.

The second approach has been to obtain erythrocytes from a normal subject and aliquot it into five fractions. Four fractions are incubated with high therapeutic plasma concentrations of different neuroleptics (chlorpromazine, haloperidol, fluphenazine, or thioridazine) chosen because they represent the predominant medications received by the schizophrenic subject population. The fifth fraction is incubated with water (control). Each fraction is then treated and assayed in the standard manner.

The in vitro incubation of the erythrocytes with neuroleptics resulted in a consistent increase in the Km of the enzyme with no apparent effect on the Vmax. The in vivo approach demonstrates a remarkably consistent increase in MAT Vmax associated with two weeks of neuroleptic therapy - all patients studied thus far have increased. The magnitude of the increase is variable depending, it seems, upon how low the Vmax is in the first sample. The effect on the Km with this approach is less consistent but most paired patient samples show an increase in the Km as well.

While still preliminary, the results, taken together, suggest that neuroleptic therapy, rather than being the cause of the observed decrease in enzyme activity in schizophrenics, may actually be minimizing the differences.

The question of the possible relationship of this neuroleptic effect to clinical efficacy of these compounds remains to be explored.

127.9 ALTERATION OF THE AMPHETAMINE RESPONSE BY ATYPICAL AND TYPICAL NEUROLEPTICS IN RATS WITH AMYGDALA LESIONS. A. Robertson and C. MacDonald*. Dept. of Psychology, McGill Univ., Montreal, Quebec, Canada H3A 1B1.

Atypical and classical neuroleptics differ according to their ability to block the various components of the behavioral response to dopamine agonists such as amphetamine. This effect is strongly correlated with their tendency to produce extrapyramidal side effects clinically or in the laboratory. Although both types of neuroleptics are dopamine antagonists, the basis for their differential effects on motor output is unclear. It has been observed, however, that atypical neuroleptics such as clozapine have selective effects on neuronal activity in the amygdala when compared to classical neuroleptics such as pimozide (Gelman et al, Neurosci. Abstr., 8:469, 1982). As the amygdala has efferent connections to the basal ganglia (eg. Yim & Mogenson, Brain Res., 239:401, 1982), it is possible that this structure mediates some of the atypical effects of such drugs. Accordingly, the effects of small electrolytic lesions, limited primarily to the central nucleus and adjacent stria terminalis, on the ability of clozapine and pimozide to alter the behavioral effects of amphetamine were studied.

Rats were injected with saline, 1 or 5 mg/kg D-amphetamine, IP, and placed in large wood and plexiglass test chambers. Their behavior was observed and categorized every 6 sec for 2 min periods, at 6 intervals during a one hr test period. In both non-lesioned control and lesioned rats, 1 mg/kg amphetamine produced a strong increase in locomotion, but did not produce any stereotyped behavior. 5 mg/kg produced strong stereotyped behavior - primarily sniffing. In control rats, pimozide (0.25 and 0.50 mg/kg, SC) produced a dose-dependent decrease in both locomotion and stereotypy. Clozapine (10 and 20 mg/kg, SC) produced a decrease in amphetamine-induced locomotion but did not alter stereotypy. In rats with amygdaloid lesions, the effectiveness of pimozide in blocking amphetamine-induced behavior (both locomotion and stereotyped behavior) was unchanged. Similarly, the effectiveness of clozapine in blocking amphetamine-induced locomotor activity was unaltered. However, in rats with lesions, clozapine markedly enhanced the ability of amphetamine to produce stereotypy. The results demonstrate that the amygdaloid lesions and clozapine have synergistic actions on stereotypy produced by amphetamine.

127.10 THE EFFECT OF AMINOPYRIDINES ON THE RELEASE OF ^{3}H-ACETYLCHOLINE FROM RAT BRAIN SLICES. R. D. Schwarz, C. J. Spencer*, A. A. Bernabei* and T. A. Pugsley*. Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, MI 48105.

Aminopyridines (APs) are currently under considerable investigation due to their suggested clinical use in such areas as anesthesia, curare poisoning, botulism and myasthenia gravis. In addition, APs may be of therapeutic use in CNS diseases which are characterized by a loss of neuronal function, e.g. Parkinsonism and senile dementia of the Alzheimer type. Treatment of these disorders with APs may be beneficial due to their ability to increase neurotransmitter release. Since many investigators have studied the effects of APs on peripheral cholinergic function, our purpose was to examine in vitro the effect of a series of APs on the release of ^{3}H-acetylcholine (^{3}H-ACh) from neurons in those brain regions with high cholinergic innervation.

The rat striatum, cortex or hippocampus was removed, sliced (0.3 x 0.3 mm) and incubated with ^{3}H-choline (0.01 uM) for 15 min at 37°C in Krebs-Ringer Hepes buffered medium, pH 7.2. After washing, the slices were further incubated for 15 min in both low K^+ medium or high K^+ medium in the presence or absence of AP (10^{-7} to 10^{-4} M). At the termination of the incubation, slices were separated from medium by rapid centrifugation and radioactivity was determined in both fractions by scintillation counting.

The APs tested were mono substituted (2-, 3-, 4-AP), di substituted (2,3-, 2,6-, 2,5-, 3,4-AP) and the related 3-phenoxypyridine (CI 844). At 10^{-6} to 10^{-4} M, 3,4-, 2,3- and 4-AP significantly increased basal ^{3}H-ACh release from striatal slices. Since 3,4-AP showed the greatest effect, it was also tested in the cortex and hippocampus. Among the three brain regions, the order of response to 3,4-AP was: striatum> hippocampus>cortex. The presence of Ca^{++} was necessary for the increase in basal release from striatal slices observed with both 3,4- and 4-AP. Further, these same APs markedly reduced K^+-stimulated release at 10^{-5} and 10^{-4} M. These latter results support the concept that APs may act in part by blockade of K^+ channels which would prolong repolarization of the nerve terminal. As a consequence, extracellular Ca^{++} entry would increase and result in enhanced release of ^{3}H-ACh.

In summary, our results show that of eight APs tested, three caused a significant increase in the basal release of ^{3}H-ACh from rat striatal slices with the order of potency being 3,4-AP> 4-AP = 2,3-AP.

127.11 RADIOIMMUNOASSAY OF FLUPHENAZINE IN HUMAN PLASMA. J. L. Browning and C. M. Davis*. Analytical Neurochemistry, Tex. Res. Inst. of Mental Sci., Houston, TX 77030.

Fluphenazine has become one of the most widely used agents in the treatment of schizophrenia. Accurate measurement of plasma levels would facilitate control of dosage and clinical management. Since fluphenazine is a very potent neuroleptic it is given in low dosages especially when given as the long acting decanoate or enanthate esters. These lower dosages result in very low plasma levels requiring either extraction of a large volume of plasma or a very sensitive assay procedure. We therefore have developed a radioimmunoassay for fluphenazine that is specific and very sensitive.

The plasma sample is extracted with 1% isoamyl in heptane with ^3H fluphenazine added as internal standard. The organic phase is passed through a small (.5 ml) silica gel column which is subsequently eluted with 6 ml CH_2Cl_2 containing 1.5% triethylamine and 2% ethanol. The eluate is dried and reconstitued in ethanol to the original plasma volume. Fifty µl aliquots are assayed for fluphenazine by RIA and 200 µl is assayed for ^3H recovery. Handled in this way a technician can assay 40-50 samples per day.

The sensitivity of the assay is less than 10 pg per 50 µl aliquot. Column treatment completely removes the only known metabolite which cross reacts significantly, the N-oxide. Other metabolites including 3-OH-fluphenazine, 2-OH-fluphenazine and fluphenazine sulfoxide do not cross react significantly and are retained on the column.

Comparison of 12 patient values determined by a HPTLC method (Davis and Fenimore, *J Chromatogr 272:*157, 1982) and RIA gave a correlation coefficient of r = .974. The RIA gave values of 0.6 to 14.5 ng/ml whereas HPTLC values were 0.5 to 9.2 ng/ml. Because of the sensitivity and selectivity of this assay, accurate measurement of fluphenazine in tissue samples as well as small plasma samples is now possible.

127.12 USE OF A CLONIDINE DRUG DISCRIMINATION TASK TO IDENTIFY ALPHA$_2$ AGONIST AND ANTAGONIST PROPERTIES OF VARIOUS COMPOUNDS: A COMPARISON OF TWO RAT STRAINS. D.A. Bennett, L. Noreika*, D. Wilson* and J.M. Liebman. Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

It has been previously shown that the interoceptive discriminative stimuli associated with clonidine (CLO-IDS) are mediated by alpha$_2$ adrenoceptor agonism (Bennett and Lal, J. Pharmacol. Exp. Ther., 223:642, 1982). The purpose of the present experiments was to: 1) further evaluate the usefulness of CLO-IDS in identifying the alpha$_2$ properties of reported antagonists; 2) investigate the possible alpha$_2$ agonist properties associated with dopamine agonists, the alpha$_1$ agonist ST-587 and low doses of the alpha$_2$ antagonist RX 781094; and, 3) compare the suitability of two rat strains in this model.

Rats of both the Long Evans hooded and Sprague Dawley albino strains were trained to discriminate clonidine from saline in two-lever operant chambers. All rats learned the discrimination over a comparable time course. The discriminative cue was dose-dependent in both Long Evans (ED_{50} = 0.011 mg/kg) and Sprague Dawley (ED_{50} = 0.018 mg/kg) animals. Yohimbine, piperoxane, RX 781094 and CGS 7525A each dose-dependently antagonized the CLO-IDS in both rat strains; although in some instances, Sprague Dawley animals exhibited shallower dose-response curves than those generated by Long Evans rats. Mianserin produced up to 70% antagonism in Long Evans rats but only 43% antagonism in Sprague Dawley rats. Rauwolscine (α-yohimbine) was more effective than coryanthine (ß-yohimbine) in antagonizing the clonidine discrimination, consistent with its greater potency at alpha$_2$ adrenoceptors. The alpha$_1$ antagonists WB 4101 (1-10 mg/kg), prazosin (10 mg/kg) and phentolamine (3-10 mg/kg) failed to block CLO-IDS. The putative dopamine autoreceptor agonist, TL-99 (3-10 mg/kg) produced dose-dependent generalization to clonidine, but no generalization resulted from 3-PPP (3-10 mg/kg). This finding supports previous evidence that TL-99, but not 3-PPP, has alpha$_2$ agonist properties (e.g., Pastor et al., Eur. J. Pharmacol., 87:459, 1983). The alpha$_1$ agonist ST-587 and the peripherally acting alpha agonist DPI produced no generalization at doses up to 10 mg/kg. Low doses of the alpha$_2$ antagonist, RX 781094, were also tested for agonist effects as proposed by a previous report (Goldstein et al., Neurosci. Proc., 8:104, 1982). No generalization of RX 781094 was seen in either strain (0.0003-0.01 mg/kg). These results indicate that CLO-IDS are useful in both identifying compounds exhibiting a primary alpha$_2$ antagonist profile and in identifying the presence or absence of alpha$_2$ agonist potential in a variety of other compounds. Although both strains are suitable for this discrimination, some differences were noted that favor the use of Long Evans rats.

128.1 BEHAVIORAL SEQUELAE IN CAT PRODUCED BY COMPOUNDS CLAIMED OR SHOWN TO BE ANXIOLYTIC AND BY RO 15-1788. S. Furman*, K. L. Keim. (SPON. W. Schlosser) Dept. of Pharmacology I, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

Cats were used to ascertain a minimum effective dose (MED; all doses mg/kg I.G.) for separate endpoints which together produced an overt symptom/effect profile. Drug-experienced 1 yr old female cats, fasted 18 hrs, were treated with selected compounds. The onset, duration and intensity of ataxia (AT; altered gait), muscle relaxation (MR; reduced abdominal tone and extensor reflex), and change in motor activity (↑MA or ↓MA) were recorded.

The MED for diazepam (0.25 to 10 mg/kg, = tested dose range) was 0.5 for producing AT & MR, and 1 for ↑MA (15 min duration); ↓MA and sleep occurred post 10 mg/kg. This benzodiazepine dose-dependently affected the frequency, intensity and duration of the motor impairments. Alprazolam (0.01 to 20) was 5x more potent in causing AT but 4x less active in producing MR; ↑MA was markedly intense and followed by ↓MA and very deep sleep. (We previously reported that in cat, EEG effects produced by alprazolam were phenobarbital-like rather than diazepam-like [Keim & Zavatsky, 1982. Fed Proc 41: 1067]). The MED for AT following phenobarbital or meprobamate was 10 and 80 mg/kg, respectively, and at these doses the AT duration exceeded 4 hrs; MR and loss of righting occurred at higher doses.

The behavioral profiles of buspirone (0.1 to 20), fenobam (0.1 to 40), and tracazolate (0.1 to 160), differed from that of classical anxiolytics; none of these produced AT or MR. Buspirone ↑MA and caused miosis (MEDs = 2.5); a dose of 20 mg/kg sustained the miosis for 4+ hrs. Fenobam at 20 mg/kg caused salivation and ↓MA. Tracazolate at a dose of 20 mg/kg caused emesis and ↑MA. The triazolopyridazine CL 218,872 (5 to 40) produced marked AT, lasting 24 hr (MED 40 mg/kg); CL 218,872 at this dose also caused ptosis, emesis, and relaxation of the nictitating membrane - the latter effect typically seen with neuroleptics in cat.

Tested at 20 mg/kg, the benzodiazepine antagonist Ro 15-1788 did not cause AT or MR but an ↑MA lasting 4 hrs was recorded.

That alprazolam has apparent hypnotic activity in the free-roaming cat corroborates our previous findings on the EEG. Most striking is the lack of AT or MR produced by most of the non-BZ anxiolytic substances in cat - a species known to be particularly sensitive to these measures. CL 218,872 is known to cause AT and ↓MA in rodents. Therefore, these behavioral profiles differ from the more classical anxiolytics.

128.2 A PRECLINICAL EVALUATION OF BUSPIRONE IN NEUROPHARMACOLOGIC, EEG, AND ANTICONFLICT TEST PROCEDURES. J.W. Sullivan*, K.L. Keim, and J. Sepinwall, Dept. Pharmacology I, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Buspirone HCl (B) has been reported to produce a variety of psychotropic actions including anxiolytic, antidepressant and antipsychotic effects. It has recently been suggested that B exerts an anxioselective effect mediated through dopamine receptors [Taylor et al., Pharmac Biochem Behav 17(1):25-35, 1982].

The pharmacologic effects of orally administered B were studied in several standard preclinical tests (mice, cats) and in several conflict tests (rats, squirrel monkeys). In addition, we determined the effects of B on the EEG recorded from the anterior cortex in squirrel monkeys working on a food-maintained lever pressing (VI60") schedule.

In mice, B (100 mg/kg) caused ataxia and a 70% reduction in locomotor activity. B also produced ethanol-primed loss of righting reflex (ED50 = 26 mg/kg) and deficits in rotarod performance (ED50 = 144 mg/kg). In female cats, miosis and hyperactivity were seen at 2.5 mg/kg of B, but neither ataxia nor muscle relaxation were observed up to 20 mg/kg.

In thirsty rats in a water-lick conflict test (shock for each 20th lick), B was inactive at 1.25-20 mg/kg. In food-deprived rats trained to bar press on a single lever during alternating components of a VI30" (food)/FR10 (food+shock) schedule, B produced a small (37% over control), significant increase in punished responding at the single dose of 10 mg/kg but was without anti-punishment activity at other dose levels ranging from 1.25-20 mg/kg. At 40 mg/kg, significant decreases in both punished and unpunished responding occurred. In food-deprived squirrel monkeys trained on a two lever concurrent VI1.5'(food)+VR24(shock)/VI6' (food) schedule of reinforcement, B failed to produce an anti-punishment effect at 1.25 or 2.5 mg/kg, and then produced significant decreases in both punished and unpunished responding at 5 mg/kg. Our conflict results, therefore, are in disagreement with the findings of Geller and Hartmann (J Clin Psychiat 43:12 (Sec. 2) Dec. 1982), which indicated B to be equipotent to diazepam as an anxiolytic in rat and cynomolgus monkey.

In the EEG/lever-press experiment with squirrel monkeys, B (2-16 mg/kg) also reduced food-reinforced responding while producing only minor changes in the frequency distribution of the EEG. At the higher doses, the monkeys clung to the cage (8 mg/kg), exhibited ataxia, tremors, and were prostrate (16 mg/kg).

Thus B does not produce antianxiety effects in several conflict paradigms or changes in EEG activity consistent with those seen with more classical anxiolytic drugs (benzodiazepines, meprobamate, phenobarbital).

128.3 ADAPTATION OF CONDITIONED TASTE AVERSION TO SCREENING ANXIOLYTIC DRUGS. Gregory Ervin* and Barrett R. Cooper, Department of Pharmacology, Wellcome Research Labs, Res. Triangle Park, NC 27709

When rats are exposed to a novel flavor, such as that produced by saccharin, and then made ill by injection of certain drugs, the animals subsequently display greatly reduced consumption of solutions containing that flavor. This effect, "conditioned taste aversion," was used in our laboratory to develop a conflict (thirst vs taste aversion) as a screen for anxiolytic drugs. After male Long Evans rats (240-300 gm) were acclimated to a schedule of drinking for 15 min a day, they were offered 0.25% saccharin solution instead of water. Fifteen minutes after this first exposure to saccharin, rats were treated with 25 mg/kg l-5-hydroxytryptophan (5-HTP) i.p. This dose of 5-HTP was used because it produces a mild aversion which appears to be due to an action peripheral to the blood brain barrier (Ervin et al., unpublished data). Forty-eight hours after the initial exposure to saccharin, rats that had been treated with 5-HTP drank only 6 ml while control rats (those receiving the vehicle for 5-HTP on the initial conditioning exposure to saccharin) consumed 12 ml of saccharin solution. This 5-HTP-induced conditioned reduction in saccharin consumption could be antagonized by treatment with clinically effective benzodiazepine and non-benzodiazepine anxiolytic drugs 1 hr before the second saccharin exposure. The ED_{50} values for representative drugs are lorazepam ($ED_{50} \approx 0.3$ mg/kg i.p.), diazepam ($ED_{50} \approx 1$ mg/kg i.p.), chlordiazepoxide ($ED_{50} \approx 5$ mg/kg i.p.), oxazepam ($ED_{50} \approx 6$ mg/kg i.p.), phenobarbital ($ED_{50} \approx 15$ mg/kg i.p.), chlormezanone ($ED_{50} \approx 36$ mg/kg i.p.), and meprobamate ($ED_{50} \approx 65$ mg/kg i.p.). These ED_{50} values are approximately the same as values reported in the literature using other conflict procedures. Representative agents which do not antagonize the taste aversion are d-amphetamine, dilantin, atropine, morphine, imipramine, chlorpromazine, naloxone, muscimol, apomorphine, cyproheptadine (and others). In conclusion, the taste aversion conflict procedure appears to be as sensitive to benzodiazepine and non-benzodiazepine anxiolytic drugs as other experimental conflict procedures, yet does not require elaborate operant equipment or programming. Its simplicity and specificity suggest that it will have application to screening for anxiolytic drugs.

128.4 INTERACTIONS OF THE ANXIOSELECTIVE AGENT BUSPIRONE WITH CENTRAL SEROTONIN SYSTEMS. Michael S. Eison, Cam P. VanderMaelen, G. Keith Matheson[1], Arlene S. Eison, and Duncan P. Taylor. CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville, IN 47721, and [1]Department of Anatomy, Indiana University School of Medicine, Evansville, IN 47732.

Although nonbenzodiazepine in both structure and pharmacology, buspirone (Buspar™; Mead Johnson Pharmaceutical Division) has demonstrated clinical efficacy in the treatment of anxiety neuroses comparable to diazepam and clorazepate (Goldberg & Finnerty, J. Clin. Psych. 43: 87,1982). While the mechanisms responsible for buspirone's anxiolytic efficacy in the absence of the ancillary pharmacological properties which characterize the benzodiazepines remains elusive, some authors have hypothesized a role for serotonin in the mediation of buspirone's effects (Hjorth & Carlsson, Eur. J. Pharm. 83: 299, 1981). We have therefore investigated buspirone's interactions with central serotonin systems in the rat behaviorally, neurochemically, and electrophysiologically.

Rats were made supersensitive to 5HT agonists by intraventricular administration of 5,7-dihydroxytryptamine (5,7-DHT). When dosed with L-5-hydroxytryptophan, they demonstrated a 5HT syndrome defined by the presence of forepaw tread, tremor, headwave, straub tail, rigidity, and abducted posture at doses 10-20 fold less than unlesioned controls. Buspirone did not induce all of these signs when administered i.p. (up to 10 mg/kg) or orally (up to 50 mg/kg). Rather, buspirone only induced mildly straub tail and a splayed, as opposed to abducted, posture. In other experiments, oral administration of buspirone to rats unilaterally lesioned in the medial forebrain bundle with 5,7-DHT did not result in consistent rotational behavior. While in binding studies buspirone is essentially devoid of activity at $5HT_1$ sites exhibiting less than 1 percent of the potency of 5HT itself upon rat cortical membranes, it does displace [^{3}H]spiperone from $5HT_2$ sites with an IC_{50} value of 740 nM. Preliminary results from single unit recordings in chloralhydrate anesthetized rats indicate that buspirone administered i.p. (2.5 - 10 mg/kg) or orally via intragastric intubation (1.0 -10 mg/kg) causes inhibition of spontaneous firing of serotonergic dorsal raphe neurons. The role of serotonin in buspirone's anxiolytic activity as reflected in its anticonflict effects will also be discussed.

128.5 CHRONIC TREATMENT WITH BUSPIRONE DOES NOT ALTER DOPAMINE RECEPTOR BINDING. Deborah K. Hyslop*, Jerry A. Becker*, Margaret Crane*, L. A. Riblet, and Duncan P. Taylor. Preclinical CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN 47721.

Buspirone (Buspar™, Mead Johnson Pharmaceutical Division) is a new antianxiety agent with clinical efficacy comparable to that of the benzodiazepines (Am. J. Psychiat. 136: 1184-1187, 1979; J. Clin. Psychiat. 43 12 [Sect. 2]: 81-86, 87-91, 92-94, 1982). Buspirone is not only chemically distinct from the benzodiazepines, but it also presents a clinical pharmacologic profile which is "anxioselective": it relieves anxiety but lacks the accompanying ancillary properties of benzodiazepines (sedation, muscle relaxation, seizure control). No conclusive interaction with the benzodiazepine-GABA-chloride ionophore complex has been demonstrated. Buspirone does interact with dopamine receptor binding sites in vitro, and some of its pharmacologic properties are shared either by dopamine agonists or dopamine antagonists (J. Clin. Psychiat. 43 12 [Sect. 2] 11-16, 1982). To identify potential concerns associated with long-term clinical usage, we administered anxiolytically-relevant doses (two times the MED value from Vogel conflict testing: 2 mg/kg for buspirone, 0.5 mg/kg for trifluoperazine) orally three times a day for 29 days to male Sprague-Dawley rats. One day later, regions of the brain were removed and analyzed for in vitro radioreceptor binding. The results of this study (see table below) show that buspirone did not alter dopamine receptor binding. Similar results are reported by McMillen (this volume). Unlike dopamine antagonists, buspirone does not induce catalepsy in rats. In fact, buspirone reverses catalepsy induced by dopamine antagonists. It also reduced type 2 serotonin binding in the cerebral cortex by a selective decrease in the number of binding sites. Taken together, these data suggest that buspirone will not produce extrapyramidal side effects or tardive dyskinesia following clinical use. Furthermore, the alteration in $5\text{-}HT_2$ binding suggests a potential mechanism for the clinically-observed decrease in symptoms of depression in patients following treatment with buspirone (see Taylor et al., this volume).

Ligand:	Spiperone	Muscimol
Brain Region:	Striatum	Substantia Nigra
Binding Site:	Dopamine	GABA
Treatment		
Vehicle	16.2 ± 1.9 (14)	8.3 ± 1.1 (12)
Buspirone	14.8 ± 1.3 (13)	7.1 ± 1.4 (11)
Trifluoperazine	21.3 ± 1.6 (12)*p<0.05	9.0 ± 1.2 (11)

The data are mean fmol bound/mg protein ± SEM (number of animals).

128.6 CHRONIC TREATMENT WITH BUSPIRONE REDUCES TYPE TWO SEROTONIN BINDING SITES. Duncan P. Taylor, Jerry A. Becker,* Margaret Crane,* Deborah K. Hyslop,* L. A. Riblet, and D. L. Temple, Jr. CNS Research, Pharmaceutical Research and Development Division, Bristol-Myers Company, Evansville, IN 47721.

Buspirone (Buspar™, Mead Johnson Pharmaceutical Division) is a new antianxiety agent with clinical efficacy comparable to the benzodiazepines. However, buspirone enjoys chemical and pharmacologic distinction from this class of drugs; it is truly "anxioselective" (J. Clin. Psychiat. 43 12 [Sec. 2] 11-16, 1982). It has been suggested that antianxiety agents given in the proper therapeutic regimen may be efficacious not only in the treatment of anxiety but in the treatment of depression as well. Clinical trials with benzodiazepine anxiolytics to substantiate this suggestion have been equivocal (Feighner, Mod. Probl. Pharmacopsychiat. 18 196, 1982). In addition to its efficacy in the relief of symptoms of anxiety neurosis, clinical trials of buspirone demonstrated antidepressant effects as well (Am. J. Psychiat. 136: 1184, 1979; J. Clin. Psychiat. 43 12[Sec. 2] 81, 87, 1982). To investigate the biochemical mechanism of this clinical observation, we administered anxiolytically relevant doses (two times the MED value from Vogel conflict testing; 2 mg/kg) orally three times a day for 29 days to male Sprague-Dawley rats. One day later, cerebral cortices were removed and analyzed for in vitro radioreceptor binding. The results of this study (see table below) show that buspirone treatment decreased β-adrenergic and type 2 serotonin (S_2) binding. This effect was due to a decrease in the number of binding sites. Chronic treatment with antidepressants has been reported to decrease either β-adrenergic or S_2 receptors (J. Clin. Psychopharmacol. 1 [6, supplement] 17S-22S, 1981). The molecular changes observed here after chronic buspirone treatment suggest a potential mechanism for the clinically-observed antidepressant effects of this unique new drug.

Ligand:	Dihydroalprenolol	Spiperone
Binding site:	β-adrenergic	$Serotonin_2$
Treatment		
Vehicle	26.8 ± 0.5 (14)	20.0 ± 1.1 (14)
Buspirone	25.0 ± 0.7 (13)*	16.9 ± 0.7 (13)*

The data are mean fmole bound/mg protein ± SEM (number of animals)
*$p < 0.05$ vs vehicle.

128.7 INDICATIONS OF SEROTONERGIC INVOLVEMENT IN THE ACTIONS OF A POTENTIAL NONBENZODIAZEPINE ANXIOLYTIC: MJ 13805. Arlene S. Eison, Michael S. Eison, L. A. Riblet, and D. L. Temple, Jr. . CNS Research, Pharmaceutical Research and Development Division of the Bristol-Myers Company, Evansville, IN 47721.

MJ 13805, known chemically as 4,4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione hydrochloride was originally synthesized by W. Lobeck and D. L. Temple, Jr. While nonbenzodiazepine in both structure and pharmacology, its potential utility as an anxiolytic drug is reflected in its potent anticonflict effects and inhibition of shock-elicited aggression in pairs of mice selected for their propensity to fight. Understanding the mechanism of action of potential antianxiety drugs such as MJ 13805 whose pharmacology represent dramatic departures from those of the prototypical class of anxiolytic drugs, the benzodiazepines, can add much to our understanding of the neural substrates which subserve anxiety states.

Recent studies suggest that central serotonin may play an important role in MJ 13805's diverse pharmacological effects. In animals unilaterally lesioned in the medial forebrain bundle with the serotonin neurotoxin 5,7-dihydroxytryptamine and administered MJ 13805, the number of turns observed in animals was low, but the direction of rotation was invariably contralateral. Neurochemical determinations revealed the animals to have at least 90% depletions of serotonin and 5-HIAA while dopamine turnover was relatively unaffected. MJ 13805 administration induces a serotonin syndrome in rats depleted of central serotonin by intraventricular 5,7-dihydroxytryptamine and allowed sufficient time to become supersensitive. However, the MJ 13805-induced syndrome differs somewhat in its topography from that induced by L-5-hydroxytryptophan in that it results in a splayed posture as opposed to an abducted one, minimal head weaving, but robust straub tail, tremor, treading, and rigidity. While not inherently altering vocalization threshold in rats, such lesions alter the effects of MJ 13805 upon this nociceptive measure. Whereas in unlesioned animals, MJ 13805 shifts the vocalization threshold towards hypergesia at anxiolytically relevant doses, MJ 13805 in serotonin lesioned rats is without effect upon vocalization threshold. That the serotonergic activity suggested by the above results is relevant to the anxiolytic effects of MJ 13805 is demonstrated by the loss of MJ 13805's potent anticonflict effects in animals depleted of central serotonin by these lesions. These data suggest that serotonin may play an important role in the behavioral effects of this nonbenzodiazepine anxiolytic candidate.

128.8 EFFECTS OF ANTIDEPRESSANTS, ANXIOGENIC SUBSTANCES AND BENZODIAZEPINE ANTAGONISTS ON THE SELF-REGULATION OF ICSS DURATION. S. Gerhardt* and J.M. Liebman (SPON: M. Roffman). Res. Dept., Pharma. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Rats self-regulate the duration of intracranial self-stimulation (ICSS) when they are placed in a shuttlebox where the interruption of an infrared beam initiates a continuous train of stimulation pulses through an indwelling electrode. They terminate the stimulation by interrupting another beam at the opposite end of the shuttlebox. Latency to initiate stimulation (the ON latency) is considered to correlate inversely with reward value of stimulation. For example, it was previously found that this latency was reduced by two stimulant drugs (d-amphetamine, pipradrol) that enhance release of dopamine (Liebman, Neurosci. Biobehav. Rev., 7:45, 1983). The latency to terminate stimulation (the OFF latency) appears to correlate inversely with stimulation-induced aversiveness, and is preferentially elevated by anxiolytic drugs (Gerhardt et al., Pharmacol. Biochem. Behav., 16:795, 1982). However, to date no drug has been reported to decrease the OFF latency preferentially. Such an effect might suggest anxiogenic activity by analogy with the opposite effects of anxiolytics. In order to explore this hypothesis, and at the same time verify the apparent association between dopamine facilitation and ON latency decrements, a wide variety of antidepressant and psychomotor stimulant drugs was evaluated.

Rats with lateral hypothalamic electrodes were tested in 10 min sessions, during which ON and OFF latencies were automatically compiled. Drugs were administered intraperitoneally 30 min before testing, except that RO 15-1788 was given orally 15 min prior to testing.

Amineptine (3-30 mg/kg) and nomifensine (3 & 10 mg/kg), which block dopamine reuptake, reduced the ON latency, as does bupropion (Liebman, 1983). Caffeine (10 & 30 mg/kg), an adenosine antagonist that enhances catecholamine levels, also reduced the ON latency. None of these substances reduced the OFF latency, a finding that excludes nonspecific behavioral excitation as an explanation of the observed effects. These findings strengthen the observed inverse relationship between drug-induced enhancement of dopamine neurotransmission and reduction of the ON latency. Neither viloxazine (10 & 30 mg/kg) nor the $alpha_2$ adrenoceptor antagonist, CGS 7525A (3-30 mg/kg), reduced either latency. Pentylenetetrazol, an anxiogenic substance, failed to reduce the OFF latency at subconvulsive doses (2.5-15 mg/kg). The benzodiazepine antagonist, RO 15-1788, also failed to alter either latency at doses of 0.3-100 mg/kg; 300 mg/kg elevated both latencies non-selectively. Thus, the hypothesis that an anxiogenic substance or a benzodiazepine antagonist would selectively decrease the OFF latency remains to be experimentally verified.

128.9 POSITIVE CORRELATION BETWEEN SEROTONIN ANTAGONISM AND ANTICONFLICT ACTIVITY. Boast, C., Popick, F.*, Stone, G.* and Kalinsky, H.* Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Previous reports have shown that certain drugs with serotonin (5HT) antagonist properties increase punished responding (Stein et al., Am. J. Psychiat., 34:665, 1977). The present study examined the relationship between the degree of selective serotonin antagonism and the degree of anticonflict effect shown by various drugs. The drugs selected were: spiperone, cyproheptadine, mianserin, cinanserin, metitepine, methysergide, quipazine, pipamperone, pirenperone and CGS 7525A. In addition, the novel 5HT agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Hjorth et al., J. Neural Trans., 55:169, 1982), was studied for possible selective presynaptic agonist activity. All drugs were assessed for their ability to displace ^{3}H-5HT (7 nM) from membrane preparations of calf caudate (Bennett & Snyder, Molec. Pharm., 12:323, 1976). Activity in this assay was used as an index of 5HT agonism (Peroutka & Snyder, Molec. Pharm., 16:687, 1979). The drugs were also assessed for their ability to displace ^{3}H-ketanserin (0.4 nM) from membrane preparations of rat prefrontal cortex. Activity in this assay was used as an index of 5HT antagonism (Leysen et al., Molec. Pharm., 21:301, 1982). A measure of the degree of selectivity as a 5HT antagonist was obtained by calculating the ratio of the agonist and antagonist IC_{50} values.

Anticonflict effects were assessed using a lick-suppression paradigm (Vogel et al., Psychopharmacologia, 21:1, 1971) and/or a paradigm using alternating unpunished and punished operant schedules (Davidson & Cook, Psychopharmacologia, 15:159, 1969). Relative anticonflict efficacy was determined by activity in one or both tests, dose response relationships and magnitude of effect.

Of the eleven drugs tested, seven showed some degree of anticonflict activity. These seven active compounds showed a positive correlation between degree of anticonflict effect and degree of selective 5HT antagonism. The four inactive compounds were quipazine, 8-OH-DPAT, pipamperone and spiperone. Quipazine showed only weak 5HT antagonist activity, consistent with the view that this action may be responsible for anticonflict effects. It was confirmed that 8-OH-DPAT is an agonist which did not appear to manifest selective presynaptic agonism at low doses. Both spiperone and pipamperone are dopamine antagonists which may mask the anticonflict effect. This study confirms previous reports that 5HT antagonists can manifest anticonflict activity and extends that finding to a large number of drugs. In addition, the degree of specificity of 5HT antagonism is correlated with the degree of anticonflict effect.

128.10 INTERACTION OF ETHANOL WITH ANXIOLYTICS OR HYPNOTICS IN MICE ON THE INCLINED SCREEN: AN ANIMAL MODEL OF DRUG POTENTIATION. K. L. Keim and S. Furman* (SPON: L.R.Klevans) Pharmacology I, Hoffmann-La Roche Inc., Nutley, N. J. 07110

Using isobolograms we demonstrated that ethanol (ETOH) potentiated the effect of diazepam or lorazepam in causing the loss of righting reflex (LRR), whereas, these interacted agents were additive when tested for impairment on the traction wire test [Davidson, Furman & Keim. Soc Neurosci Abstr 8: 470, 1982]. We expanded these studies with the following experiments using different endpoints. As before, ETOH was given to fasted CF1 male mice per os 20 min after oral administration of a test compound and performance was measured 10 min later in two procedures: rotarod (RR; 16 rpm, 5 cm dia) and inclined screen (IS; 8" x 11" wire mesh field; 70° angle). Isobols were constructed as mentioned previously [also see Loewe, S. Pharmacol Rev 9: 237, 1957]. We found that the diazepam-ethanol isobol for RR impairment indicated the interaction to be one of additivity, whereas, the IS impairment endpoint is one of potentiation. Therefore, selected drugs were evaluated in the latter test to estimate possible ethanol potentiation:

Compound	I IS ED50 Alone mg/kg PO	E IS ED50 with ED5 ETOH	I/E Ratio
Alprazolam	>400	0.16	>2500
Lorazepam	168	5	34
Diazepam	72	5.6	13
Phenobarbital	120	31	4
Buspirone	>200	>100	*
Fenobam	>200	>100	*
Zopiclone	>200	>260	*
Triazolam	>400	0.25	>1600
Temazepam	5	2.2	2.3
Midazolam	15	7.2	2.1
Flurazepam	200	27	7.4

A range in the degree of potentiation (I/E ratio occurred with compounds that alone impaired IS. The effect of nonbenzodiazepines (BZ) on IS was not altered by ETOH. In contrast, IS impairment by the triazolo-BZs was markedly potentiated by ETOH. While the large I/E ratio may be a numerical artefact, alprazolam and triazolam are quite distinct from the other BZs tested in their interaction with ETOH in these and other tests.

128.11 YOHIMBINE: A BETA CARBOLINE WITH BEHAVIORAL AND NEUROCHEMICAL PROPERTIES COMMON TO ANXIOGENIC DRUGS. Harbans Lal, Gary Shearman,* Debra Bennett,* and Agnes Horvat* Dep. of Pharmacology, Texas Col. Osteopathic Med., Fort Worth, TX 76107, & Univ. of Rhode Island, Kingston, R.I. 02881.

Yohimbine is an alpha-2 adrenoreceptor-antagonist and is reported to be anxiogenic in humans. It has, therefore, been postulated that the alpha-2 adrenoreceptor modulation may be the basis of the anxiogenic activity of yohimbine. Structurally, yohimbine may be considered to be a beta carboline derivative, a class of drugs known for their anxiogenic action and for interaction with the benzodiazepine-GABA receptor system. We, therefor, investigated the possibility of yohimbine producing anxiogenic action related to its beta carboline structure, by comparing its anxiety-related pharmacological profile with those of other beta carbolines. Anxiogenic drugs produce interoceptive discriminable-stimuli (IDS) which generalize to those of pentylenetetrazol (PTZ) in a drug-discrimination paradigm and are selectively antagonized by anxiolytic drugs (Lal and Shearman, Ann. Rep. Med. Chem. 15: 51-58, 1980). Beta carboline-type anxiogenics resemble other anxiogenics in this respect, and also displace benzodiazepines from binding sites in the brain. In the present experiment beta carboline-3-carboxylate (BCC) methyl ester and harmane generalized to PTZ in a dose-dependent manner in rats trained to discriminate PTZ from saline. yohimbine (0.16-10 mg/kg) and BCC ethyl ester also generalized to PTZ; however, BCC ethyl ester was toxic at the higher doses necessary for complete generalization and yohimbine produced a bell-shaped dose-response curve. The IDS produced by these drugs, including yohimbine, showed pharmacological characteristics related to anxiety since they were antagonized by diazepam dose-dependently. In addition, similar to the known neurochemical action of beta carbolines, yohimbine inhibited 3H-flunitrazepam binding to cerebral cortex synaptosomes with potency similar to that of harmane (IC50, 7 uM). Clonidine was ineffective in either antagonizing PTZ-IDS or in displacing flunitrazepam from its binding site. Based upon the resemblence in structure and pharmacological profile characteristic of anxiogenic beta carbolines, we suggest that anxiogenic action of yohimbine is mediated through neurobiological mechanisms underlying the beta carboline action, i.e., interaction with benzodiazepine-GABA system. Our hypothesis is further supported by our observation that neither behavioral nor neurochemical actions described above were sensitive to a well established alpha-2 adrenoreceptor agonist.

128.12 ONTOGENY OF THE ANXIOLYTIC PROPERTIES OF CHLORDIAZEPOXIDE. Elizabeth Ochoa* and Gordon A. Barr*, Dept. of Psychology Hunter College, CUNY and Dept. of Psychiatry, Albert Einstein College of Medicine, New York, New York (SPON: W.H. Bridger).

A hallmark action of benzodiazepines is to release suppressed behavior. This effect correlates well with benzodiazepine's anxiolytic actions in humans. Previous research has shown that the benzodiazepines interact with at least two high affinity benzodiazepine (Type 1, Type 2) receptors. Further, in the rat, the ontogenesis of these benzodiazepine receptors differs, with the Type 2 receptor present at birth and the Type 1 receptor developing during the second week. We conducted a parallel behavioral study in infant rats to those used in adult rats, to determine at what age the behavioral effects of these drugs become apparent.

Rat pups were tested with chlordiazepoxide (CDZ) at either 10 or 14 days of age in one of two conditions: milk tainted with quinine or milk only (Half & Half). Quinine, which is bitter to the taste, suppresses milk intake in rat pups. All pups were isolated from their mothers and littermates for 8 hours but kept at 33° C. Anterior tongue cannulas were implanted and the pups' weights, following voiding, were recorded. CDZ was dissolved in distilled water and one of three doses (0.3, 1.0 or 3.0 mg/kg) or the water vehicle was injected intraperitoneally in a volume of 1.0 mg/100 g body weight. Milk or the adulterated milk was infused immediately after injection for one hour. The pups were reweighed, and the increase over the hour expressed as grams or as percent of preinfusion weight.

We found that CDZ produced no significant alteration of intake of either milk or milk + quinine in the younger 10 day old pups. In the 14 day olds, however, CDZ produced a dose dependent increase in ingestion of the adulterated milk (ANOVA, $p < .005$) with both the 1.0 and 3.0 mg/kg doses significantly enhancing intake. In the milk only condition, the drug effect was not significant ($p > .05$).

Our behavioral data correspond to the ontogeny of the Type 1 but not the Type 2 benzodiazepine receptor. Prior to the development of the Type 1 receptor release from suppression was not found. However, with the rapid development of the Type 1 receptor, occurring during the second week, was found the rapid onset of release from suppression. These data provide further evidence for the role of the Type 1 receptor in the anxiolytic actions of the benzodiazepines, and offer a model for studying the physiological roles of these two receptor types. Further, the data correlate strongly with the onset of the ability of opiate antagonists to modify milk ingestion, adding support to the speculation of opiate-benzodiazepine interactions in food and water intake.

128.13 INTERACTION BETWEEN HARMANE AND BETA-RECEPTORS, B. Delbarre, G. Delbarre and A. Ferger*. Lab. Ch. Exp., Faculté de Médecine 37032 TOURS, FRANCE.

Harmane is a very potent inhibitor of specific (^{3}H)-flunitrazepam binding to rat brain and bovine retina membranes, two tissues containing brain specific BDZ receptors (Braestrup C. et al., Br. J. Psychiatr. 133 : 249, 1978). In contrast to several other ß-carbolines, the CNS excitatory and convulsive properties of harmane are much more pronounced (Sigg E.B. et al., Arch. Int. Pharmacodyn. 149 : 164, 1964). Thus, if harmane acts endogenously at the BDZ receptor it elicits - rather benzodiazepine antagonistic effects. Anticonvulsivant effect of BDZ are highly correlated with their anxiolytic properties (Paul S.M., Psycho pharma. Bulletin, 16, 1 : 9, 1980). Recently V.H. Sethy and al. (Soc. Neurosci. Abstr. Vol. 8 : 376, 1982) demonstrated role of ß-adrenergic receptors in the antidepressant activity of some BDZ. In harmane convulsions in mice, we have compared Diazepam and a $ß_2$-stimulant agent, Clenbuterol with antidepressant activity (Delbarre G. and al., Soc. Neurosci. Abstr. Vol. 5 : 553, 1979). Diazepam (1 $mgkg^{-1}$ IP) and Clenbuterol (0,5 $mgkg^{-1}$ IP) antagonize harmane convulsions in mice. These results suggest that antidepressive activity of some BDZ may be correlated with their activity on harmane receptors.

129.1 REGIONAL DISTRIBUTION OF DYNORPHIN AND NEO-ENDORPHIN PEPTIDES IN RAT BRAIN, R.I. Cone[1], E. Weber*, J.D. Barchas and A. Goldstein. Addiction Research Foundation, Palo Alto, CA 94304 and Stanford University, Stanford, CA 94305

Five products of the dynorphin gene -- α-neo-endorphin (α-Neo), β-neo-endorphin (β-Neo), dynorphin A (Dyn A), dynorphin A-(1-8) (Dyn A-8), and dynorphin B (Dyn B) -- were measured in various regions of rat brain by RIA. Specific antisera were used, supplemented by gel permeation analysis and HPLC.

Antisera directed against α-Neo, β-Neo or Dyn A-8 recognize single classes of peptide which co-elute on HPLC with the respective synthetic peptides. On the other hand, antisera directed against Dyn A or Dyn B recognize larger sized dynorphins in addition to the respective synthetic peptides. For example, in brain, there were three gel-sizing peaks of immunoreactive (ir)-Dyn A and ir-Dyn B which either overlapped (2-kDal) or coincided exactly (4- and 7-kDal). On HPLC, the 2-kDal ir-Dyn A and ir-Dyn B eluted in the same positions as synthetic Dyn A and Dyn B, respectively and the 4-kDal peaks eluted in the same position as synthetic dynorphin-32 (ie. another gene product with Dyn A at the amino terminus followed by the "processing signal" Lys^{18} - Arg^{19}, and then by Dyn B at the carboxyl terminus). The 7-kDal peaks were not characterized further.

In order to compare tissue amounts of the five gene products, estimates of Dyn A and Dyn B required the subtraction of the 4- and 7-kDal ir-Dyn A and ir-Dyn B. In whole brain, α-Neo, Dyn A-8 and Dyn B were present in greater amounts than β-Neo and Dyn A. Although a general parallelism was found in the regional distribution of the five peptides, there were also noteworthy exceptions, suggesting that differential processing may occur.

Some dynorphin gene products are mutually exclusive because they contain the same sequence. (ie. one molecule of the precursor, pro-dynorphin, could yield Dyn A-8 or Dyn A but not both). In some brain regions, we found a reciprocal relationship between the amounts of some mutually-exclusive products. If one assumes that the peptides are present in approximate proportion to their relative rates of production, it would follow that the processing of pro-dynorphin may take different pathways in different regions. Since the dynorphin gene products have different degrees of opiate receptor selectivity and biologic activity, differential processing might therefore be a mechanism for regulating function in the dynorphin system. Supported by NIDA grants DA-01207; DA-1199, NIH grant NS-18098 and NSF grant BNS-91-07237.

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129.2 STORAGE SITE OF LOW-MOLECULAR WEIGHT MET-ENKEPHALIN IMMUNOREACTIVITY IN BLOOD PLATELETS. G. B. Picotti*, A. M. Di Giulio, L. Borriero*, M. D. Galva* and M. Da Prada*°. Dept. of Pharmacol., Univ. of Milan, I-20129 Milan, Italy and °Pharm. Res. Dept., Hoffmann-La Roche, CH-4002 Basle, Switzerland.

A met-enkephalin immunoreactive material (ME), comigrating with synthetic met-enkephalin on TLC, gel permeation and HPLC, has recently been detected in human and animal blood platelets (Di Giulio et al., Life Sci. 30, 1605, 1982). Moreover, indirect evidence has been obtained suggesting a common subcellular localization for human platelet ME and 5-hydroxytryptamine (5-HT), i.e., the 5-HT organelles.

We have further investigated the subcellular distribution of platelet ME by direct assessment of ME concentrations in subcellular fractions of rabbit platelets isolated by urographin density gradient centrifugation. The results obtained indicate that ME, like 5-HT and catecholamines, is concentrated to a much grater extent in the 5-HT organelles than in any other subcellular fraction or in intact platelets. BioGel P-2 chromatography of granular ME revealed that it behaves identically with synthetic met-enkephalin. As in intact human and rat platelets (same ref.), no high-molecular weight ME could be detected in 5-HT granules from rabbit platelets, even after trypsin and carboxypeptidase B hydrolysis, using two antisera which are able to recognize some of the putative met-enkephalin precursors present in the adrenal gland or striatum. The apparent lack of ME precursors in platelets, together with the fact that platelets do not take up met-enkephalin in vitro from plasma or physiological media might indicate either that there is a different synthetic pathway for ME from those in other tissues (e.g., the adrenal medulla), a carry over mechanism from the megakaryocyte stage, or other less-likely alternatives.

Chronic administration of the 5-HT precursor 5-hydroxytryptophan to headache patients significantly increased both 5-HT and ME platelet concentrations. A 14-day treatment with the amine-uptake inhibitor amitriptyline also increased platelet ME concentrations while decreasing 5-HT content.

Additional results provide evidence that human and animal platelets are rich in enkephalin-degrading enzymes, i.e., aminopeptidase, but also the possibly more specific enkephalinases, mainly of the B type.

129.3 IMMUNOCYTOCHEMICAL LOCALIZATION OF ACTH IMMUNOREACTIVE PERIKARYA IN NUCLEUS TRACTUS SOLITARIUS. S.A. Joseph and W.H. Pilcher*. The Neuroendocrine Unit, Univ. of Rochester, Rochester, NY 14642.

To date numerous investigations have indicated that neuronal perikarya containing opiocortin peptides including ACTH, β-endorphin, β-lipotropin and αMSH reside within the arcuate and periarcuate region of medial basal hypothalamus. An extensive fiber distribution emanating from this hypothalamic neuronal pool has been shown to project to forebrain, limbic diencephalic and brainstem structures. In the brainstem opiocortin immunoreactive fibers have been observed in periaqueductal gray (PAG), various catecholamine containing regions, specific cranial nuclei and autonomic centers. Our preliminary lesion and hypothalamic deafferentation studies suggest that the origin of immunoreactive fibers within several caudal brainstem centers may be derived from an additional pool of opiocortin containing neurons.

In this report we reveal a previously unidentified group of opiocortin containing neurons in the nucleus tractus solitarius (NTS). These immunocytochemically demonstrable ACTH-neurons are confined to the caudal medulla and reside within the commissural portion of NTS. This medullary neuronal pool could be visualized only in the brains of animals in which colchicine had been infused into the lateral or 4th cerebral ventricle. Using the ABC method of immunocytochemistry on Bouin's fixed Vibratome sections, these medullary perikarya were immunoreactive with antisera generated against $ACTH^{1-39}$ and the 16K fragment (Mains and Eipper) of the pro-opiocortin molecule.

In this medullary region ACTH-ir fibers were observed within all divisions of NTS, as well as tractus solitarius throughout their rostral-caudal extent. Additional fibers were observed in the parabrachial nucleus, nucleus ambiguus and various portions of the medullary reticular nuclei.

The presence of this medullary opiocortin neuronal pool suggests that a more complex organization of the central opiocortin system exists with a separate component apparently associated within the NTS brainstem autonomic center.

129.4 ENKEPHALINS IN THE PITUITARY GLAND: IMMUNOHISTOCHEMICAL AND BIOCHEMICAL CHARACTERIZATION. P. Panula*, I. Lindberg*, H.-Y.T. Yang and E. Costa (SPON: Joan S. McIntosh) Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Antisera against $[Met^5]$-enkephalin, $[Met^5]$-enkephalin-Arg^6-Phe^7, $[Met^5]$-enkephalin-Arg^6-Gly^7-Leu^8, Phe-Met-Arg-Phe-NH_2 and β-endorphin were used to localize these immunoreactivities in the rat pituitary gland using indirect immunofluorescence. $[Met^5]$-enkephalin- and $[Met^5]$-enkephalin-Arg^6-Gly^7-Leu^8-like immunoreactivities (ME-LI and MEAGL-LI) were found only in nerve terminals in the perimeter of the posterior lobe (PL). $[Met^5]$-enkephalin-Arg^6-Phe^7-like immunoreactivity (MEAP-LI) was found in the same location in the PL. In addition, most cells in the intermediate lobe (IL) exhibited MEAP-LI. Intermediate lobe MEAP-LI was not due to cross-reactivity of the antiserum with β-endorphin, since preabsorption of the antiserum with MEAP but not with β-endorphin diminished the staining. When consecutive sections were processed for MEAP-LI and Phe-Met-Arg-Phe-NH_2-like immunoreactivity, different immunofluorescence patterns were observed. Phe-Met-Arg-Phe-NH_2-like immunoreactivity was distributed throughout the PL. The specificity of all immunocytochemical procedures was confirmed using appropriate absorption controls. Enkephalin-immunoreactive peptides were characterized in acid extracts of fresh pituitaries by gel filtration on Bio-Gel P-30. While more than 90% of ME-LI and $[Leu^5]$-enkephalin-like immunoreactivities were recovered in the elution positions of the authentic pentapeptides, only 17% and 25% of the MEAGL-LI and MEAP-LI, respectively, eluted in the positions of the authentic peptides. High molecular weight peptide(s) with MEAGL-LI eluted in a region of 8,000 daltons, while high molecular weight peptide(s) with MEAP-LI eluted at ca. 10,000 daltons. Considerably more MEAP-LI than MEAGL-LI was found in extracts of rat pituitaries. Since our MEAP antiserum cross-reacts with Phe-Met-Arg-Phe (but not with Phe-Met-Arg-Phe-NH_2), the possibility remains that the immunoreactivity detected in the IL is related to this tetrapeptide but does not contain MEAP. We are conducting studies to determine if the proenkephalin mRNA is present in the cells of the IL using in situ hybridization.

129.5 IMMUNOCYTOCHEMICAL DISTRIBUTION OF OPIOID PEPTIDERGIC NEURONS IN RAT NEOCORTEX. J. F. McGinty. Dept. of Anatomy, East Carolina University School of Medicine, Greenville, NC 27834.

Radiochemical evidence has implied the presence in the cerebral cortex of a widespread opioid peptide system to mediate a variety of opiate effects on cerebral cortical functioning. However, immunocytochemical detection of opioid peptides in the cortex has been limited. This study concerns the characterization of the regional and laminar distribution of a widespread population of murine neocortical cell bodies and fibers immunoreactive for pro-enkephalin-A and B.

Our preliminary observations (McGinty et al., Anat. Rec. 202: 125Z, 1982) were limited to olfactory and medial cortical regions where clusters of neurons in layers II-III containing enkephalin immunoreactivity (ir) were revealed in colchicine-treated rats. Further analysis with anti-leu^5-enkephalin as well as an anti-serum raised against the proenkephalin-A fragment, bovine adrenal medullary peptide (BAM22P), in colchicine and kainic acid-treated rats has revealed a morphoheterogeneous population of neocortical cells containing proenkephalin A-ir, including bipolar and multi-polar stellate cells and pyramidal cells. These cells reside in layers II-III and V-VI of medial and lateral neocortex. The regional and laminar distribution of the neurons containing en-kephalin-ir and BAM-ir was more easily detected, and thus, more extensive than that of cortical cells containing dynorphin A-ir which have been detected to date. The bimodal distribution of immunopositive neurons in layers II-III and V-VI of neocortex and layers II and III of paleocortex is similar to the distribution of mu-type opiate binding sites in rat cortex (Herkenham, M. and Pert, C., J. Neurosci. 2:1129, 1982). The morphoheterogeneity of cells containing pro-enkephalin-A and B immunoreactivity suggests that opioid peptides are in a position to influence the function-ing of local, commissural, and projection neurons of murine cerebral cortex.

(Supported by NS 18837.)

129.6 ENKEPHALIN-LIKE AND SUBSTANCE P-LIKE IMMUNOREACTIVITY IN THE EYESTALK NEUROENDOCRINE COMPLEX OF THE FIDDLER CRAB, UCA PUGILATOR. M. Fingerman, M. M. Hanumante* and †L. L. Vacca. Dept. of Biology, Tulane Univ., New Orleans, LA 70118 and †Dept. of Anatomy, Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

The presence and distribution of Leu-enkephalin (LE)-like, Met-enkephalin (ME)-like and Substance P (SP)-like peptides were determined in the eyestalk neuroendocrine complex of Uca pugilator using light microscopic peroxidase-antiperoxidase immunocytochemistry. Immunoreactivity for all three substances was observed in the optic nerve, sinus gland and lamina ganglionaris. LE-like and ME-like immunoreactive material was also found in the portion of the neuropile of the medulla terminalis abutting the neurosecretory cells (NSC) in the medulla terminalis X-organ. Extensive ME-like immunoreactivity was also evident in the NSC of the medulla terminalis X-organ and in axons of NSC in the medulla externa. SP-like immunostainable material was also found in the epineurium enclosing the ganglia of the eyestalk, just proximal to the retina. Interestingly, none of the somata of the NSC in this neuroendocrine complex exhibited LE-like or SP-like immunoreactivity.

129.7 COMPARATIVE STUDIES OF OPIOID PEPTIDES: ENKEPHALIN DISTRIBUTION IN TURTLE CENTRAL NERVOUS SYSTEM. Anton Reiner. Dept. Anatomy and Cell Biology, Univ. of Michigan, Ann Arbor, Mich. 48109.

High levels of opioid receptor binding sites have been reported in the CNS of all vertebrates studied, including reptiles (Pert et al., Brain Res., V75, 1974). Thus, opioid peptide neurotransmitter systems are presumably an ubiquitous feature of the vertebrate nervous system. The present study used immunohistochemical techniques to determine the distribution of enkephalin in the CNS of red-eared and painted turtles.

An antiserum against leucine-enkephalin (A206, courtesy K.J. Chang) was used initially to localize enkephalin-like immunoreactivity (ELI). The distribution of ELI observed was similar in many respects to that observed in mammalian CNS with antiserum A206 (Sar et al., JCN, V182, 1978). Numerous ELI+ neurons were consistently found in: 1) telencephalon - "striatum", septum and superficial one-fifth of the medial half of the cortex; 2) dien-cephalon - rostral two-thirds of the hypothalamus and mammillary region; 3) midbrain - deep tectal layers, medial tegmentum and the dorsal nucleus of the posterior commissure; and 4) hindbrain and spinal cord - lateral to the caudal descending tract of the trigeminus and in the dorsal horn and Lissauer's zone of the spinal cord. Dense accumulations of ELI+ fibers were observed in: 1) telencephalon - "striatum", septum, deep four-fifths of the medial half of the cortex, globus pallidus and ventral paleostri-atum; 2) diencephalon - hypothalamus, neurohypophysis, dorsomed-ial/dorsolateral thalamic nuclei and medial habenula; 3) midbrain - tectum, medial tegmentum, periventricular gray and area pretec-talis; and 4) hindbrain and spinal cord - raphe region, ventro-lateral pons and medulla, locus coeruleus, parabrachial region, nucleus of the solitary tract, vagal motor nucleus, lateral to the descending tract of the trigeminus and in the dorsal horn and Lissauer's zone of the spinal cord.

Although all ELI described above could be blocked by 10μM synthetic leucine-enkephalin, antiserum A206 has a slight crossreac-tivity with dynorphin. Accordingly, antisera against two other proenkephalin-derived opioid peptides, methionine-enkephalin (A900, courtesy K.J. Chang) and BAM-22P (#66-B16, courtesy S.J. Watson) were used. The distributions of ELI observed with all three antisera were highly similar and (particularly in the case of the BAM-22P antiserum) readily distinguishable from that ob-tained with a dynorphin A antiserum (3CS, courtesy A. Goldstein). With this dynorphin antiserum, dense staining was observed in the strionigral pathway and its ventrolateral tegmental terminal field. The similar staining patterns observed with the three en-kephalin antisera suggests that various enkephalin-like peptides may be present in turtle CNS and derive from a common precursor similar to proenkephalin of mammals. (Supported by NS-19620)

129.8 IN SITU HYBRIDIZATION OF OPIOID PEPTIDE CONTAINING CELLS IN NERVOUS TISSUES USING SPECIFIC cDNA PROBES. S.J. Watson, J. Roberts*+, R. Thompson*, S. Burke*, and H. Akil. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109; +Columbia Univ., New York, N.Y.

Recent advances in molecular genetics, opioid peptide biochemistry, and histochemical methods have made it possible to study the mRNA's coding for the opioid peptides, in their cells of origin. DNA sequences which are the compliment of portions of specific mRNA's (i.e. cDNA) coding for all three opioid peptide families have been produced in several laboratories. Sequence specific labelled cDNA probes can form a stable double stranded hybrid with a specific region of a particular mRNA under conditions which can allow identification of that mRNA in an anatomical context.

We have been focusing on the several techniques and controls necessary for the use of cDNA probes as markers for their matching mRNA in their cells of origin. After the mRNA sequence has been cloned into a cDNA form (from the lab of J.R.) a sequence of steps are requried to insert the cDNA probe into a proper bacterial phage cloning vector system, grow the phage, extract it, label the cDNA, and prepare it for use as a hybridization probe. A further series of steps are necessary to prepare tissue for in situ hybridization and then to combine the probe and tissue for visualization of the specific mRNA producing cell. Finally, before the data can be interpreted, a series of technical and logical issues need to be considered. Our use of such probes will be presented along with the aforementioned methods.

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129.9 ANATOMICAL RELATIONSHIP OF OPIATE RECEPTORS TO β-ENDORPHIN, ENKEPHALIN, AND DYNORPHIN-POSITIVE NEURONAL SYSTEMS IN RHESUS MONKEY BRAIN. M.E. Lewis*, H. Khachaturian, and S.J. Watson (SPON: C.M. Butter). Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109

The functional significance of opioid systems in primate brain is poorly understood, although some clues have been provided by previous studies of opiate receptor distribution (e.g. Lewis et al, Science, 211:1166-69, 1981). To obtain further information, we have compared the anatomical distribution of opiate receptors to that of the three opioid systems containing β-endorphin (β-END), [Leu]enkephalin (ENK), or dynorphin A (DYN)-like peptides in rhesus monkey brain. Using adjacent section PAP immunocytochemistry and in vitro receptor autoradiography (with [^{3}H]naloxone) as described for rat brain (Lewis et al., Life Sci., 31:1347-50, 1982), we have observed that binding site density varies with different opioid peptide systems in different brain regions, as summarized in the list of structures below (density: + = light, ++ = medium, +++ = heavy).

	[^{3}H]nal	POMC	ENK	DYN
bed nuc. stria terminalis	++	+++	+++	+
periventricular hypothal.	+++	+++	++	+
supraoptic nucleus	+++	0	+	+++
periaqueductal grey	++	++	+++	+
interpeduncular nucleus	+++	0	++	+
parabrachial nuclei	+++	+	++	+
nucleus tractus solitarius	++	++	+++	++
spinal treigeminal nucleus	+++	0	++	++

This partial list reflects the complex relationships of the three opioid systems in rhesus monkey brain, as well as the difficulties in anatomically relating opiate receptors to individual opioid systems. Although it is unlikely that [^{3}H]naloxone is binding to δ sites (virtually all specific [^{3}H]D-Ala^2, D-Leu^5-enkephalin binding is abolished by perfusion fixation), we are exploring possible binding site heterogeneities which may correspond to differences in the regional distribution of the three opioid systems in brain.

This work was supported by Theophile Raphael Fund to S.J. Watson.

129.10 ONTOGENY OF OPIOID AND RELATED PEPTIDES IN THE RAT BRAIN AND PITUITARY. H. Khachaturian, N.E. Alessi, M.E. Lewis*, N. Munfakh*, and S.J. Watson. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

The embryonic (E) and postnatal (P) development of the proopiomelanocortin (POMC) peptides (16K fragment, ACTH, β-endorphin, and α-MSH) were compared to that of [Leu]enkephalin (ENK) and dynorphin A (DYN) in the Sprague-Dawley rat. Further, to determine the time of origin of neurons immunoreactive$_3$(i.r.) for each peptide, timed-pregnant rats were injected with [^{3}H]thymidine at different stages (E11-17) and their progeny were allowed to develop into young adults prior to PAP immunocytochemistry combined with [^{3}H]thymidine autoradiography. All postnatal animals were treated with colchicine prior to sacrifice.

The POMC peptides (except α-MSH) appeared first on E12 in small i.r. perikarya in the hypothalamic ridge. At E15, a few i.r. POMC cells also appeared in the pituitary anterior lobe (AL). By E16, i.r. POMC fibers had reached brain stem and spinal cord. Also at E16, scattered i.r. POMC cells appeared in the intermediate lobe (IL). At E17, faintly i.r. 16K perikarya were seen in the nucleus tractus solitarius. By E19-21, all POMC perikarya appeared darker, and their projections more widespread. The pituitary appeared adult-like except for the intense immunoreactivity (i.r.) for all POMC peptides (especially α-MSH) in AL. Postnatally, brain POMC i.r. increased gradually, reaching adult-like patterns by P21-28. Conversely, α-MSH i.r. in the AL declined gradually, and appeared very faint by P28. Furthermore, the so-called "α-2" neurons of the lateral hypothalamus were not detectable with our α-MSH antiserum during embryonic stages, but were visible starting at P1, with colchicine pretreatment.

ENK and DYN i.r., on the other hand, appeared later in embryonic development. At E16, both ENK and DYN i.r. fibers could be seen in the ventral brainstem and spinal cord. By E17, diffuse DYN i.r. could be detected in the posterior pituitary. Faintly i.r. ENK perikarya were detected in the brainstem at E18-19. Also at E18-19, diffuse DYN i.r. was seen in the magnocellular supraoptic neurons. At P1, ENK i.r. perikarya were visible in many brain areas, and DYN i.r. magnocellular neurons could be seen in the hypothalamus. From P7 to P28, both ENK and DYN i.r. perikarya/fibers increased in number/density to resemble adult-like patterns.

Preliminary data gathered from [^{3}H]thymidine autoradiographic studies of immunostained neurons indicate that a majority of arcuate (POMC) and magnocellular (DYN) hypothalamic neurons are "born" at E12-14. Accordingly, we are attempting to determine the time of origin of other DYN and ENK neuronal systems.

This work was suported by NIMH grants MH15794, MH36851 to and HK and SJW.

129.11 THE IMMUNOCYTOCHEMICAL DISTRIBUTION OF ENKEPHALIN IN THE THORACOLUMBAR SPINAL CORD OF THE RAT: COINCIDENCE WITH SYMPATHETIC PREGANGLIONIC NUCLEAR REGIONS. M.A.Romagnano and R.W.Hamill, Dept. of Neurology, Monroe Community Hospital/Strong Memorial Hospital, Rochester, N.Y. 14603

The distribution of enkephalin-like(Enk-like) immunoreactive fibers was examined in thoracolumbar segments of the spinal cord of adult Sprague-Dawley rats using the unlabeled antibody method of immunocytochemistry. Normal and colchicine treated rats were perfused with Zamboni's fixative. The spinal cords were removed and 30-40μ horizontal serial sections were cut on a Vibratome. Sections were incubated for 48 hours in anti-Enk serum at a dilution of 1/2000 (Sundberg et al, Endocr.Abs.106:153,1980) or 1/500-1/1000(Immunonuclear Inc,18H2T). Immunoreactive Enk-like fibers were found in the thoracolumbar spinal cord localized in nuclear regions known to contain preganglionic sympathetic neurons. Moderate to dense numbers of Enk-like fibers were interspersed between or outlined cells of the nucleus intermediolateralis thoracolumbalis,pars principalis(Ilp). Labeled fibers were localized in a thin column along the gray-white border of the lateral funiculus connecting adjacent Ilp cell nests. Moderate numbers of Enk-like fibers also were found in the nucleus intermediolateralis thoracolumbalis, pars funicularis extending laterally from Ilp to the pial surface,perpendicular to the course of lateral funiculus fibers and often juxtaopposed to blood vessels. Immunoreactive fibers in moderate numbers were located in nucleus intercalatus spinalis (IC) forming a series of transverse bands linking the clusters of neurons of Ilp with the more medial central autonomic areas. Labeled fibers were found both dorsal and dorsolateral to the central canal. The dorsal group of Enk-like fibers formed a continuous column running the entire rostro-caudal length of the thoracolumbar cord while the fibers located dorsolateral to the central canal appeared to form arches interconnecting adjacent IC nuclear groups. These regions correspond to the dorsal commissural nucleus and the nucleus intercalatus, pars paraependymalis, respectively. At lumbar segments there is an increase in the number of Enk-like fibers found in the dorsal commissural nucleus as compared to thoracic segments. Enk-like fibers and cells were observed in the lateral funiculus, dorsal and ventral horns of thoracolumbar segments and in the cervical and sacral horn. The distribution at these locations corresponds to previously published reports.

Our results demonstrate that the distribution of Enk-like fibers in the rat thoracolumbar spinal cord parallels the distribution of autonomic nuclear regions containing sympathetic preganglionic neurons. In addition, our findings provide anatomical evidence to suggest a possible involvement of the opioid peptide, enkephalin in sympathetic functions. BRSG #5-27238.

129.12 COMPARISON OF THE DISTRIBUTION OF PRODYNORPHIN AND PROENKEPHALIN DERIVED PEPTIDES IN THE RAT CENTRAL NERVOUS SYSTEM. N. Zamir*, M. Palkovits* and M. J. Brownstein (SPON: M. C. Wilson). Lab. of Cell Biology, NIMH, Bethesda, Maryland 20205.

The distributions of prodynorphin derived peptides (α and β-neo-endorphin, dynorphin A, dynorphin A (1-8), dynorphin B) and proenkephalin derived peptides (Leu-enkephalin and Met-enkephalin-Arg-Gly-Leu) were determined in 100 microdissected rat brain areas and in the neurointermediate lobe of pituitary gland using highly specific radioimmunoassays. Data for selected brain nuclei will be presented. The data illustrate several points: 1) All opioid peptides have a widespread and uneven distribution in the neuraxis. a) Especially high levels of prodynorphin derived peptides were found in the posterior pituitary. b) Among brain nuclei the substantia nigra and the anterior lateral hypothalamus have abundant prodynorphin derived peptides except for dynorphin A. In addition, the nucleus accumbens, bed nucleus of stria terminalis, hippocampus, dentate gyrus, anterior hypothalamic nucleus, median eminence, periaqueductal central gray, parabrachial nuclei and some other telencephalic and diencephalic nuclei are rich in α-neo-endorphin and dynorphin B. High levels of dynorphin A, dynorphin A (1-8) and β-neo-endorphin were found in hypothalamic nuclei, e.g., anterior hypothalamic, dorsomedial, ventromedial, and ventral premamillary nuclei, in the median eminence and others. 2) The molar excess of α and β-neo endorphin over dynorphin A, dynorphin B and their derivatives suggests that a) not all derivatives are accounted for. Indeed, Leu-enkephalin may be derived from dynorphin A and/or dynorphin B in certain brain areas as well as in the neurointermediate lobe. The existence of Leu-enkephalin in two separate precursors may explain why the ratio of Leu-enkephalin to Met-enkephalin-Arg-Gly-Leu, and between some prodynorphin peptides varies in different brain regions, e.g., substantia nigra. b) selective release or degradation of dynorphin A, dynorphin B and their derivatives. 3) Although partial anatomical overlap exists, the pattern of distribution of proenkephalin derived peptides are distinct from that of the prodynorphin derived peptides.

129.13 CO-DISTRIBUTION OF LEU-ENKEPHALIN AND SUBSTANCE P IN THE BRAIN: MAPPING WITH RADIOIMMUNOHISTOTOCHEMISTRY. S. McLean, L.R. Skirboll, and C.B. Pert. Sec. on Brain Biochem., NSB, NIMH, Bethesda, MD 20205.

Immunohistochemical mapping of the medulla and spinal cord has demonstrated substance P-like immunoreactivity (SP) and enkephalin-like immunoreactivity (ENK) to have a similar distribution (Hokfelt et al., PNAS 74:3081-3085). Although providing morphological detail, fluorescence or PAP immunohistochemistry does not provide an easy assessment of peptide distribution. Radioimmunohistochemistry, however, facilitates examination of whole brain sections without magnification, allowing one to discern with relative ease the pattern of distribution of a compound throughout the brain. Using radioimmunocytochemistry the distribution of SP and ENK in more rostral structures will be reported.

Sprague-Dawley rats, untreated or given colchicine intraventricularly, were perfused intracardially with 4% paraformaldehyde. Every 300 µm, 2 to 4 10-µm-thick, frozen sections were cut and incubated (40 hrs) with SP antisera (diluted 1:10,000) or leu-ENK antisera (1:10,000). Control sera were absorbed with 50 µg/ml of the respective peptide. Incubation with ^{125}I goat-anti rabbit antibodies were used to label the primary antibody (McLean et al., Brain Res., submitted). Visualization of the primary antibody was carried out by autoradiography using LKB Ultrofilm or emulsion-coated slides (Herkenham and Pert, J. Neurosci. 2:1129-1149).

There is a striking concordance in the distribution of SP and ENK. In almost all areas displaying ENK immunoreactivity SP could be found: medial wall of the frontal cortex, caudate, ventral pallidum, lateral septal nuclei, periventricular thalamus, hypothalamus, habenula, central and medial nuclei of the amygdala, and the periaqueductal gray. Some exceptions to this overlap are: 1) the olfactory bulb where most of the SP is confined to the granular layer, while the strongest ENK labeling is found in the plexiform and glomerula layers; 2) the posterior portion of the lateral septum exhibits SP but no ENK, 3) the ventromedial nucleus of the hypothalamus without colchicine treatment is almost devoid of SP but shows moderate staining for ENK, with colchicine treatment this difference disappears; 4) the pars compacta of the substantia nigra displays both SP and ENK but the reticulata has only SP; 5) the entopeduncular nucleus shows strong SP labeling but only weak ENK labeling; 6) the CA3 and CA4 layers of the hippocampus display ENK immunoreactivity but no SP; and 7) there is a cortical labeling by ENK and little by SP. Overall, the striking neuroanatomical overlap of SP and ENK suggests a functional interrelationship between these peptides that may be similar to that documented for the spinal cord.

129.14 NONESTROUS FEMALE HAMSTERS HAVE ELEVATED OPIATE RECEPTOR DENSITY IN A NUMBER OF DISCRETE BRAIN LOCI. B.E. Morton[1], C. Chan[1], R.P. Hammer, Jr.[2], and C.B. Pert[3]. [1]Univ Hawaii, Honolulu, HA 96822; [2]Lab of Neurophysiol., and Sec. on Brain Biochem., NIMH, Bethesda, MD 20205

Brains from three groups of hamsters were processed according to Herkenham and Pert (J. Neurosci., 2:1129, 1982) for visualization of type 1 opiate receptors with tritiated naloxone on tritium-sensitive film. Females were characterized as sexually receptive if they produced the classical estrous stringy vaginal discharge and assumed a lordotic posture when placed briefly with a male. Nonestrous females, estrous females, and males (9 in each group) were processed in a balanced design at all stages from decapitation and freezing of the brain to placement of slides in cassettes for visualization on film. After 10 wks, a fiber optic densitometer (Morton et al., J. Neurosci. Methods, 6:113, 1982) was used to quantify optical density in 86 discrete loci of hamster brains. Following normalization to the mean optical density of all (27 x 86) recorded densities, analysis of variance revealed that at all loci where a significant ($p < 0.001$) difference was demonstrated, nonestrous females had more opiate receptors than estrous females and males. The latter two groups were not significantly different from each other. These data are consistent with a rather large body of literature, suggesting that increased opiatergic tone produces inhibition of sexual activity while the administration of opiate antagonists heightens libido. Detailed analysis of the preoptic area showed a significant difference in nonestrous females vs. males and estrous females at 4 of the 6 points surveyed (medial and dorsal regions but not the lateral/ventral region). This is consistent with the fact that certain characteristics of the dorsomedial preoptic area in the hamster have been shown to exhibit sexual dimorphism (Greenough et al., Brain Res., 126:63, 1977).

The present study suggests that rather rapid (4 day) cyclical changes in opiate receptor density may be related to sexual receptivity in the female. Opiate effects could modulate sexual behavior by direct or indirect inhibition of sexual activity. The biochemical mechanism of these effects is unknown; however, the notion of steroid hormones influencing receptor density is not without precedent.

129.15 THE DISTRIBUTION AND ORIGIN OF PROOPIOMELANOCORTIN (β-END & αMSH) FIBERS AND TERMINALS IN THE CENTRAL NUCLEUS OF THE RAT AMYGDALA (CNA). T.S. Gray, M.D. Cassell*, and J.Z. Kiss*. Departments of Anatomy, Loyola University Medical School, Maywood, IL 60153 and University of Iowa Medical School, Iowa City, IA 52242.

The central nucleus of the amygdala (CNA) appears to play a critical role in mediating opioid-opiate influences on emotive behavior and learning and memory (Gallagher & Kapp, in Endogenous Peptides & Learning & Memory, 1981). The CNA receives a dense enkephalinergic innervation though this is differentially distributed relative to the four subdivisions of the CNA which represent clear cytological, hodological and histochemical differences within the nucleus (Cassell et al., in prep.). Proopiomelanocortin peptide (β-END, αMSH) terminals have been identified in the CNA though their precise distribution is uncertain. To identify the neuronal sub-populations receiving proopiomelanocortin peptide input, we have studied the distribution and origin of β-END and αMSH terminals in the CNA using immunocytochemistry and retrograde fluorescent dye transport.

β-END and αMSH immunoreactivity was visualized on 30 µ rat brain sections using the avidin-biotin immunoperoxidase technique. Similar distributions of β-END and αMSH immunoreactive terminals and fibers were identified in the medial nucleus, CNA, and intercalated cell masses in that order of decreasing density. Within the CNA, β-END and αMSH fibers and terminals had very similar distributions and were confined mainly to its medial subdivision. This distribution is complementary to the distribution of enkephalinergic terminals which are densest in the lateral, ventral and central divisions. Fibers reaching the CNA and appearing to originate in the basal hypothalamus could be traced over the optic tract and, more rostrally, through the substantia innominata. In a preliminary set of experiments animals received injections of true blue into the CNA. After survival times of 6-14, animals were given intraventricular colchicine 48 hrs prior to sacrifice. Retrogradely labeled neurons were observed in the lateral and mediobasal hypothalamus, notably the arcuate nucleus. Some of the retrogradely labeled arcuate neurons were found using an immunofluorescence technique, to be immunoreactive for β-END.

The data demonstrate that the distributions of the proopiomelanocortins β-END and αMSH in the CNA are very similar suggesting terminal co-localization and possible common origin in the arcuate nucleus. The proopiomelanocortin terminals are restricted to the medial subdivision of the CNA where the bulk of CNA efferents to brain stem autonomic nuclei originate. The differential distribution of proopiomelanocortins and enkephalins may be of physiological significance.

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129.16 COEXISTENCE OF IMMUNOREACTIVE ENKEPHALIN (I-Enk) AND DYNORPHIN (I-Dyn) IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT. J. Mulcahy*, H.S. Lee* and A.I. Basbaum. Depts. of Anatomy and Endocrinology, University of California San Francisco, CA 94143.

Several studies have described significant overlap in the CNS distribution of the two endogenous opioid peptides, Enk and Dyn. Since marked and varied opiate effects are exerted in the nucleus of the solitary tract (NTS), and since Enk and Dyn are associated with different opiate receptor subtypes, we decided to examine the NTS for local differences in opioid peptide distribution. Adult rats, some pretreated with intracisternal colchicine, were perfused with 4% paraformaldehyde. Fifty micron frozen sections of the medulla were cut and reacted for immunoreactive Enk and Dyn. To identify I-Enk we used an antiserum directed against the proEnk product, met-Enk-arg-gly-leu; for I-Dyn, we used an antiserum directed against the C-terminal proDyn peptide, dynorphin B (Weber E.). Absorption controls established that the Dyn antiserum does not cross react with Enk and vice-versa.

While there is considerable overlap in the I-Enk and I-Dyn terminal and cellular distribution in the NTS caudal to the obex (including the commissural nucleus), rostral to the obex, there is a marked segregation. Small round I-Enk cells are found in the medial NTS, medial to the tract. Large, multipolar I-Dyn cells are concentrated lateral to the tract. To assess possible peptide coexistence in neurons caudal to the obex, we next examined serial 3-4u cryostat sections of colchicine-treated rats; this allowed staining of the same neurons in several adjacent sections. While the majority of neurons in this region stain for only one of the peptides, we found numerous examples in the caudal ventrolateral NTS in which I-Enk and I-Dyn are colocalized. Double-labelled cells were also found in areas associated with the control of pain, including the ventrolateral medulla, the midline raphe and the dorsal horn.

The medial and lateral nuclei of the solitary tract receive baroreceptor and lung stretch receptor afferents, respectively. Thus our data suggest that Enk and Dyn may exert selective actions on the different visceral afferent inputs to the rostral NTS. Our data further indicate that some CNS neurons synthesize more than one opioid peptide precursor. Since these double labelled neurons are found in that part of the NTS which receives convergent baroreceptor and lung stretch receptor input, the coexistence may underlie a dual opioid control by single medullary neurons.

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129.18 DYNORPHINS LOCALIZED IN SPINAL CORD AND DORSAL SENSORY NEURONS BY IMMUNOHISTOCHEMISTRY.
P.M.Sweetnam, J.R.Wrathall[1], E.Weber*[2], J.D. Barchas[2], A.Goldstein[3] and J.H. Neale. Departments of Biology and Anatomy[1], Georgetown University, Washington, D.C. 20057, Departments of Psychiatry and Behavioral Sciences[2] and Pharmacology[3], Stanford University, Stanford, CA 94305.

Using peroxidase-antiperoxidase(PAP) immunohistochemistry as well as an immunofluorescence method, we have studied the cellular distribution of dynorphin- and dynorphin B-immuno reactivity(IR)in spinal cord and dorsal root ganglion neurons. Using antibody which is specific for the midportion of dynorphin, we have previously reported(PNAS 79:6742, 1982) the presence of dynorphin-IR in both spinal and sensory neuronal cell bodies with very little, if any, PAP reaction product in neurites. We report here the detection of dynorphin B-IR and dynorphin (9-17)-IR in spinal cord cell neurites as well as perikarya, a distribution similar to that observed with enkephalin antisera. Distal neurite IR was particularly apparent when fluorescein-conjugated secondary antisera were used. The observation of dynorphin (9-17)-IR in the neurites, while the midportion dynorphin-IR is relatively absent, suggests that this peptide is split in the midregion and that the non-opioid fragment is distributed within the neurites in a manner similar to the amino terminal opioid fragment.

We have followed our initial observation of dynorphin-IR in dorsal root ganglion neurons which developed from fetal tissue in dissociated cell culture with studies of freshly dissociated neurons from adult mouse ganglia. We have observed dynorphin-IR in a few percent of the adult sensory neurons, both immediately after dissociation and after brief periods in cell culture. Sensory neurons which are obtained from fetal mice develop dynorphin-IR after several weeks in culture. Neither increased concentrations of nerve growth factor nor coculture with spinal cord cells for several weeks changes the percentage (about 4%) of these neurons which develop high levels of dynorphin-IR. Preliminary fluorescence immunohistochemistry further suggests the presence of a small but significant number of neurons with dynorphin-IR in sections of adult mouse dorsal root ganglia.

129.PO IMMUNOREACTIVE BETA ENDORPHIN-SPECIFIC PERIKARYA IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI OF THE HORSE AND PONY.
K.M. Knigge, P.A. Melrose*, and W.H. Pilcher* (SPON: J. Paterson). The Neuroendocrine Unit, Univ. of Rochester, Roch., NY 14642.

Unlabeled antibody techniques were used to map the distribution of beta-endorphin (β-E) in the equine diencephalon. Brains from 3 horses and 2 ponies were fixed by immersion in buffered 4% paraformaldehyde containing 0.1% picric acid. Serial 50μm sections of the diencephalon were cut on a freezing sliding microtome in frontal and parasagittal planes. Immunoreactive (ir) β-E specific cell bodies were present in the arcuate, supraoptic, and paraventricular nuclei. The topography and cross-immunoreactivity of these cells in supraoptic and paraventricular nuclei was distinctly different from ir-cells of the arcuate opiocortin formation. Immunoreactive cells in the supraoptic and paraventricular nuclei stained with antisera generated against human and camel β-E but not with antisera against human ACTH 1-39, alpha-MSH, enkephalin, or dynorphin. All immunoreactivity was abolished by incubating anti-camel β-E antisera (1:1000) with 1μg/ml of camel β-E for 48 hrs. at 4°C. Staining was not affected when antisera was incubated in a similar manner with 1-2μg/ml of ACTH 1-39, oxytocin, or arginine vasopressin.

Results from this study suggest that neurons in the supraoptic and paraventricular nuclei of the equine brain contain β-E or a β-E-like peptide. Further, preliminary data suggest that this population of ir-cells is separate from the oxytocin and vasopressin magnocellular cell populations.

PEPTIDES: PHYSIOLOGICAL EFFECTS III

130.1 GASTRIC HYPOSECRETION AND HYPERGLYCEMIA INDUCED BY BOMBESIN INJECTION NEAR THE PARAVENTRICULAR NUCLEUS.
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Bombesin inhibits gastric acid secretion and causes hyperglycemia when given intracisternally. Bombesin immunoreactivity is localized in the forebrain as well as the lower brainstem, with particularly numerous terminals and cell bodies in the hypothalamic paraventricular nucleus. This nucleus has direct outputs to lower brainstem autonomic centers. The possible role of this region in gastric secretory and blood glucose regulation was therefore evaluated by direct injection of bombesin into this area.

Male Sprague-Dawley albino rats (200-290 g) were used; surgery was under ether anesthesia. Simultaneous bilateral infusions of bombesin-14 (50 ng/side) or vehicle (0.1% BSA/0.9% NaCl, 1 μl/side) were made over 2 min, with the 30 g stainless steel injection cannulae left in place an additional 2 min before withdrawl. The pylorus was immediately ligated through an abdominal incision; exactly 2 hr postinjection the animals were decapitated and blood and gastric contents collected.

Injections near the dorsomedial aspect of the hypothalamic paraventricular nucleus caused marked suppression of gastric acid output and pronounced hyperglycemia. Acid secretion after bombesin dropped to 2 μmol/2 hr; vehicle at the same sites gave 103 μmol/2 hr. As expected, pH and serum gastrin rose, and secretion volume dropped, after effective injections. Serum glucose levels rose to 298 mg/dl after bombesin; vehicle gave 125 mg/dl. Bombesin did not affect these measures when injected into the lateral hypothalamus (output: bombesin 91 μmol/2 hr, vehicle 129 μmol/2 hr; glucose: bombesin 137 mg/dl, vehicle 127 mg/dl).

These data suggest that the paraventricular nucleus is involved in the regulation of both gastric exocrine secretion and blood glucose levels, and that bombesin may have a physiological role in these processes. Further, it appears that the linkage between gastric secretion and blood glucose seen with intracisternal injections also occurs with intrahypothalamic administration.

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130.2 AGE-DEPENDENT CHANGES IN LHRH NEUROSECRETORY RESPONSE OF SUPERFUSED MALE RAT HYPOTHALAMI TO CYCLIC AMP DELIVERY.
Daryl E. Hartter and V. D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL. 61801.

Previous studies in several laboratories have suggested that the adenyl cyclase/cyclic AMP-generating system is involved in the control of in vitro release of LH-releasing hormone (LHRH) from adult rat hypothalamus. In the present study, we have investigated the effect of intermittent (10-min. on, 40 min. off) delivery of dibutyryl cyclic AMP (dbcAMP) on LHRH release from superfused hypothalamic fragments taken from intact male rats of 25, 30, 45 or 60-75 (adult) days (d.) of age.

Animals in each experimental group were sacrificed by ether anesthesia-hemorrhage, and mediobasal hypothalamus (MBH) fragments were dissected out and prepared for superfusion as previously described (Hartter & Ramirez, Endocr. 107:375, 1980), using either 4 (in 25, 30 or 45 d. groups) or 2 MBH fragments/chamber (in adult group). Each age group was replicated 4 times, with experimental (dbcAMP, 5 x 10^{-8}M) and control (butyrate, 5 x 10^{-8}M) groups being run simultaneously in a single superfusion experiment (of 240-min. duration). Superfusion samples were assayed for LHRH content by radioimmunoassay.

Our results indicate that at 25 d. of age, male rat MBHs were quite sensitive to intermittent dbcAMP (mean post-dbcAMP infusion LHRH release = 162% of pre-infusion basal release); at 30 d., the dbcAMP effect decreased to 120% of basal. At 45 d., the effect of dbcAMP on LHRH release was inhibitory (only 78% of basal); however, by adulthood, this cyclic nucleotide exerted negligeable effect on LHRH release (92% of basal). Similar intermittent infusions of butyrate resulted in less dramatic modifications of in vitro LHRH release, with post-butyrate mean LHRH release of 116, 82, 68 and 94% of basal at 25, 30, 45 d., and adult, respectively. Thus, differences in relative LHRH release between dbcAMP and butyrate conditions equalled 46, 38, 10 and -2% in the 4 respective age groups, indicating a true age-dependent decrease in responsiveness of the LHRH apparatus to cyclic AMP. In addition, this study showed that while spontaneous basal LHRH release increased dramatically (over 8-fold) and in a near-linear fashion with increasing age of intact male rat donor, MBH-LHRH concentration reached near-maximal levels of 175 pg/mg tissue by 45 d. of age.

In sum, it can be concluded that 1.) sensitivity of the male rat neural LHRH apparatus to cyclic AMP delivery changes in an age-dependent manner and 2.) distinct maturational changes in LHRH release from and LHRH concentration in superfused male rat MBH fragments are observed using this in vitro system.

130.3 THE ROLE OF PROSTAGLANDINS AND cAMP IN IN VITRO PROGESTERONE-STIMULATED LHRH RELEASE FROM SUPERFUSED RAT HYPOTHALAMIC FRAGMENTS. K. Kim and V. D. Ramirez. Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, IL 61801.

The present studies attempt to elucidate the possible role of Prostaglandin E_2 (PG-E_2) and Adenosine 3',5'-monophosphate (cAMP) in in vitro Progesterone (P)-stimulated LHRH release from superfused rat hypothalamic fragments. Immature female rats (day 28) were ovariectomized and implanted with Silastic capsules containing Estradiol-17β. On day 30, mediobasal hypothalamic-anterior hypothalamic-preoptic area (MBH-AHA-POA) units were removed and superfused in vitro. Effluents were collected on ice at 10 min-intervals. LHRH was determined by a specific and sensitive LHRH radioimmunoassay. Each experimental group was replicated four times. Intermittent P infusion (10 ng/ml; 10 min-on, 20 min-off) is effective in stimulating LHRH release in vitro as previously shown (Kim and Ramirez, Endocrinol., 111:750, 1982). Continuous in vitro infusion of a PG synthesis inhibitor, indomethacin (100 μM) blocked completely P-induced LHRH release (mean post-P LHRH release between Control and Indomethacin groups: 1.09 + .07 vs .31 + .02 pg/min/mg x 10^{-2}; $p < .01$). Following a 50 min-control period, a step-wise increment in several infusion doses of PG-E_2 (each dose for a 50 min-interval) evoked dose-related increases in LHRH release. PG-E_2 induced significant ($p<.01$) increments in LHRH release at doses of 5.7 x 10^{-7}, 5.7 x 10^{-6}, and 5.7 x 10^{-5} M respectively. Intermittent infusion of dibutyryl cAMP (10^{-7} M; 10 min-on, 20 min-off) resulted in rhythmic LHRH secretion. Following a 50 min-control period, a dose-related increase in LHRH release was obtained when adenylate cyclase activators, such as Forskolin and Cholera Toxin were infused in a step-wise manner (each dose for a 50 min-interval). Forskolin and Cholera Toxin significantly ($p<.01$) stimulated LHRH release at doses of 10^{-4} and 5.4 x 10^{-10} M respectively. These stimulatory effects of Forskolin and Cholera Toxin on LHRH release were no longer present when hypothalamic fragments were superfused in calcium-free plus EGTA (10 mM) containing medium.

It appears that PG-E_2 and cAMP may mediate P-stimulated LHRH release in vitro. It is tempting to postulate that P directly or indirectly (perhaps thru Norepinephrine stimulation) enhances PG-E_2 activity which then activates the adenylate cyclase system to increase cAMP level which in turn triggers the release of LHRH.

130.4 AN ELECTROPHYSIOLOGICAL ANALYSIS OF CHOLECYSTOKININ-DOPAMINE INTERACTION. D. Hommer*, L. Skirboll and M. Palkovits*. Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20205.

Immunohistochemical studies have shown that there is a coexistence of dopamine(DA) and cholecystokinin (CCK) in a subpopulation of mesencephalic neurons which in the rat project primarily to limbic areas (Hökfelt et al., Neurosci. 5, 2093). Previously, we have reported that these DA/CCK containing cells are excited by either systemically and iontophoretically administered CCK (Skirboll et al., Neurosci. 6, 2111). This raises the question of the functional significance of DA/CCK coexistence. To investigate this question we used extracellular single unit recording techniques in male albino rats. Four ug/kg of CCK or its analogue ceruletide were administered systemically and their effect on the ability of the DA agonist, apormorphine, to inhibit medial A9 neurons was evaluated. These neurons are in a region which has a high proportion of cells containing both CCK and DA. Pretreatment with either CCK or ceruletide lead to a significant shift to the left of the dose response curve for apomorphine induced inhibition of firing. The ED_{50} of apomorphine for both CCK and ceruletide was 4 ug/kg while the ED_{50} for the control group was 8 ug/kg. This suggests that systemically administered CCK-like peptides can induce a DA autoreceptor supersensitivity. Iontophoretically applied CCK also is able to potentiate the inhibitory action of iontophoretically applied DA on medial A9 neurons.

Although we have found that systemically and iontophoretically administered CCK have similar effects on CCK/DA neurons several behavioral effects of systemically administered CCK are not present in vagotomized animals (Crawley et. al., Eur. J. Pharmacol. 73, 379) suggesting that CCK's behavioral effects may be mediated by an action outside the CNS. In an effort to determine whether the excititory effects of CCK on DA neurons were mediated centrally or peripherally the effect of CCK on medial A9 cells was examined in three groups of rats: 1) acutely vagotomized animals; 2) rats with a unilateral lesion to the nucleus tractus solitarius or 3) rats given a C-1 transection prior to recording. None of these procedures altered CCK's excititory effect on medial A-9 cells suggesting that the ability of CCK to enhance the activity of DA neurons is not mediated through a peripheral action but rather represents a central action of the peptide.

We also examined DA-CCK interactions in limbic DA terminal areas. In the olfactory tubercle CCK excited only those cells which were inhibited by apomorphine. The effects of CCK antagonists in pre- and post-synaptic regions will also be reported.

130.5 CNS ACTIVITY OF N-TERMINAL FRAGMENTS OF CHOLECYSTOKININ OCTAPEPTIDE. C.A. Tamminga, M. Knight, M. Beck,* S. Cohen,* L. Steardo,* T.N. Chase. Maryland Psychiatric Research Center, University of Maryland, Baltimore, Maryland 21228; NINCDS, ETB, Bethesda, Maryland 20205.

Biologic activity of cholecystokinin, a 33 amino acid neurally active peptide, resides in the carboxyl-terminal octapeptide sequence, CCK 26-33 (CCK8); most actions require no more than CCK 29-33. Modifications of CCK8 at the carboxyl end have been reported to eliminate or reverse peripheral activity and modify behavioral actions of CCK8. In an effort to define the structure-activity relationships of other portions of the peptide and to identify fragments of specific pharmacologic interest, we have prepared and tested an N-terminal analogue and N-terminal fragments of CCK8. N-acetylated peptides of the sequences Asp^{26} to Gly^{29} inclusive through Asp^{26} to Asp^{32} amide and $(Lys)^{31}$ CCK 26-31 were synthesized by solid phase techniques and sulfated. Purification of the fragments was carried out using countercurrent chromatography on the coil planet centrifuge and HPLC techniques. The acetylated fragments CCK 26-29, CCK 26-30 amide, CCK 26-31 amide, and CCK 26-32 amide and the $(Lys)^{31}$ CCK 26-31 analogue were tested for CCK receptor affinity and biologic activity in mammalian brain. The $(Lys)^{31}$ CCK 26-31 analogue has been shown to bind to the pancreatic CCK receptor and to antagonize the action of CCK8 on dispersed pancreatic acinar cells; the analogue demonstrates no affinity at the CNS CCK receptor at molar concentrations up to 10^{-3}. The N-terminal CCK fragments were similarly tested for affinity at brain CCK receptors. Using ^{125}I CCK-33 and guinea pig cortical membranes, we have shown that CCK 26-32 amide displaces CCK-33 binding with an IC_{50} of 2 x 10^{-5}M; dibutyryl cyclic GMP in this system displaces ligand binding at a potency of 5 x 10^{-4}M. The three shorter CCK fragments failed to displace labeled ligand binding when tested at concentrations up to 10^{-3}M. Neither the analogue nor the fragments demonstrate CCK-like activity in rat avoidance paradigms. These fragments may provide a pharmacologic tool for distinguishing central from peripheral actions of CCK. In addition, once central activity of CCK 26-32 is studied, this centrally-active fragment may be of value as a specific pharmacologic probe for examining the physiologic role of CCK in brain. Results of behavioral experiments with the N-terminal fragments, testing for CCK antagonist activity will be reported.

130.6 EVIDENCE FOR NEURON-SPECIFIC PROTEIN PHOSPHORYLATION FOLLOWING PEPTIDERGIC BAG CELL ACTIVITY IN APLYSIA. M. Schaefer, P.H. Brownell, and P. Shirk*. Dept. of Zoology, Oregon State Univ., Corvallis, Oregon 97331

The peptidergic bag cell neurons in the abdominal ganglion of Aplysia californica strongly influence the activities of other ganglionic neurons for periods of minutes to hours. We are investigating the role of protein phosphorylation in mediating these long-term responses. Two types of bursting pacemaker neurons in the abdominal ganglion are affected differently by bag cell activity (BCA). The left upper quadrant (LUQ) cells L2-L6 undergo long-lasting inhibition, while burst activity in neurosecretory cell R15 is augmented. We reasoned that since the responses to BCA in the LUQ's and R15 are essentially opposite, a comparison of the protein phosphorylation patterns in these cells might allow us to identify phosphoproteins involved in long-term modulation of neuronal activity.

Protein phosphorylation was monitored by intracellular injection of [γ-^{32}P] labeled ATP (NEN, >2900 Ci/mmole) following a modification of techniques described by Lemos et al. (Nature 298, 64-65, 1982). Microelectrodes were filled with labeled ATP (1 μCi/nl) in 300 mM potassium phosphate buffer (pH 6.8), and the solution was pressure injected into a single neuron per ganglion. In control ganglia, label was incorporated for 1 hr and the ganglia were frozen in liquid nitrogen. In experimental ganglia, BCA was evoked by brief localized electrical stimulation of a bag cell cluster and a maximal response was allowed to develop prior to freezing. Ganglia were homogenized and the proteins were resolved by electrophoresis on a sodium dodecyl sulfate polyacrylamide gradient gel (8-15%) (O'Farrell, J. Biol. Chem. 250, 4007-4021, 1975). The labeled proteins were visualized by autoradiography at -70°C using a Cronex (Dow) intensifying screen.

Our results indicate considerable similarity in the patterns of protein phosphorylation between neurons in the ganglion as well as neuron-specific and BCA response-specific changes in protein phosphorylation. Quantitative similarities in phosphorylation of various proteins ranging in molecular weight from approximately 250 kd to 10 kd were noted in all of the neurons examined. The two types of bursting pacemaker neurons could be distinguished by at least one phosphoprotein (in the molecular weight range of 20-30 kd) which was labeled in R15 but not in LUQ's. Finally, the BCA-induced responses of R15 and LUQ cells were correlated with changes in their patterns of phosphorylation. The R15 response was associated with phosphorylation of two 130-150 kd proteins while inhibition of LUQ cells was associated with phosphorylation of a 55 kd protein.

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130.7 ACTIONS OF N-ACETYLASPARTYLGLUTAMATE ON MAMMALIAN NEURONS. J. M. H. ffrench-Mullen*, R. Zaczek*, K. J. Koller, J. T. Coyle and D. O. Carpenter, NYS Dept. of Health, Albany, New York 12201 and Johns Hopkins University, Baltimore, Maryland 21205

While L-glutamate (Glu) and L-aspartate (Asp) are considered possible excitatory transmitters there are reasons to believe that at some sites the true transmitter is related to but distinct from either Glu or Asp (cf. Hori, et al., J. Neurophysiol. 48:1289, 1982). N-acetylaspartylglutamate (NAAG) is a dipeptide present in mammalian brain which a) demonstrates a high affinity binding (420 nM) to receptor sites labelled by ^{3}H-Glu and b) is a potent convulsant (Zaczek, et al., PNAS 80:1116, 1983). We have applied NAAG by single and multiple barrel ionophoresis to neurons in both rat prepyriform and sensory-motor cortex brain slices in order to determine whether NAAG has direct electrical actions on neurons and, if so, to compare the physiological and pharmacological properties of the NAAG response to that of the natural transmitter and the excitatory amino acids. NAAG was a potent excitant of 24 of 25 neurons in prepyriform and 20 of 20 neurons in sensory-motor cortex. The response was of relativly short latency, consisting of a high frequency burst of spikes. With higher ionophoretic currents the response was prolonged, often leading to irregular spontaneous discharge and ultimately to spike inactivation and cell death. Although it is difficult to determine precise potency in ionophoretic studies, NAAG was clearly more potent than Glu, Asp or N-methyl-D-aspartate (NMDA), but was equal to or less potent than quisqualate (Quis). NAAG caused a prolonged potentiation of the responses to the other agonists. It was more neurotoxic than Glu, NMDA and Quis but less so than kainic acid.

There are differences in the pharmacologic sensitivities of the amino acid responses in these two areas of cortex, in that the traditional Glu antangonist glutamate diethylester (GDEE) and the Asp antagonist alpha amino adipate (αAA) have the expected actions in sensory motor cortex but are both without apparent effect in prepyriform cortex. Furthermore the excitatory actions of the lateral olfactory fibers on prepyriform neurons are blocked by aminophosphonobutyric acid (ABP), but not by GDEE or αAA. In preliminary studies the NAAG response in prepyriform cortex was depressed by APB but not GDEE or αAA, but in sensory-motor cortex it was unaltered by any of these agents. These studies suggest the possibility of a physiological role for NAAG as a neurotransmitter in the rat central nervous system, and also indicate that there are regional differences in pharmacologic sensitivities to NAAG and other excitatory agents in the cortex.

130.8 CARDIOVASCULAR ACTIONS OF BRADYKININ INJECTED INTO SPECIFIC HYPOTHALAMIC AND PREOPTIC NUCLEI. D. I. Diz* and D. M. Jacobowitz, Lab. of Clinical Science, NIMH and NIGMS, Bethesda, MD 20205.

Bradykinin-like immunoreactive neuronal cells and fibers are reported to be present throughout the rat hypothalamus (Corrêa, F.M.A. et al., Proc. Natl. Acad. Sci., 76:1489, 1979), and bradykinin (BK) has been reported to induce changes in blood pressure (BP) when administered into the lateral cerebral ventricle or pars ventralis of the lateral septal area (Corrêa, F.M.A. and F.G. Graeff, J. Pharmacol. Exp. Ther., 192:670, 1975). In the present study, we monitored BP and heart rate (HR) during microinjections of BK in discrete hypothalamic (HT) nuclei in order to determine the HT sites of cardiovascular (CV) actions of the peptide. Initial BP and HR in 51 halothane-anesthetized rats was 88 ± 1 mm Hg and 352 ± 4 beats/min (b/m), respectively. BK (5 nmol) or saline vehicle was injected (100-300 nl) via double-barreled glass micropipettes (O.D. 20-70 μm). In the HT paraventricular nucleus (PVN; n = 6), BK, but not saline, decreased HR 42 ± 10 b/m (-12%) without change in BP. In contrast, increases in HR of 9% and 13% occurred at sites within the medial preoptic nucleus (POM; n = 7) and the preoptic suprachiasmatic nucleus (POS; n = 5), respectively; BP was unchanged. In the HT dorsomedial nucleus (DMN; n = 10), BK increased both BP (13%) and HR (15%). Similarly, increases of 8% and 11% in BP and HR were observed with BK injections into the posterior HT nucleus (PH; n = 7). Variable responses occurred with BK in the anterior HT nucleus depending upon the rostrocaudal localization of injections. The general characteristics of all the responses include an onset of less than 1 min with peak activity occurring at 6 ± 1 min and a duration of about 40 min. Tachyphylaxis to repeated BK injections was present for approximately 60 min following a prior exposure to the peptide.

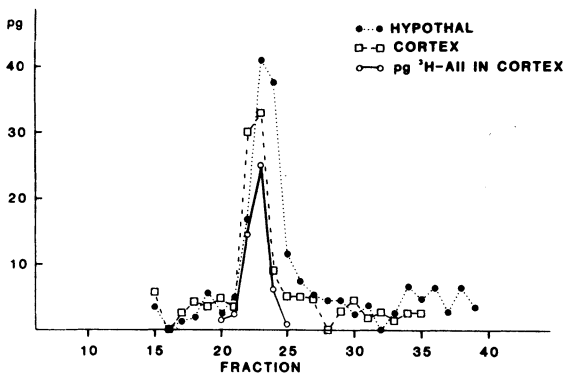
A preliminary study in which the CV effect of bradykinin potentiating factor (BPF; angiotension converting enzyme inhibitor) were investigated suggests BPF (5 nmol) has minimal CV actions of its own in the POS, POM or PVN and may increase BP and HR in the DMN and PH. Further experiments will evaluate the effects of BPF injections 10-15 min prior to BK injections to determine if discrete inhibition of kininase II alters the CV responses to HT injections of BK.

In summary, the data indicate that the characteristics of the CV response to BK depend upon the specific site of injection and that these CV sites of action correspond well with the previously observed localization of BK-like immunoreactive nerve cells and fibers. Bradycardic actions occur within the PVN, tachycardic and pressor actions occur within the DMN and PH, and tachycardia only occurs within the POS and POM.

130.9 ANGIOTENSIN II AND III IN RAT HYPOTHALAMUS AND CORTEX SHOWN BY HPLC. M. Ian Phillips and Birgitta Stenstrom. Department of Physiology, College of Medicine, University of Florida, Gainesville, FL 32610.

To answer the question of whether angiotensin II exists in the brain independently of peripheral angiotensin, a sensitivie radioimmunoassay with high recovery rates was used to measure fractions eluted from high pressure liquid chromotography (HPLC) assay of male adult rat brains.

Twenty three rats were nephrectomized bilaterally and 24 hrs later the brains were extracted in acetic acid. The hypothalamus block had consistently higher levels of Ang II than cortex. The range of Ang II concentration measured by RIA (93% recovery) was 63 pg to 873 pg/g tissue in hypothalamus and 14-179 pg/g tissue in cortex. Fifteen HPLC assays were run with the hypothalamic and cortex tissues and in every case a clear peak was reliably found in the same fractions that authentic ^{5}(Ile) angiotensin II migrated to. The recovery rate in the HPLC column was 90%. Further tests with the ^{3}H-Ang II confirmed the position of the peak fraction. The results shown in Figure 1 demonstrate the presence of a brain peptide which comigrates with the octapeptide angiotensin II. The results indicate that the antibody which was previously used in our immunocytochemical localization of angiotensin in the hypothalamus detects authentic angiotensins. [Supported by NIH and SF grants to M.I.P.]



130.10 EXCITATORY EFFECTS OF ANGIOTENSIN II AND INHIBITORY EFFECTS OF INSULIN ON HIPPOCAMPAL CA1 PYRAMIDAL NEURONS. R.A. Palovcik, M.I. Phillips, and M.K. Raizada*. Department of Physiology, College of Medicine, University of Florida, Gainesville, FL, 32610.

Recent immunocytochemical and receptor binding studies show that the brain contains angiotensin II (AII) and insulin and their receptors. The physiological significance of the angiotensin system appears to be in the regulation of blood pressure and drinking responses but the role of insulin in brain function is not clearly understood. The present investigation was undertaken to study the effect of these peptides on the spontaneous firing of hippocampal neurons in brain slices.

The hippocampus was removed from Sprague-Dawley male rats (150-300 g) and sectioned transversely into 0.4 mm thick slices. These were maintained in a chamber at 34°C with 95% O_2, 5% CO_2 under continuous perfusion (30 ml/hr) of Yamamoto's solution. Robust activity could be recorded as long as 35 hours; however, all experiments were done between 2 and 10 hours post setup. Peptides were applied into the medium by slow infusion to avoid mechanical artifacts, extracellular recordings were made in most cases but some intracellular recordings were also used. Application of AII, into the perfusion medium resulted in the excitation of 50% of CA1 pyramidal neurons, no effect in 43% and inhibition in 7%. This excitatory effect was observed over a wide dose range of AII with 3 cells responding to as low as 10^{-15} M and increased with each increasing dose. Saralasin, an AII receptor antagonist caused attenuation of the excitatory effect of AII. Intracellular recording from CA1 pyramidal neurons showed an increase in excitatory synaptic activity at low doses of AII.

In contrast to AII, insulin inhibited firing of CA1 pyramidal neurons (in 38% and no effect in 62%) in a dose dependent manner, producing significant inhibition with as low as 10^{-9} M. For comparison carbachol was tested. Excitatory effects occurred at 10^{-7} M and above. This indicates that CA1 pyramidal cells are highly sensitive to insulin and AII.

The results are the first demonstration that insulin directly affects the electrophysiological activity of neurons. These results suggest that peptides may be effective in altering neuronal activity in concentrations that are comparable to those detected in vivo and are consistent with the existence of receptors for these peptides in the brain. [Supported by NSF grant to M.I.P. and NIH Fellowship 1F32HL06709-01 to R.A.P.]

130.11 ENALAPRIL, INTRACEREBROVENTRICULAR BUT NOT PERIPHERAL ADMINISTRATION, BLOCKS DRINKING EVOKED BY ANGIOTENSIN I GIVEN INTRAVENTRICULARLY. G.E. Martin, T. Naruse,* N.L. Papp* and S.L. Gaul.* Merck Institute for Therapeutic Research, West Point, PA 19486.

Blockade of the enzyme which converts angiotensin I (AI) to AII should block the dipsogenic action of centrally administered AI. Blockade of AI-induced drinking by a peripherally administered angiotensin converting enzyme (ACE) inhibitor such as enalapril would provide strong evidence for a CNS action for that compound. These experiments were conducted to determine whether enalapril given peripherally could reduce drinking produced by the ICV injection of AI in normotensive rats.

All ICV injections were made via an implanted 24-gauge guide tube in male Sprague-Dawley rats. In the antagonism studies, a crossover design was used in which half of the rats were first given the vehicle and half enalapril before AI. One week later the pretreatments were alternated. Enalapril was given 10 minutes before AI when given ICV, but 30 minutes prior to AI when given p.o. or i.p.

The ICV administration of AI was found to produce a dose-related drinking response which reached asymptote at 25 ng. Pretreatment with a potent hypotensive dose (3 mg/kg) of enalapril p.o. failed to reduce the fluid consumption induced by 5, 15, or 25 ng (n=5 or 6/group) of AI given ICV. The drinking produced by the intracerebral infusion of 500 ng of AI was not significantly reduced by the prior i.p. administration of enalapril (1 mg/kg i.p.), it was significantly reduced from a mean of 11.5 + 1.1 (SEM) ml/hr to 4.5 + 1.7 ml/hr following enalapril given ICV (100 µg). Furthermore, the ICV infusion of 50 µg of enalapril significantly reduced drinking produced by 25 ng of angiotensin from 7.9 + 0.7 to 3 + 1 ml/hr. Although p.o. pretreatment with 9 mg/kg of enalapril did reduce fluid consumption from 9.2 + 1.0 to 5.4 + 0.8 ml/hr, the reduction was not statistically significant.

These results in the normotensive rat corroborate those reported previously for the spontaneously hypertensive rat (Sweet et al., Fed. Proc. 41: 1663, 1982) and suggest enalapril does not efficiently cross the blood-brain barrier to block ACE in the ventricles. One might infer from these data that enalapril's hypotensive action may be exerted primarily in the peripheral nervous system.

130.12 POTENTIATION OF APOMORPHINE-INDUCED EMESIS IN THE DOG BY CENTRALLY ADMINISTERED ANGIOTENSIN II, J. Kucharczyk, M. Wu* and R.K. Harding*, Dept. of Physiology, School of Medicine, Univ of Ottawa, Ottawa, Ont. K1H 8M5.

Emesis can be induced by intravenous (Carpenter, SAM-RE-82-28) and intracerebroventricular (Wu & Kucharczyk, Proc. Can. Fed. Biol. Soc. 26, 1983) administration of angiotensin II (AII) and other vasoactive peptides, such as neurotensin and arginine vasopressin. Ablation of the area postrema (AP) blocks the neurogenic pressor response to AII (Ferrario et al., Hypertension 1: 235, 1979) as well as emesis induced by the dopaminergic agonist, apomorphine (APO) (Borison, Life Sci. 14: 1807, 1974).

In order to study whether APO and AII act on the same central receptors to initiate emesis, five adult mongrel dogs with chronically implanted lateral cerebroventricular (LV) cannulae received repeated injections of various doses of the drugs and their antagonists. Administration of 5 µg APO + 500 µg AII induced vomiting in all four dogs tested with a mean latency of 7' 45", whereas the same dose of APO given alone was totally ineffective. Two dogs tested with LV injections of 5 µg APO + 250 µg AII and 5 µg APO + 100 µg AII also responded. At higher doses of APO (20 and 40 µg), the main effect of combined APO-AII injections was to reduce the response latency and to increase the number of bouts of vomiting. Pretreatment of the LV with the dopaminergic antagonist, spiroperidol (500 µg), blocked vomiting in three dogs tested with a combined injection of 40 µg APO + 500 µg AII delivered into the same intracranial cannula. Pretreatment of the LV site with 100-900 µg of the AII antagonist, 1-Sar, 8-Ala AII, significantly reduced the number of emetic episodes elicited by injections of 40 µg APO.

Finally, in acute blood pressure recording experiments in chloralose-anesthetized dogs, LV administration of 40 µg APO produced a small and transient decrease in mean arterial blood pressure (MAP) (5-23 mmHg), whereas injection of 500 µg AII produced an increase in MAP of 33 mmHg. Combined LV treatment with 40 µg APO + 500 µg AII, on the other hand, induced a large and sustained increase in MAP (peak increase of 78 mmHg 5 min after injection). These data suggest that APO and AII may induce vomiting through a vasoactive mechanism in which the AP plays an important receptor or integrative function. (Supported by Govt. Canada Contract 2SU81-00190).

130.13 VASOPRESSIN RELEASE IN THE RAT SEPTUM IN RESPONSE TO SYSTEMIC STIMULI, F. Rodriguez*, J. Demotes-Mainard*, J. Chauveau*, D. Poulain* and J.D. Vincent. Unité de Neurobiologie, U.176 INSERM Domaine de Carreire, Rue Camille-Saint-Saëns, 33077 BORDEAUX-Cedex (France).

Immunohistochemical studies have shown that the lateral septum is a main target area for arginin-vasopressin (AVP) counta-ining fibers and synapses (Buijs, R.M. and Swaab, D.F., Cell Tissue Res., 204 : 355, 1979). Furthermore, a calcium-dependent release of AVP was evoked from septal slices in vitro by potassium and veratridine (Buijs, R.M. and Van Heerkluize, J.J., Brain Res., 252 : 71, 1982). In order to confirm such a release in vivo and to study its physiological significance we have used push-pull cannulae implanted stereotaxically in the lateral septum to mesure the local release of AVP as rats were submitted to systemic stimuli known to affect the plasma level of AVP.

Push-pull cannulae (1.4mm diameter) were implanted in the lateral septum (LS), caudate nucleus (CN) and lateral ventricule (LV) of male rats anaesthetized with urethane. The cannulae were continuously supplied with artificial cerebrospinal fluid (1ml/15min). Radioiummunological assay of AVP was then carried out on superfusate samples collected every 15 min.

After 30 min of superfusion, a stable release of AVP (1pg/ml/15min) was measured in the LS and the LV. AVP was not detected in the CN. Osmotic stimulation (4ml IP, NaCl 2M) induced a dramatic increase of AVP release from the LS (30-55pg/ml/15min) which paralleled the increase of plasma osmotic pressure. Only a small increase (2pg/ml/15min) was observed in the LV and none was observed in the CN. The release from the LS was calcium-dependent since it was inhibited when the cannulae were supplied with Ca-free artificial CSF. Hypovolemic stimulation (polyvynil-pyrrolidone 40% in NaCl 0.15M, 2ml/100g IP) also induced a dramatic increase in AVP release in the LS (25-30pg/ml/15min) which paralleled an increase in plasma AVP. The fact that elevated levels of AVP in response to osmotic and hypovolemic stimulations were noted only in the LS argues against a non specific diffusion of plasma AVP through the blood brain barrier. Furthermore electrical stimulation of the pituitary stalk (0.2mA, 15Hz, 20sec trains, 40min) which induced an increase in plasma level of AVP had no effect on septal release of AVP. On the other hand electrical stimulation of the supraoptic nucleus augmented septal release of AVP.

Our results demonstrate that a Ca-dependent release of AVP occurs within the lateral septum. This release is affected by the same stimuli that induce the liberation of AVP into the blood, suggesting that AVP within the septum may contribute to the central regulation of body fluid homeostasis.

130.14 CENTRAL ACTION OF ARGININE VASOPRESSIN ON RENAL FUNCTION. C. L. Riphagen* and Q. J. Pittman. Dept. of Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada.

The posterior pituitary hormone, arginine vasopressin (AVP) is well known for its peripheral antidiuretic and pressor effects. Immunocytochemical studies have established the presence of immunoreactive AVP in neuronal fibres within the spinal cord and evidence now suggests that AVP may function as a neurotransmitter in the central nervous system. In the present study we have tested the hypothesis that AVP may affect renal function through a central action on neurons within the spinal cord.

In inactin anaesthetized rats a polyethylene (PE10) cannula was introduced through the cisterna magna and threaded down the spinal cord in the sub-arachnoid space to the region of the T_{12}-T_{13} vertebrae. Blood pressure was monitored using a carotid cannula. A jugular infusion of 0.7% NaCl/5 mM glucose was infused at a rate of 5.1 ml/hour to maintain a stable urine flow and a cannula leading to a conductivity meter and a fraction collector drained urine from the bladder. Urine volume and osmolality were calculated over 10 minute periods. 3 µl 10^{-6} M to 10^{-7} M AVP in Krebs or artificial CSF [or the same volumes of the analogues arginine vasotocin (AVT), oxytocin and DDAVP (a molecule having antidiuretic, but not pressor activity)] were injected into the T_{12}-T_{13} region.

After the injection of 10^{-6} M AVP or the analogue AVT into the spinal cord, urine output consistently dropped to less than 50% of the control levels and the conductivity and osmolality of the urine increased. Similar injections of 10^{-7} M AVP or oxytocin did not produce consistent results. Spinal application of DDAVP appeared to have little effect on urine flow.

The observed antidiuretic effects of the centrally applied AVP was not likely due to its diffusion into the peripheral circulation as intravenous application of the antidiuretic antagonist d(CH_2)$_5$-D-Tyr-VAVP (10^{-6} M) did not have a significant effect on the responses to central application of the AVP.

These studies suggest a central action of AVP on renal function and raise the possibility that AVP (or a related molecule) may act within the CNS in a manner consistent with one of its peripheral effects.

Supported by MRC. Antagonist supplied by Dr. M. Manning.

130.15 NEUROPEPTIDE PROFILES IN POSTPARTUM PSYCHIATRIC SYNDROMES. C.S. Sebastian*, R.G. Nathan*, M. Hack*, A. Vekovius* and A. Lassen*. (Spon: G. Trapp). Psychiatry Research Unit, Department of Psychiatry, Louisiana State University Medical Center, Shreveport, LA 71130.

Increased activity of the hypothalamus-pituitary-cortisol axis is found in a number of patients with primary depression. The peptides that control the discharge of the pituitary hormones are known as release or release-inhibiting hormones. Many of these peptides are considered to have effects on neurotransmission in other parts of the brain. For particular control peptides such as the hypothalamus releasing factor, degradation is under extensive feedback control. On this basis, it has been proposed that several psychiatric disorders may be due to a peptidase insufficiency. Increased strain on the peptidergic system regulated by such a peptidase could reach a point where peptide release would exceed breakdown, resulting in pathology and peptide excretion. Trygstad et al (1980) demonstrated a pathological carrier-protein-pattern upon Sephadex G 25 gel filtration of urine samples obtained from patients suffering from acute affective psychosis. The pathological pattern was normalized by treatment with tricyclic antidepressants.

Our prospective study was designed to investigate the patterns of urinary neuropeptides occurring in pospartum psychiatric syndromes (PPS). A minor form of PPS, characterized by mild depression, anxiety and clouding of consciousness occurs in 50-80% of the women after childbirth. Accordingly, urine samples, and responses to the Zung's Self-Rating Depression Scale (SDS) and the Depression Adjective Checklist (DACL) were obtained from 50 women during the eighth month of pregnancy (samples A), day one postpartum (samples B) and six weeks postpartum (samples C). Preliminary computer analyses of the psychometric data indicate that the women had started out with highest scores of depression before delivery which fell off significantly by the sixth week. Thus, contrary to popular belief, women appear to report depressive features even prior to childbirth. On the SDS ($n=22$), the mean scores were A=54.716; B=52.102 and C=46.40 (A and B were significantly >C, $p<.001$). On the DACL ($n=35$), A=13.714 was significantly > both B=12.257 and C=11.829 ($p<.0180$).

The urinary peptides ($n=27$) were precipitated with benzoic acid, washed in ethanol and chromatographed on 2.5 x 97 cm Sephadex G-25 column. Optical density (280 nM) on 20 ml fractions were monitored and the elution profiles plotted. The mean peak heights occurring between fractions 30 to 45 were compared. The mean peak heights in samples A & B were significantly lower than C ($p<.001$).

130.16 DIFFERENTIAL SENSITIVITY OF HOME-CAGE SEEKING BEHAVIOR OF NEONATAL RATS TO ACTH PEPTIDES. J. Berran*, G.R. Acker* and F.L. Strand. Lab. of Physiology, New York University, N.Y.C., N.Y. 10003.

The role of brain neuropeptides, specifically those of the ACTH family, in modulating learning and memory function, has been widely investigated in the adult mammalian system, while their effects in the immature animal have had little attention. Therefore, we investigated the effects of ACTH 4-10 and ACTH 4-9 (Org 2766) on learning acquisition and extinction in neonatal animals less than three weeks of age, using a home-cage seeking test as motivation and reinforcement. Animals were given (SC) either ACTH 4-10, ACTH 4-9 (Org 2766) (10 μg/kg) or saline every day starting at birth. When the animals were 16 days of age, learning trials were given by placing one animal at a time at the start of a T maze and allowing it to run either to a left or right goal box, one of which contained the mother. After 24 hours the rats were subjected to extinction trials (running the maze without the presence of the mother as reinforcement). The number of trials needed before learning or extinction was reached, the number of errors made, and the time it took to reach the goal box were measured. Our results show that those animals treated with ACTH 4-10 demonstrated a significantly greater delay in extinction of learning of the T maze ($p<0.02$), as compared to ACTH 4-9 (Org 2766) or saline. In addition, ACTH 4-10 treated animals ran the T maze more quickly, but this response was not significant. The acquisition phase was not affected by the ACTH peptides at the dosage given. We conclude that, at this early stage of development, the retention of a learned task is more sensitive to the neuromodulatory action of ACTH than the initial learning itself, and perhaps this is due to ACTH acting on the organizational process of the developing central nervous system.

This research was partially supported by Grant BRSG RR07062 awarded by the Biomedical Research Support Grant Program Division of Research Resources, National Institutes of Health.

130.17 CRANIAL MOTONEURONS IN VITRO: MEMBRANE PROPERTIES AND ACTIONS OF TRANSMITTERS AND TRH. R. A. Gregg and D. O. Carpenter, New York State Department of Health, Albany, NY 12201

Antidromically identified motoneurons of the hypoglossal nucleus (XIIth) and neurons of the dorsal motor vagal (DMV) nucleus (Xth) were studied in submerged, perfused brain stem slices of the rat at 35°C. Intracellular recordings were made using either single or double barreled electrodes. Hypoglossal motoneurons had resting potentials averaging -57.3 $\pm$ 1.68 mV and input resistances of between 40-70 MΩ. When antidromically activated by stimulation of the XIIth nerve as it exits from the ventral brain stem with a bipolar electrode, the motoneurons show clear initial segment (IS) and soma-dendritic (SD) spikes, similar to those characteristic of spinal motoneurons (Brock, et al., J. Physiol. 122:429, 1953). The SD spike followed frequencies up to 100 Hz, while the IS spike followed faithfully at frequencies $\geq$ 250 Hz. The majority of neurons penetrated in the XIIth nucleus could be antidromically activated. Neurons in the DMV could not be antidromically activated in the plane of the slice, but had average membrane potentials of -55.6 $\pm$ 1.63 mV and input resistances similar to those of XIIth neurons. The current-voltage relations of both types of motoneuron were linear in the potential range -50 to -80 mV.

L-glutamate (Glu), gamma aminobutyric acid (GABA), glycine (Gly) and thyrotropin releasing hormone (TRH) were applied to DMV and XIIth neurons by ionophoresis. Glu excited every cell, causing a rapid depolarization which was not associated with a measureable conductance change. GABA and Gly both hyperpolarized DMV neurons, causing a conductance increase of approximately 80%. However in the XIIth nucleus both GABA and Gly depolarized the cells. The response was associated with a conductance increase of approximately 25%, and reversed in polarity with about 10 mV depolarization. In spite of the depolarization with both extra- and intracellular recordings the response to each was inhibitory. TRH elicited a direct excitatory response in approximately half of the cells in both nuclei. The response was relatively slow in onset and long in duration, and was associated with an approximately 20% decrease in membrane conductance. Perfusion of 4 mM BaCl elicited a response very similar to that induced by TRH, suggesting the possibility that TRH is acting through effects on "M" channels.

130.18 PROTECTIVE EFFECT OF TRH IN ANAPHYLACTIC SHOCK: SITE AND MECHANISM OF ACTION. S. Amir, A. Schachar*, M. Harel* and D. Samuel*. Dept. of Isotope Res., Weizmann Inst. Sci., Rehovot, Israel.

The tripeptide thyrotropin releasing hormone (TRH) has been shown to improve survival in experimental endotoxic or spinal shock. In these studies, the effect of TRH appeared to be secondary to its ability to reverse the cardiovascular depression associated with endotoxin administration or spinal trauma. Here we report the beneficial action of TRH in another model of fatal shock, systemic anaphylaxis, and provide evidence that this effect may be mediated through central activation of the adrenal medulla and consequent stimulation of β-adenoceptive sites.

Subjects were ICR mice immunized with 2 mg bovine serum albumin (BSA) in 0.2 ml aluminium hydroxide gel. Fatal shock was induced by challenging the mice IV with 25 ug BSA 10 days after immunization. Administration of 125 or 250 ug TRH IV together with the challenge dose of BSA decreased the mortality rate of shocked mice by 57 and 65%, respectively ($p<0.05$). IV administration of 5 or 25 ug TRH was ineffective. Similar doses of TRH decreased the mortality rate by 55 to 70%, respectively ($p<0.05$), when administered ICV 5 min after induction of anaphylaxis. 'Acid' TRH, a deamidated metabolite of TRH was ineffective in improving survival following IV administration (125 or 250 ug); ICV 'acid' TRH, 5 ug, decreased the mortality rate by 60% ($p<0.05$). The involvement of the sympatho-adrenal system in the effect of TRH was studied by evaluating the effect of TRH following pretreatment with the ganglionic blocker chlorisondamine chloride (2.5 mg/kg IP) or after bilateral adrenal gland denervation (4 weeks before experimentation). Both chlorisondamine, which diminishes sympathetic outflow, and adrenal denervation reversed the protective effect of IV (125 ug) or ICV (5 mg) TRH. Pretreatment with 6-OHDA (50 mg/kg IV), which selectively destroys sympathetic nerve endings did not block the effect of IV of ICV TRH. Finally, mice were pretreated with 2.5 mg/kg phentolamine, an α-adrenergic receptor blocker or 2.5 mg/kg propranolol a β-adrenergic receptor blocker, or with 2.5 mg/kg domperidone, a peripherally acting dopamine antagonist, prior to TRH administration. In these experiments propranolol blocked the protective effect of IV or ICV TRH whereas phentolamine or domperidone had no demonstrable effects. Collectively, these results suggest that TRH exerts its protective effect in anaphylactic shock by acting in the CNS to increase sympathetic outflow to the adrenal medulla. Released adrenal catecholamines may act through β-adrenoceptive sites, most probably in the heart, to improve cardiocriculatory performance and thus to increase survival.

130.19 BENEFICIAL ACTIONS OF TRH IN NALOXONE-RESISTANT SHOCK, W. E. Lux, Jr.*, G. Feuerstein and A. I. Faden (SPON: A.H. Hassen). Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Both thyrotropin-releasing hormone (TRH) and naloxone have previously been shown to improve experimental shock due to endotoxemia, hypovolemia or spinal injury, and the beneficial actions of TRH in these models have been attributed to "physiologic" antagonism of endogenous opioid (endorphin) systems. We utilized an unanesthetized guinea pig model to examine the therapeutic effects of TRH and naloxone in four additional forms of experimental shock involving vasoactive lipids. Leukotriene D_4 (LTD_4) (5 µg/kg IV) and platelet-activating factor (PAF) (1 nmol IV) each produced profound hypotension in this model. Similar hypotension was produced by ovalbumin-induced anaphylaxis, a condition in which endogenous LTD_4 and/or PAF may be implicated. In addition, the hypotension produced by 15-hydroperoxy eicosatetraenoic acid (15-HPETE) was examined by administration of the enzyme responsible for its formation, soybean lipoxygenase (150 mg/kg IV). In all four instances, the hypotension was significantly improved by TRH (2 mg/kg IV), whereas naloxone (2 mg/kg IV) was without effect. In the LTD_4 and PAF models, intracerebroventricular (ICV) administration of TRH reversed the hypotension at doses which were ineffective when administered systemically (0.02 mg/kg). In these same models, the synthetic TRH analog MK 771 (2 mg/kg IV) produced a beneficial effect which was comparable to that of TRH. In the LTD_4 model, moreover, the TRH effect was dose-dependent at doses between 0.2 mg/kg IV and 2 mg/kg IV. These data suggest that TRH may be of therapeutic benefit in naloxone-resistant, lipid-related experimental shock models through receptor-mediated central interactions which do not involve antagonism of endorphin effects. Whether TRH exerts similar non-endorphin-related actions in naloxone-sensitive shock models remains to be determined.

FUNCTIONS OF GLIA

131.1 ELECTROPHYSIOLOGY OF ASTROCYTES IN PRIMARY CULTURES. W. Walz and L. Hertz*. Dept. of Pharmacology, Univ. of Saskatchewan, Saskatoon, Sask. S7N OWO Canada.

In the past 15 years there has been a growing controversy whether glial cells are involved in K^+ homeostasis of the central nervous system and whether they function as spatial buffers (distributing K^+ by transcellular passive K^+ currents) or by actively accumulating K^+ by a Na^+-K^+ ATPase. We studied this problem by using pure primary cultures of mouse astrocytes and intracellular glass microelectrodes for potential recording. The membrane potential was -92 mV at 3 mM external K^+ concentration. Reduction of the external Na^+ concentration from 143 to 27 mM or of the external Cl^- concentration from 125 to 9 mM for 5 min had no effect on the membrane potential. Application of 2 mM furosemide, which in our astrocytes has an inhibitory effect on Na^+ and Cl^- fluxes, resulted in no measurable alteration of the membrane potential. Hence, these experiments reveal an exclusive K^+ conductance of the astrocytic membrane. Application of ouabain (1 mM) caused a one-phase depolarization with a linear time course of 0.7 mV/min. Washing out of ouabain led to a repolarization of the membrane with a faster time course (2.3 mV/min) but no hyperpolarization below resting potential. The astrocytes were also exposed to K^+-free saline which loaded the cells with intracellular Na^+. When the K^+-free saline was replaced with the normal saline, the membrane repolarized but there was, again, no transient hyperpolarization below resting potential. These experiments show that a ouabain-sensitive Na^+-K^+ pump is necessary for a stable membrane potential, but they give no evidence for an electrogenic contribution during steady-state or stimulation of the pump. Nevertheless, the behavior of the membrane potential was found to deviate from a Nernst potential for K^+ when extracellular K^+ was changed: the slope was linear from 1.5 to 100 mM with 51 mV/tenfold change compared with 61 mV for a Nernstian behavior at 37°C. ^{42}K measurements showed that the accumulation, respectively release of K^+ as a function of the K^+ concentration showed two phases: between 1.5 and 25 mM the cells almost trippled their K^+ content and, assuming a constant cell water content of 6.5 µl/mg protein the calculated changes in internal K^+ concentration corresponds well with the deviation from the Nernst behavior of the membrane potential. Between 25 and 100 mM the K^+ content changed linearly with the extracellular K^+ concentration and the change in the calculated internal K^+ concentration was higher than could be expected from the deviation from the Nernst behavior, possibly because cell swelling increases cell water. Thus, our experiments reveal an exclusive K^+ conductance and an accumulation of K^+ by astrocytes. The astrocytes therefore express membrane properties which enable them to act both as spatial buffer and as accumulators of K^+.

131.2 FAMILIAL AND SOCIAL INTERRELATIONSHIPS OF ASTROCYTES AND OLIGODENDROCYTES. M. Noble (SPON: W.I.McDonald). Dept. Clinical Neurology, Institute of Neurology, Queen Sq., London WC1N 3BG England.

Recent studies have (i) identified protoplasmic and fibrous astrocytes as two distinct populations,with different antigenic phenotypes and seemingly not interconvertible (Raff, et.al., J. Neurosci., in press;Raff and Miller, submitted) and (ii)identified a glial progenitor cell in the optic nerve of 7d old rats which can be induced in vitro to differentiate into either an oligodendrocyte or a fibrous astrocyte (Raff, Miller and Noble, Nature, in press). When grown in the prescence of fetal calf serum (FCS), the bipotential oligodendrocyte-astrocyte progenitors (OAPs) all expressed Glial Fibrillary Acidic Protein (GFAP) and became fibrous astrocytes;in the absence of FCS all OAPs expressed galactocerobroside and became oligodendrocytes. In these first studies, in vitro differentiation of all OAPs took place in 3 days, with little or no associated cell division.

These findings present several puzzles, for it's known that,both in vivo and in other in vitro systems, oligodendrocyte differentiation takes place over a span of up to several weeks, and is associated with at least limited cell division. We have begun our investigations into regulation of OAP development by examining the effect of purified protoplasmic astrocytes on in vitro development of the OAP. We have found (1)Medium conditioned by astrocytes can maintain the OAP in an undifferentiated state for several days and (2) associated with this lack of differentiation, the OAP now divides in vitro, as judged by ^3H-Thymidine incorporation.

We are currently attempting to characterize the factor(s) produced by astrocytes, and to examine the effects of axons on OAP development. In addition, the ability to maintain the OAP in an undifferentiated state now allows us to investigate the possible existence of positive signals capable of promoting differentiation of the OAP into an oligodendrocyte.

131.3 REACTIVE ASTROGLIOSIS IS A FOCAL PROCESS. P.S. Fishman*, G. Nilaver, and J.P. Kelly* (SPON: C. Noback). Dept. of Neurology and VA Res. Serv., Univ. of Maryland Sch. of Med., Balt., Md. 21201 and Depts. of Neurology and Anatomy and Cell Biology, Coll. of P&S, Columbia Univ., N.Y., N.Y. 10032

Reactive astrogliosis, the sequence of changes that occurs in astrocytes after brain trauma, is characterized by the proliferation of intermediate cytoskeletal filaments and increased immunoreactivity with antibodies against glial fibrillary acidic protein (GFAP). There are two principal hypotheses that have been advanced to explain reactive gliosis. The first states that diffusable substances emanating from the site of injury activate astrocytes nearby eventually leading to an expanding zone of gliosis. The other hypothesis states that for an astrocyte to become reactive, it must be in direct contact with neuronal or myelin debris. We have tested these two conflicting hypotheses directly by examining the pattern of gliosis that appears in the optic chiasm of the mouse after unilateral eye enucleation. Using this model system, we could compare the responses of astrocytes to fascicles of degenerating axons or normal axons lying adjacent to one another. After survival times ranging from three days to two months following enucleation, the animals' brains were processed for immunohistochemistry. Reactive astrocytes were identified in sections by their intense immunoreactivity with anti-GFAP. Nerve fibers undergoing Wallerian degeneration were identified by their pathological appearance and by their enhanced immunoreactivity to anti-neurofilament (NF) antibody (courtesy of Dr. D. Dahl). The distribution of reactive astrocytes was compared to the distribution of degenerating axons within the optic chiasm and optic tract in alternate sections.

Following enucleation, all seven experimental animals showed an increase in anti-GFAP immunoreactivity that peaked at survival intervals of two to three weeks. The regions of increased GFAP immunoreactivity were strictly confined (usually within 50 um) to corresponding regions of enhanced NF immunoreactivity in all animals examined. Regions containing reactive astrocytes and their processes were quite uniform in outline, so that the borders between anti-GFAP positive and negative regions were remarkably sharp. There was no spread in the distribution of anti-GFAP immunoreactivity with increasing time after enucleation. In summary, reactive astrocytes in the optic chiasm are restricted in distribution to the immediate environment of degenerating axons and myelin, and it appears that intimate contact with degenerating debris is necessary to provoke and maintain reactive astrogliosis. Supported by an Associate Investigator Award from the VA Research Service.

131.4 AUTORADIOGRAPHY OF HIGH AFFINITY UPTAKE OF CATECHOLAMINES BY PRIMARY ASTROCYTE CULTURES. D. Semenoff* and H.K. Kimelberg, Division of Neurosurgery, Albany Medical College, Albany, N.Y.

We have previously studied uptake of catecholamines by primary astrocyte cultures prepared from the cerebral hemispheres of 2 day old rats (Pelton et al. Life Sci. 28:1655, 1981). Uptake of ^{3}H labelled DL-norepinephrine (^{3}HNE) or dopamine (^{3}HDA) at around 10^{-7}M was very sensitive to inhibition by tricyclic antidepressants or omission of Na^+. The apparent K_m for the uptake of ^{3}HNE was in the range of 2-3 x 10^{-7}M (Kimelberg and Pelton, J. Neurochem. 40:1265, 1983). We have now examined the cellular localization of this uptake using autoradiography. Uptake of 7.5 x 10^{-7}M ^{3}HNE representing 1μCi/35mm diameter plastic dishes was studied at an external $[K^+]_o$ of 4.5mM. The dishes were developed after 1-4 weeks exposure to emulsion. Grain density above background was seen in almost all the cells, both the flat epithelial-looking cells and cells with numerous radiating processes. In previous studies we have shown both types of cells to be glial fibrillary acidic protein (+). In about 20% of the total area the grain density was very intense, and again this high density occurred in both flat and process-bearing cells. Omission of Na^+ or addition of the antidepressant desmethylimpramine (10^{-7}M) reduced the grain density of all the cells to that seen for the lowest density in the non-inhibited cultures, or to background levels. Autoradiography after exposure to ^{3}HDA, in this case at 27mM $[K^+]_o$, gave essentially the same results. Inhibitors of catechol-O-methyltransferase and monoamine oxidase were always present to prevent metabolism of catecholamines. Omission of either inhibitor, and especially both, markedly lowered grain density after exposure to ^{3}HDA. Uptake studies using ^{3}HNE resulted in continually increased uptake of ^{3}HNE as $[Na^+]_o$ was increased from 0 to 140mM. Increasing $[K^+]_o$ up to 40mM did not seem to inhibit ^{3}HNE uptake. The presence of either the α- and β-receptor antagonists phentolamine or propranolol (10^{-5}M) did not inhibit Na^+-dependent uptake indicating that we are measuring uptake and not receptor binding. These results further confirm that astrocytes in primary cultures contain a high affinity uptake system for catecholamines. The autoradiographic studies also show that astrocytes prepared from whole cerebral hemispheres show differences in uptake intensity suggesting some functional heterogeneity that may depend on the region of origin within the cerebral hemisphere, even at a relatively early age. (Supported by NIH grant NS 19492 from NINCDS to H.K.K.).

131.5 "PERMISSIVE EFFECTS" IN C_6 GLIOMA CELLS. H. Nakamura*, P. E. McKeever, B. H. Smith and P. L. Kornblith. Surgical Neurology Branch, NINCDS, National Institutes of Health, Bethesda, MD 20205.

Glucocorticoids provide the opportunity for agents which increase cAMP to work more effectively. Since these "permissive effects" of glucocorticoids have been well documented in many tissues other than central nervous system, we wondered whether they occurred in glial cells. For this reason, the modulation of the 1-isoproterenol induction of enzyme, protein and RNA by dexamethasone in C_6 glioma cells was studied. Cells pretreated with 10^{-6}M dexamethasone for 24 hours were compared with untreated cells in assays for stimulation by 10^{-5}M 1-isoproterenol. While 1-isoproterenol for 24 hours increased lactate hydrogenase (LDH) enzyme activity from 2247 ± 47 units/mg protein (N=3) to 4071 ± 22 units/mg (68%) in non treated cells, LDH increased from 2624 ± 40 units/mg protein to 7185 ± 29/units/mg protein (178%) in dexamethasone pretreated cells.

RNA and protein synthesis were compared at 0, 1, 2, 3, 4, 5, and 6 hr. after adding isoproterenol. Stimulation of ^{3}H leucine incorporation into protein by isoproterenol was greater (67% peak) and more prolonged (at least 6 hr.) in the dexamethasone pretreated group than in the control group (27% peak and 3 hr. stimulation). Isoproterenol increased ^{3}H uridine uptake by 107% in the dexamethasone pretreated group but did not increase uridine uptake in the group without dexamethasone. Dexamethasone has a "permissive effect" on induction of LDH in C_6 glioma cells by isoproterenol. This effect is associated with earlier "permissive effects" on RNA and protein synthesis.

131.6 VERATRIDINE CAUSES ASTROCYTES IN PRIMARY CULTURE TO BECOME EXCITABLE. C.L. Bowman*, C. Edwards and H.K. Kimelberg (SPON. P.A. Hanson), Neurobiology Research Center, SUNY-Albany and Albany Medical College, Albany, N.Y.

Astrocytes in primary culture have large negative resting membraine potentials (-64 ± 10 mV, n = 32 cells) which are predominantly K^+ diffusion potentials. 90% of the cells stain for glial fibrillary acidic protein . The cells are electrically silent since their current-voltage curves are approximately linear (Kimelberg et al. Neurosci. Abst. 8:238, 1982). Veratridine, or the combination of veratridine and α-scorpion toxin (II) causes either a series of transient depolarizations or a single long-lasting depolarization (Bowman et al. Biophysical J. 41:386a, 1983). The depolarizations are sensitive to tetrodotoxin (TTX) (15nM) and to a reduction in the external Na^+ concentration (replacement with Tris, choline or arginine).

We have extended these studies to test if astrocytes become electrically excitable in the presence of veratridine. Using two microelectrodes (one to pass current and one to monitor membrane potential), the membrane of the cells was hyperpolarized to between -90mV and -150mV for at least 10 seconds. In the absence of veratridine the membrane potential returned to its resting value upon cessation of the hyperpolarizing current (7 cells). In the presence of veratridine (10^{-4}M) termination of the hyperpolarization produced a transient depolarization more positive than the original resting potential (amplitude 2 to 20mV, lasting 5 to 15 sec., n = 7 cells). The amplitude of this transient depolarization was reduced but not completely eliminated by TTX (60nM) (4 cells). This effect of veratridine was reversible upon washout of the drug (7 cells).

These results show that astrocytes in primary cultures, which are normally electrically silent, have a chemically inducible Na^+ channel and voltage-sensitive gate.

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131.7 NOREPINEPHRINE INDUCED DEPOLARIZATION OF ASTROCYTES IN PRIMARY CULTURE. H.K. Kimelberg, C.L. Bowman*, H. Hirata, C. Edwards, R. S. Bourke and N.T. Slater*. Div. of Neurosurgery, Albany Medical College; Neurobiology Research Center, SUNY-Albany and Center for Laboratories and Research, NYS Dept. of Health, Albany, N.Y.

Astrocytes in primary monolayer cultures from the cerebral hemispheres of 2 day old rat pups have large negative membrane potentials which are predominantly K^+ diffusion potentials (Kimelberg et al. Soc. Neurosci. Abst. 8:238, 1982). Previous biochemical studies of these cells have demonstrated the presence of both α- and β-adrenergic receptors[1,2]. We have recorded from these cells using conventional intracellular techniques. Norepinephrine (NE) and the α-agonist phenylephrine were applied either in the bath or by ionophoresis. Both agonists evoked depolarizing responses in these cells. These responses were reversibly antagonized by the α-antagonist phentolamine, but not by the β-antagonist propranolol. Repeated applications of NE by ionophoresis often resulted in a decline in the amplitude of the depolarization, suggestive of desensitization.

In an effort to determine the ionic dependence of this response, we have also examined the effect of ionic substitution on the amplitude of the response to bath-applied NE. In this series of studies bath application of NE (10^{-5}M) caused a depolarization in 17 out of 18 cells tested. The amplitudes of the depolarizations ranged from 4 to 30mV. Replacement of external Cl^- with methanesulfonate appeared to have no effect on the NE response (1 cell), while reduction of external Na^+ (replaced with arginine) reduced, but did not completely eliminate, the NE response in 4 out of 7 cells tested. We have also studied the effects of long-term (∿ 10 min.) application of NE. Following the initial depolarization, there was a slow repolarization in the continued presence of NE (10 cells). The rate of repolarization ranged from 0.8 to 1.6mV/min and this rate appears to be reversibly reduced by ouabain (0.5mM; 2 cells). However, whether this ouabain-sensitive repolarization represents desensitization of the depolarizing response, or the appearance of a slower hyperpolarizing response remains to be determined. These results demonstrate that NE evokes depolarization of astrocytes in primary culture through activation of an α-adrenergic receptor. Further studies are required to determine the precise ionic basis of this response and the α-receptor subtype involved. (Supported by NIH grant NS19492 and NSF grant BNS 8213873 to H.K.K. and by NIH grant NS0681 to C.E.).

1. Van Calker and Hamprecht, in: Advances in Cellular Neurobiology, Vol. 1, Academic Press, 1980, pp 31-67.
2. Ebersolt et al. J. Neurosci. Res. 6:643, 1981.

131.8 REACTIVE RESPONSES OF ASTROCYTES IN CULTURE TO EPILEPTOGENIC AGENTS. E. Tiffany-Castiglioni* and A.J. Castiglioni. Depts. of Veterinary Anatomy and Biology. Texas A&M University, College Station, TX 77843.

Gliotic scar formation is a prominent characteristic of human epilepsy and is associated with subchronic or chronic models of focal epilepsy induced in animals by alumina, iron or cobalt. Reactive astrocytes form a glial scar by hypertrophy and accumulation of densely packed glial filaments. Although cortical gliosis accompanies pathological conditions other than epilepsy, its almost invariable presence in chronic focal epilepsy has prompted several investigators to propose a physiological role for glia in the epileptic process. Moreover, other workers have described metabolically "activated" cortical astrocytes in the anatomical region of epileptic foci, both in humans and animal models, which are not present in gliotic scars unrelated to epilepsy. The cells have elevated levels of glutamate dehydrogenase, glucose-6-phosphate dehydrogenase, and lactate dehydrogenase. In cobalt-induced foci in the rat, activated astrocytes appear to be a subset of morphologically reactive astrocytes. Since neurons and glia interact intimately on molecular and cellular levels, a deficiency of one cell type may affect the other. Thus primary damage to astrocytes could result in secondary lesions to the neurons. A cell culture system was used in the present study to observe primary effects of epileptogenic agents on astroglia. Objectives were: (1) to establish cultures of astroglial cells from 1-3 d old rat cortex that resemble normal, non-reactive protoplasmic astrocytes morphologically and histoenzymatically and (2) to induce cells to become reactive in response to exposure to alumina or ferrous chloride. Results will be discussed.
Supported by the Epilepsy Foundation of America and Texas A&M University grant ORR-6-83.

131.9 **CYCLIC AMP STIMULATION OF CYTOSKELETAL PROTEIN PHOSPHORYLATION AND MORPHOLOGIC ALTERATION IN INTACT CULTURED RAT ASTROCYTES.** **Edward T. Browning, Monica Ruina*, and Beverly Poelstra*, Dept. of Pharmacol., Rutgers Medical School, Piscataway, NJ 08854.**

Past studies of cAMP stimulated protein phosphorylation in the C-6 glioma cell line showed an increased cytoskeletal intermediate filament protein (GFA and vimentin) phosphorylation following treatment of the cultures for 5' with norepinephrine. Present studies with primary cultures of rat astrocytes examined the relationship between cyclic AMP content, protein phosphorylation and changes in cellular morphology following exposure of the cultures to forskolin, an activator of adenylate cyclase. Cultures were prepared from neonatal rat cerebrum and were grown in Eagles medium plus 15% fetal bovine serum. Forskolin (20 uM) produced a 250-fold increase in the cAMP in 10'; the elevation was largely maintained over 120' of treatment. Following forskolin treatment, the ^{32}P-content of GFA was increased 4-, 12- and 3-fold after 5', 30' and 120', respectively; ^{32}P-content of vimentin was increased 70%, 240%, and 30% after 5', 30', and 120', respectively. Prior to forskolin treatment, the astrocytes were irregularly shaped and highly spread on the growth surface. Following addition of forskolin the cells retracted their cytoplasm toward the nucleus forming several well defined narrow processes. The initiation of cytoplasmic retraction was evident by phase contrast microscopy within 10 min of forskolin addition, progressed during the next 90 min or so and was essentially complete by 120 min of forskolin treatment. Therefore the period of active cytoplasmic retraction correlated in time with the period of greatest intermediate filament protein phosphorylation. (Supported by NSF Grant BNS 81-10564.)

131.10 PC12 CELLS ARE MITOGENIC FOR RAT SCHWANN CELLS. N. Ratner, R. Bunge and L. Glaser. (SPON: F. Mithen). Departments of Anatomy & Neurobiology and Biochemistry, Washington University Sch. Med., St. Louis, MO.

Previous studies from this laboratory have shown that normal rat Schwann cells derived from primary cultures of dorsal root ganglia are stimulated to proliferate by neurites in culture (Wood, Brain Res. 115:361, 1976), that the neurite-derived mitogen is a trypsin and heat-sensitive factor on the neurite surface (Salzer et al., J. Cell Biol. 84:753, 1980) and that contact between neurite and Schwann cell is required for its action. We have now shown that the pheochromocytoma cell line (PC12) can also stimulate Schwann cell division, as assayed by autoradiography of co-cultures of Schwann cells and PC12 cells in the presence of NGF. Under these conditions PC12 cells extend neurites and Schwann cells in contact with these neurites can be demonstrated to incorporate ^3H-thymidine. Schwann cell cultures to which crude PC12 membranes 60-75 μg/0.6 cm^2 (60-75 μg/0.25 ml) have been added show a labelling index (LI)* of 5%; in control cultures the LI is 0.5-0.9%.

Partial purification of the PC12 cell derived mitogen has been accomplished by sucrose gradient centrifugation of a crude PC12 membrane preparations followed by 0.2 M Na_2Co_3 extraction (pH 10-11) of gradient purified membranes. 75% of total membrane protein is extracted by Na_2CO_3 treatment; the 25% residue at a concentration of 20 μg/0.6 cm^2 (20 μg/0.25 ml) stimulates Schwann cell division approximately 100-300 X over background. The 22%-30% LI in these cultures is equivalent to the stimulation observed with crude dorsal root ganglion neurite membranes at a concentration of 8 μg/0.6 cm^2 (8 μg/0.25 ml). The PC12 cell mitogen and the neurite mitogen are similar that both mitogens:

1. are inactivated by heating to 60°C for 10'
2. are inactivated by incubation with 0.05% trypsin for 20' at 37°C (0.2 mg Na_2CO_3 extracted membranes/ml or 10^7 intact PC12 cells/ml)
3. stimulate the production of intracellular cAMP in Schwann cells 2.5-3.5 fold 16h after addition of membranes, while heat treated membranes are neither mitogenic nor do they elevate cAMP levels
4. are partially inactivated by freezing overnight at -80°C

The mitogens differ in that while both are active in serum-free media when co-cultures of neurons and Schwann cells or PC12 cells and Schwann cells are grown, membranes derived from PC12 cells are not mitogenic in serum-free media while membranes derived from neurons retain their mitogenicity under these conditions.

*LI = % labelled cells/total cells.

131.11 ETHANOL EFFECTS ON GLIAL CELL CULTURES: CELL PROLIFERATION AND GLUTAMINE SYNTHETASE ACTIVITY. D. L. Davies and A. Vernadakis*. Depts. of Pharmacology and Psychiatry, Univ. of Colo. School of Medicine, Denver, CO 80262.

Numerically glial cells constitute the major cell type of the brain and play key roles in establishing and maintaining the microenvironment of neighboring neurons. As part of an endeavor to assess the extent of glial cell involvement in the pathogenesis of brain damage following ethanol abuse, ethanol was administered to glial-enriched cultures and glutamine synthetase (GS) enzymatic activity was determined. GS activity is a glial cell marker and has been implicated in glutamate/glutamine compartmentation. DNA content of cultures was used as a measure of cell proliferation. Glial-enriched cultures were prepared from day 15 embryonic chick cerebral hemispheres. The nutrient medium was Dulbecco's Modified Eagle Medium (DMEM) fortified with 20% fetal bovine serum. After 15 hr the medium was aspirated and replaced with fresh medium. On culture day 6 ethanol was administered at 4 dose levels (0.1% to 2%, i.e., 22 to 435 mM) in DMEM - 10% fetal bovine serum. Thereafter, control and ethanol-containing media were changed daily until the cultures were harvested on culture day 10. Cell proliferation as assessed by DNA content was lower than for controls in cultures treated with 1% or 2% ethanol; the response was dose dependent. GS activity was measured in the supernatant from cell homogenates centrifuged at 15,000 RPM for 30 min and was expressed as μmoles γ-glutamylhydroxamate formed/mg protein/hr. Treatment levels of 1% and 2% ethanol markedly decreased GS activity. With regard to DNA content and GS activity, cultures exposed to 0.5% ethanol were slightly lower than controls, whereas treatment at 0.1% ethanol slightly increased both DNA and GS. The protein to DNA ratio was not altered by ethanol treatment at any dose level. These findings indicate that ethanol at dose levels of 1% and above impairs cell proliferation. Additionally, since the decrease in GS activity in ethanol-exposed cultures was not matched by an equivalent decrease in protein content, GS may be selectively susceptible to ethanol. Alteration in glutamate/glutamine metabolism may be a potential consequence of ethanol-induced changes in GS activity. Alternatively, ethanol may selectively inhibit the proliferation of a subpopulation of cells. Since primary cultures are heterogeneous, consisting of astrocytes, fibroblasts and endothelial cells, the discrepancy in GS to protein ratios may reflect the presence of cells refractory to ethanol exposure. In order to delineate this possibility, we are currently assessing the effects of ethanol on clones of astrocytoma cells and fibroblasts. (Supported by NRS fellowship award T32 DA 07043 and NIAAA research grant AA 03527.)

131.12 ELEVATED STEROID LEVELS SUPPRESS THE GLIAL RESPONSE TO BRAIN INJURY IN THE RAT. V. K. Vijayan and C. W. Cotman. Dept. of Hum. Anat., Sch. Med., Univ. Calif., Davis, CA 95616 and Dept. of Psychobiol., Univ. Calif., Irvine, CA 92717.

Previous studies have suggested that high levels of circulating glucocorticoid hormones interfere with the clearing of degeneration products in the rat hippocampus following an entorhinal lesion. It was speculated that these steroid effects might be mediated through glial cells by decreasing their number and by suppressing the activation of glial lysosomes.

The present study tested these hypotheses by comparing the increases in the number of glial cells and in the activity of glial lysosomal enzymes in response to a unilateral entorhinal lesion in steroid-treated and untreated adult male Sprague-Dawley rats. Animals (60-70 days old) were implanted subcutaneously with Alzet osmotic minipumps to deliver 200 μG hydrocortisone per day. Steroid administration started 5 days prior to the lesion and continued until sacrifice of animals on the 7th post-lesion day. Animals were perfused with buffered 4% paraformaldehyde and frozen sections were prepared from the brains. Glial cell number and density were evaluated from sections stained with cresyl violet. Glial cell induction in the denervated dentate outer molecular layer was expressed as a ratio of the glial cell density on the ipsilateral/the contralateral side. Acid phosphatase was stained histochemically by the method of Gomori. β-glucuronidase was examined by the method of Hayashi et al. In untreated animals (N=5), the glial cell density ratio was 2.34, indicating a substantial increase in the number of glial cells in the denervated zone in agreement with previous results. Treated animals (N=7), compared to the untreated, exhibited 33% reduction ($P < 0.001$) in the glial cell density ratio. In untreated animals, entorhinal lesion caused an enhancement in the staining of both lysosomal enzymes in the denervated zone. Steroid treatment reduced this enhancement. Computer-assisted quantitative cytophotometric measurements of individual, randomly sampled glial cells suggested that the reduction occurred, at least in part, due to a reduced enzyme staining intensity per cell.

We conclude from these results that glucocorticoid hormone treatment for brief periods prior to and following an entorhinal lesion significantly suppresses the increase in glial cell number and the activation of glial lysosomes in the rat hippocampus. It is proposed that in the steroid-treated animal, these effects slow the clearing of degeneration products after injury. Steroid hormones might exert an antagonistic effect on the neuroglial response to brain injury, much like their well-known anti-inflammatory effects on other tissues.

131.13 REACTIVE GLIOSIS OF MÜLLER CELLS IN RESPONSE TO GENETIC AND EXPERIMENTALLY PRODUCED PHOTORECEPTOR DEGENERATION. A.J. Eisenfeld*, A.H. Bunt-Milam, and P.V. Sarthy. Dept. of Ophthalmology, University of Washington, Seattle, Washington 98195.

The Müller cell is the main non-neuronal cell of the retina. It is usually classified as glial, although it does not contain glial fibrillary acidic protein (GFA). This protein is found in astrocytes and increases in brain astrocytes undergoing reactive gliosis. In this study we have demonstrated that the Müller cell expresses this protein in response to photoreceptor cell necrosis using immunofluorescence to localize GFA.

In the Royal College of Surgeons (RCS) rat with inherited retinal dystrophy, the photoreceptors begin to degenerate on day 18 and very few photoreceptors remain by day 60. In the normal rat retina at all ages only astrocytes contained GFA. This staining pattern was also found in RCS rats younger than 32 days. Beginning on day 32, a few GFA positive fibers spanned the retina from the inner limiting membrane to the external limiting membrane. By day 41 and at all later ages examined, the radial fibers, as well as the somata, of Müller cells were intensely labelled throughout the retina. To determine if the presence of GFA in Müller cells was a response to photoreceptor necrosis or might be a direct effect of the mutant gene, we maintained normal, adult Sprague-Dawley rats in constant light for 5 weeks. This produced photoreceptor degeneration comparable to a 40 day-old RCS rat. The Müller cells in the retinas damaged by constant light were positive for GFA, indicating that Müller cells express GFA reactivity in response to experimentally produced photoreceptor degeneration.

We conclude that Müller cells undergo reactive gliosis in response to both genetic and experimentally produced photoreceptor degeneration.

Anti-GFA kindly provided by Larry Eng. Supported by USPHS Grants EY-07013, EY-01311, and EY-01730.

131.14 ACTIVITY-DEPENDENT SHRINKAGE OF BRAIN EXTRACELLULAR SPACE IN RAT OPTIC NERVE: A DEVELOPMENTAL STUDY. C.L. Yamate*, B.R. Ransom, and B.W. Connors (SPON: L. Eng). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

In a previous investigation on the extracellular space (ECS) of the rat optic nerve (Connors et al., Science 216:1341, 1982) we noted activity-dependent shrinkage of the ECS in older animals. We have further characterized this phenomena and report the time course of its appearance in postnatal development.

Nerves were dissected free and bathed in physiological saline with $[K^+]_o$ of 5 mM. Supramaximal stimuli (with regard to evoked field potentials) were delivered to the nerves at different frequencies by suction electrodes. K^+ specific microelectrodes were used to measure $[K^+]_o$ or monitor the concentrations of choline or tetramethylammonium (TMA) when these cations had been added to the bathing solution. In the presence of TMA or choline, the K^+ specific microelectrode does not detect changes in $[K^+]_o$ of from 3 to 25 mM. Since cellular membranes are impermeable to choline or TMA increases in the concentration of these ions imply a shrinkage of the volume fraction occupied by the ECS. To determine the developmental sequence of activity-dependent ECS shrinkage optic nerves ranging in age from 1 to 30 days were stimulated using 10 sec. trains of different frequencies. This was done initially in normal solution allowing the evoked increase in $[K^+]_o$ to be monitored. The bathing solution was then switched to one containing choline (10 mM) or TMA (5 mM) and the same stimulus trains were repeated. Under these conditions voltage fluctuations in the ion-sensitive microelectrode could be translated into changes in the size of the ECS. The relationship between maximum activity-dependent shrinkage of ECS volume fraction and age was roughly sigmoid-shaped with little or no shrinkage evident between 1 to 5 days (av. = 1.8%), moderate shrinkage between 7 to 17 days (av. = 5.7%) and striking shrinkage in nerves older than 24 days (17-30%). The magnitudes of ECS shrinkage and increase in $[K^+]_o$ were both directly proportional to stimulus frequency in the older nerves. The time course of activity-dependent ECS shrinkage was similar but slower than the time course of the evoked increase in $[K^+]_o$ produced by the same stimulus train.

The developmental sequence described above for activity-dependent shrinkage of the ECS coincides roughly with the birth and maturational sequence of glial cells in this structure (Skoff et al., J. Comp. Neur. 169:291, 1976). This coincidence would be consistent with the hypothesis that activity-dependent shrinkage of the ECS depends upon K^+-induced electrolyte and water transport into glial cells with tissue swelling and reduction of the ECS. Further studies are underway to test this hypothesis.

Supported by NIH grants NS15589 and NS00473 from the NINCDS.

131.15 LACTATION-ASSOCIATED GLIAL PLASTICITY IN THE SUPRAOPTIC NUCLEUS OF THE RAT. A. K. Salm, K. G. Smithson* and G. I. Hatton. Neurosci. Prog. and Dept. Psych., Michigan St. Univ., E. Lansing, MI 48824.

Electron microscopical studies have shown an absence of astrocytic glial processes from between neurosecretory cells in the supraoptic nucleus (SON) during lactation, a time when an active retraction of the glial processes has been hypothesized to occur (Hatton and Tweedle, 1982; Theodosis et al., 1981). We sought to demonstrate changes in the glia within SON which might underlie this apparent morphological plasticity. Therefore, the distribution of the cytoskeletal glial fibrillary acidic protein (GFAP) as visualized by PAP immunocytochemistry, was assessed in tissue from estrous and 10 day lactating rats. Reaction product distribution in the form of a frequency histogram of 256 staining densities was determined by computerized image analysis. All measurements were made in a random order and without knowledge of experimental group membership. A split-plot design statistical analysis of the features of the frequency histogram, i.e., mean, standard deviation, skewness, and kurtosis was then performed to ascertain effects of hormonal state, location within SON, and interactions of these variables. These analyses revealed that a redistribution of GFAP had occurred in the SON of lactating subjects. Although observed in all parts of the nucleus, the change was far more dramatic in the predominantly oxytocinergic portions of the SON. In addition, the change was to a less dense, more homogeneous distribution of the GFAP, which first, is reflective of a reduction in the high density staining characteristic of that observed in glial processes, and second, has been previously shown in vitro to be associated with a state of great glial plasticity (Duffy et al., 1982). Identical measurements were taken from the same material in the lateral hypothalamic area. No changes were found to occur in that part of the hypothalamus. These results are important for a number of reasons. First, by demonstrating a redistribution of a glial cytoskeletal protein these data provide direct support for the hypothesis that glia actively retract their processes during lactation. Second, they show that the morphological plasticity often seen in vitro occurs in vivo as well. Third, the stimulus necessary for inducing this phenomenon was a physiological one. This implies the existence of a normal underlying mechanism that somehow goes awry in the aberrations of GFAP which have been observed in neuropathologies. Finally, the homogeneity of staining densities found in tissue from lactating subjects is similar to that observed in "undifferentiated" glia in vitro. The data reported here suggest that this morphological form may represent a functional state which an astrocyte might transiently assume in vivo.

Supported by NIH Grant NS09140. We gratefully acknowledge the Pattern Recognition and Image Processing Laboratory, MSU.

131.16 ISOLATION OF A FRACTION ENRICHED IN OLIGODENDROCYTES PLASMA MEMBRANE. S Szuchet, PE Polak and SH Yim, Department of Neurology, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois 60637.

A simple procedure was developed using isolated oligodendrocytes as the starting material for the separation of oligodendrocyte plasma membrane. A plasma membrane fraction was obtained that had a 21 fold enrichment in a membrane specific marker, p-nitrophenyl phosphatase (pNPase, 9.2 ± 2.2 nM/min/mgP) and a 7 fold increase in an oligodendrocyte specific marker, 2',3',cyclic nucleotide phosphodiesterase (41.5 ± 1.5 μM/min/mgP). The corresponding values for the cell homogenate were: 0.43 ± 0.08 nM/min/mgP and 6.0 ± 0.2 μM/min/mgP, respectively. In addition, nuclear and mitochondrial enriched fractions were also collected. Oligodendrocytes were isolated from ovine brains by our procedure (Szuchet et al,J Neuroscience Meth 3:7-19,1980) and maintained in culture for 4 days to allow them to recover from the trauma of isolation. After harvesting, cells were washed free of serum, resuspended in disruption medium (20mM Tris, 0.12M NaCl, 1mM $MnCl_2$, pH 7.4) and broken by passage through a cell disrupter designed by us (Szuchet and Polak, Anal Bioch,128:1983, 453-458). Disrupted cells were collected in disruption medium containing 1mM EDTA and centrifuged at 300g for 7 min. The pellet (P_1) contained mostly nuclei. The supernatant (SP_1) was applied on a self-generating gradient of 20% percoll in disruption medium + EDTA and centrifuged at 30,000g (av.) for 15 min. Three bands separated on this gradient. They were designated: F1,F2, and F3 in the centrifugal direction. F2 exhibited a 5-7 fold enrichment of pNPase whereas F3 gave a 5-7 fold enrichment in a mitochondrial marker (succinic dehydrogenase), suggesting that these fractions were enriched in plasma membrane and mitochondria, respectively. F2 was washed 3 times with 10mM Tris buffer pH 7.4, centrifuging after each wash. The final pellet was resuspended in 1 ml of 0.25M sucrose, cooled to 0°C and 2.7 ml of 0.23% saponin in the same solvent were slowly added, left for 15 min. at 0°C and centrifuged at 200,000g for 2 hours. The resulting pellet was dispersed in 0.85M sucrose, applied on a linear sucrose gradient from 0.9-1.5M and centrifuged at 200,000g (av.) for 16 hours. Three bands separated on this gradient, the middle band, F2(2), had the highest enrichment of plasma membrane markers (see above). Membrane fractions prepared in this way should prove valuable for characterizing oligodendrocyte plasma membrane and its changes over time in culture and for comparative studies with myelin. Supported by grant from the National Mutliple Sclerosis Soc RG 1223-B3.

131.17 IMMUNOCYTOCHEMISTRY OF MYELIN BASIC PROTEIN IN ADULT OLIGODENDROGLIA. Constancia del Cerro*, Nancy H. Sternberger*, Marian W. Kies*+ and Robert M. Herndon (Spon: William H. Merigan). Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642 and Laboratory of Cerebral Metabolism, National Institute of Mental Health, NIH, Bethesda, Maryland 20014+.

Myelin basic protein (MBP) has been found immunocytochemically in oligodendroglia of the developing rat and human central nervous system. However, reaction with MBP antiserum was detectable only prior to and during the early phase of myelination. The lack of reaction with still actively myelinating and mature oligodendroglia has been puzzling. We report here that by the use of mild fixation and modification of the staining technique previously used for detection of MBP on vibratome sections, staining of oligodendroglia in sections of adult rat brain has been achieved. Stained mature oligodendroglia had very fine, branched processes. Myelin staining was not intense and oligodendroglia could be detected in heavily myelinated tracts. Oligodendroglial staining was obtained with antisera to myelin, myelin basic protein and carbonic anhydrase. Antiserum to glial fibrillary acidic protein (supplied by Dr. L. Eng) reacted with astrocytic cell bodies and processes in a pattern quite different from the staining seen with antisera to oligodendroglial proteins.

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131.18 THE MYELIN-ASSOCIATED GLYCOPROTEIN AND MYELIN-FORMING SCHWANN CELL-AXON INTERACTION IN QUAKING MICE. B.D. Trapp,* R.H. Quarles* and K. Suzuki*. NINCDS, NIH, Bethesda, MD and Dept. Neuropathology, Albert Einstein College of Medicine, Bronx, N.Y. (Sponsor: M. Dubois-Dalcq)

The myelin-associated glycoprotein (MAG) is an integral membrane glycoprotein which represents approximately 0.7% of the total PNS myelin proteins. MAG has an apparent molecular weight of 100K and consists of approximately 30% sugar. Immunocytochemically, MAG has been localized in periaxonal membranes of PNS myelin. MAG is not present in compact portions of PNS myelin. In myelinated fibers from normal peripheral nerve, the Schwann cell periaxonal membrane is separated from the axolemma by a 12-14 nm gap or periaxonal space. Based on its periaxonal localization, MAG may play a role in maintaining contact between myelin-forming Schwann cells and axon. The bulk and polarity of MAG's oligosaccharide moieties could account for this 12-14 nm spacing. In contrast to compact myelin, which does not contain MAG, the cytoplasmic side of the periaxonal membrane does not "fuse" with the cytoplasmic side of the inner myelin lamellae to form a major dense line. The carboxy terminal of MAG may play a role in preventing this fusion. To test these hypotheses, the immunocytochemical localization of MAG was determined in ventral roots from adult quaking mice. These roots display several pathological alterations in Schwann cells and axons including partial and total separation of myelin sheaths from their axon. We compared the immunocytochemical localization of MAG in 1μm thick Epon sections with the ultrastructure in adjacent thin sections. Where the periaxonal space is maintained in myelinated quaking fibers, the Schwann cell periaxonal membrane is stained intensely by MAG antiserum. MAG was not detected in periaxonal membranes in regions where this 12-14nm gap is enlarged. The cytoplasmic side of these MAG negative periaxonal membranes fused with the cytoplasmic side of the inner compact myelin lamellae to form a major dense line. These results support a structural role for MAG in maintaining myelin-forming Schwann cell-axon contact and are consistent with the hypothesis that the carboxy terminal of MAG prevents the fusion of the cytoplasmic side of Schwann cell periaxonal membranes with the cytoplasmic side of the inner compact myelin lamellae. (Supported in part by grants MS 10803 and MS 03356 awarded to K.S.).

131.19 SULFATIDE: THE REGULATION OF SYNTHESIS AND ITS ROLE IN MYELINOGENESIS, G. I. Tennekoon* and M. Zaruba* (SPON: R. T. Johnson). Dept. of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

To obtain a better understanding of the assembly of myelin we undertook an investigation of the synthesis of sulfogalactosylceramide (sulfatide) and of factors affecting the regulation of this process. Sulfatide is enriched in myelin and it has been postulated that this acidic lipid interacts with myelin basic protein and aids in the stabilization of the membrane. If this were the case, sulfatide would be expected to be localized at the major dense line of myelin, where the basic protein is located.

Cerebroside sulfotransferase (CST), the enzyme catalyzing the transfer of the "active" sulfate donor 3'-phosphoadenosine 5'-phosphosulfate to the acceptor lipid galactosyl ceremide, has been localized to the Golgi apparatus and co-migrates with thiamine pyrophosphatase indicating that these two enzymes are localized to the trans-Golgi. Within the trans-Golgi, CST appears to have a luminal localization, as judged by resistance to protease digestion and the fact that galactosylsphingosine covalently linked to agarose is not sulfated unless detergents are added. Our date would indicate that sulfatide was not localized to the cytoplasmic side but was localized to the outer half of the lipid bilayer. Moreover, the "active" sulfate donor (PAPS) is synthesized in the cytosol and we shall present evidence that this compound is transported into the Golgi lumen via a "carrier" mechanism. The net synthesis of sulfatide is controlled by the activity of CST and by the degradive enzyme cerebroside sulfatase. We showed that neither PAPS, its transporter, nor galactosylceramide played a role in regulating sulfatide accumulation.

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131.20 EFFECTS OF DICYCLOHEXYLCARBODIIMIDE ON THE STRUCTURE OF THE MYELIN PROTEOLIPID. E. Ling*, T. Rounds*, M.B. Lees and V.S. Sapirstein* Depts. of Biochem., E.K. Shriver Ctr., Waltham, MA 02254 and Biol. Chem., Harvard Med. Sch. Boston, MA 02115.

The myelin proteolipid has been shown to interact covalently with dicyclohexylcarbodiimide (DCCD) when incubations are carried out either with whole myelin or the isolated proteolipid; reconstitution of the proteolipid in liposomes stimulates a proton flux which is inhibited by DCCD (PNAS 79:941, 1982). We report here the effects of DCCD on the secondary and tertiary structure of this protein using circular dichroism (CD) and fluorescence energy transfer. Our data indicate that aqueous solutions of the apoprotein prepared by delipidation on Sephadex LH-60 gives a CD spectrum consistent with a structure of 51% α helix, 25% β and 24% random coil. After incubation with with a 20 fold molar excess of DCCD for 30 minutes the helical content was reduced to 17% with a corresponding increase in random coil. However, if the proteolipid is first incorporated into asolectin liposomes, under conditions allowing for DCCD inhibitable proton flux, DCCD exerted no significant effect on the structure of the protein. Thus, the presence of lipid appears to supply sufficient constraints to retain secondary structure. Although the protein contains 4 tryptophan (Trp) residues, analysis of the Trp fluorescence in water revealed one emission peak with a maxima at 340 nm suggesting all Trp residues were in a hydrophobic environment. Addition of anilino napthol sulfonate (ANS) reduced the Trp fluorescence due to energy transfer yielding an ANS emission peak at 460 nm. By following the emission at 460 nm with serial additions of ANS a binding curve was established. The existence of two binding sites was suggested; there was one high affinity site and one low affinity site per molecule of proteolipid. Since DCCD binds to ANS, the effect of DCCD was studied after scavenging the excess DCCD with acetic acid and the protein dialyzed against water prior to fluorescence studies. Control preparations were similarly treated. Incubation with DCCD did not change the shape of the ANS titration curves but did markedly reduce the degree of energy transfer to both the high and low affinity sites. Because the energy transfer depends upon the distance between the ANS-binding site and the Trp residues, these results suggest that a significant structural change had occurred after treatment with DCCD. Thus, both CD and fluorescence studies suggest that DCCD can promote structural changes in the myelin proteolipid, presumably through breaking ionic linkages resulting from the reaction of DCCD with aspartic and/or glutamic side chains. These changes would appear to be minimized when the protein is incorporated into a lipid bilayer.

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132 SYMPOSIUM: DEVELOPMENTAL STRATEGIES FOR SELECTIVE SYNAPSE FORMATION. E. Frank, Northwestern Univ., Evanston (Chairman); J.T. Schmidt, SUNY, Albany; M.P. Stryker, UC Med Sch, San Francisco; D.E. VanEssen, Calif Inst Tech, Pasadena.

D. VanEssen will discuss the maturation of neuromuscular connections in mammalian muscle. At birth, when polyneuronal innervation of muscle fibers is extensive, fast and slow motoneurons innervate largely separate populations of muscle fibers, suggesting considerable selectivity in initial formation of connections. The elimination of synapses appears to occur in two stages. During the first two postnatal weeks most polyneuronal innervation disappears. Over the next several weeks there is a substantial reorganization of connections. The competitive basis of synapse elimination will be discussed, including its relationship to neural activity and to motor unit size, spinal postion and fast versus slow muscle type.

J. Schmidt has studied the regeneration of optic axons in goldfish. These axons initially produce diffuse arbors over wide regions of tectum, then concentrate them to produce an orderly retinotopic map recorded electrophysiologically. Correlated activity between neighboring ganglion cells is implicated in the sharpening mechanisms. Binocular projections to dually innervated tecta initially overlap, then segregate into ocular dominance patches. Binocular blockade with TTX reversibly prevents the segregation. These results can be interpreted in terms of correlated firing of neighbors within an eye versus lack of correlation between eyes. Synchronous firing may increase synaptic effectiveness (summation of EPSPs) and thereby lead to selective synapse stabilization.

M. Stryker will discuss the role of impulse activity in the progressive, eye-specific segregation of geniculate afferents to the developing visual cortex of the cat. Equal vision in the two eyes during the segregation process is necessary for their afferents to partition the terminal space equally. Recent findings are consistent with the notion that information in the pattern of spontaneous retinal impulse activity may be used in the formation of central connections: blockade of optic nerve activity prevents the segregation process; while asynchronous electrical stimulation of the two optic nerves reverses the physiological effects of the blockade.

E. Frank has studied the development of the monosynaptic stretch reflex in the spinal cord. The synaptic connections between sensory and motor neurons begin to form after these cells have contacted their peripheral targets. The synapses are highly specific from the beginning; there is no stage at which appreciable numbers of inappropriate connections are seen. Topography cannot play a key role in determining this specificity because the dendrites of appropriate and inappropriate motoneurons are intermingled with each other. Local interactions may underlie the selectivity.

133 HETEROGENEITY OF NEUROTANSMITTER AND DRUG RECEPTORS. Henry I. Yamamura, University of Arizona, Tucson, Arizona, Solomon H. Snyder, Johns Hopkins University, Baltimore, Maryland, Gavril W. Pasternak, Memorial Sloan-Kettering, New York, New York, David L. Nelson, University of Arizona, Tucson, Arizona, Ian Creese, University of California, San Diego, California, and Arnold S. Lippa, Lederle Labs, Pearl River, New York.

In the last decade, great advances have been made in the understanding of neurotransmitter and drug receptors by the direct labeling of recognition sites by the use of high specific activity radioligands. Initial studies provided the identification and characterization of receptor binding sites however, more recent studies have shown complexity in receptor binding. Receptor heterogeneity appears to exist for every neurotransmitter or drug receptor. This workshop will focus on several important neurotransmitter and drug receptors and will provide an up-to-date information on whether different interconvertible forms of a single receptor exists or if different molecular forms of the receptor exists.

134.1 IDENTIFICATION AND ELECTROPHYSIOLOGY OF THE PEPTIDERGIC NEUROSECRETORY CELLS THAT CONTAIN ECLOSION HORMONE IN MANDUCA SEXTA. P. F. Copenhaver and J. W. Truman. Dept. Zoology, Univ. of Washington, Seattle, WA 98195.

Adult development in the moth Manduca sexta culminates in the pulsatile release of eclosion hormone, an 8500 dalton peptide, which triggers adult emergence and activates the adult repertoire of behaviors (Truman JW. J.exp.Biol.54, 805, 1971). During metamorphosis, eclosion hormone is transported from the brain to its associated neurohemal organ (the corpora cardiaca) where it accumulates. The timing of its release is governed by the combined action of ecdysteroid titers and circadian input (Truman JW. Am. Zool. 21,655,1981). To examine the nature of these controlling mechanisms on a cellular level, it was necessary to identify the neurosecretory cells producing eclosion hormone.

Backfilling the nerves that run from the adult brain to the corpora cardiaca with cobalt chloride revealed discrete pools of neurons in the medial and lateral regions of the protocerebrum and a small cluster in the tritocerebrum. Subdissection of these regions and microdissection of individual cell groups localized the cells containing biological activity to the lateral cluster of protocerebral neurosecretory cells. Intracellular recording from the somata of these cells revealed broad (20-40 msec) action potentials that could be repeatedly elicited by current injection over the course of many hours. Intracellular iontophoresis of dye confirmed the axon trajectories determined by backfilling,and revealed a distinctive pattern of neurite arborization that is superficial to the major neuropilar regions of the brain.

A minimally dissected preparation of the pharate adult moth has been developed which exposes the brain-retrocerebral complex in a restrained animal while leaving most of the tracheation and innervation intact. By sequentially assaying the dissection bath for biological activity, we found that eclosion hormone was released at a developmentally appropriate time and that this release was followed after about two hours by eclosion behavior. Recording extracellularly from the nerve containing the neurosecretory cells' axons in such preparations revealed a marked increase in electrical activity correlated with the appearence of eclosion hormone-like activity in the surrounding bath. A combination of this viable semi-intact preparation and the long-term intracellular recording described above should permit an elucidation of the cellular events underlying the precise timing of release of this peptide hormone.

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134.2 DISTRIBUTION AND PROJECTIONS OF CHOLECYSTOKININ (CCK) IMMUNOREACTIVE NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) OF RAT. J.Z. Kiss*, M.C. Beinfeld*, T.H. Williams, and M. Palkovits*. Dept. of Anatomy, Univ. of Iowa, Iowa City, IA 52242; and Neuroendocrine Unit, Lab. Clin. Sci. NIH, Bethesda, MD 20205.

CCK-like peptides have been demonstrated in the magnocellular hypothalamo-hypophyseal system by immunocytochemical (IC) and radioimmunoassay (RIA) techniques. CCK has been reported to coexists with oxytocin in some neurons of the PVN and supraoptic nucleus (SON) (Vanderhaegen, J.J. et al., Cell Tiss. Res., 221: 277, 1981) although the exact distribution, morphology and projections of CCK-immunopositive cell bodies remains unclear. The present study addresses these points using IC and RIA.

Analysis of coronal sections from colchicine treated animals revealed that CCK-positive cells form a morphologically heterogenous group, since CCK-immunoreactive cells were found among magnocellular neurons in the PVN and also in substantial numbers in the parvocellular subdivisions. Magnocellular CCK-positive neurons were concentrated mainly in the antero-medial subdivisions, while more caudally they formed a ring around a core of unstained magnocellular neurons. Immunoreactive, typical parvocellular neurons predominate in the anterior, medial and, to a lesser degree the lateral parvocellular subdivisions.

Efferent projections of the CCK-immunoreactive neurons were studied after the following transections: pituitary stalk (ST), median eminence (ME), medial forebrain bundle (MFB), lateral retrochiasmatic area (RCAL); and also after bilateral lesions of the PVN. Since CCK-immunoreactivity of the pituitary and of ME fell 8 days after PVN lesions to 23% and 18% of normal levels respectively, we believe that a significant portion of the total CCK-positive cells of the PVN project into the posterior lobe of the pituitary via ME. The RCAL cut, which interrupts both PVN and SON-hypophyseal fibers, resulted in a comparable depletion, indicating that the PVN is the main source of CCK in the ME and hypophysis. Following the ME lesion, there was an accumulation of CCK-immunoreactive material in both magno- and parvocellular cells. Following the ST lesion the accumulation was seen only in magnocellular neurons. Thus magnocellular (presumed oxytocin-CCK) cells send their axons to the pituitary, whereas axons of parvocellular neurons most likely terminate in the ME. After the MFB cut, CCK immunoreactivity increased in transected fibers proximal to the knife cut and in a few perikarya in the PVN, indicating that some CCK cells may send descending fibers to the lower brainstem via the MFB.

These studies demonstrate that CCK-immunoreactive neurons in the PVN are a morphologically heterogenous population with efferent projections to ME, neurohypophysis and brainstem.

134.3 CHOLECYSTOKININ CONTAINING AFFERENTS TO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS IN RAT. L. Zaborszky[1], M.C. Beinfeld[2*], M. Palkovits[3*] and L. Heimer[1]. Clinical Neuroscience Research Center, University of Virginia, Charlottesville, VA 22908[1], Dept. Pharmacol., St. Louis Univ. Med. Ctr., St. Louis, MO[2] and 1st Dept. Anat., Semmelweis Univ. Med. Sch., Budapest, Hungary[3].

The ventromedial hypothalamic nucleus (VMN) is involved in a variety of autonomic, neuroendocrine and behavioral functions, and it has been suggested (Morley, Life Sci., 30, 497, 1982) that some of its activities are dependent on the neurotransmitter CCK, which is present in the nucleus. Since the VMN contains very few CCK-containing cells it is conceivable that the major portion of CCK is located in axon terminals of extrinsic origin. Anterograde degeneration, retrograde tracing of HRP, radioimmunoassay and immunocytochemistry were used to determine the source and topography of CCK fibers to the VMN.

The biochemical studies showed that CCK content in the VMN decreased dramatically (sham 1.96±0.13 ng/mg protein; operated: 0.22±0.20) following bilateral transections of the upper brainstem at the level of the posterior diencephalon (A=3.5mm according to Konig-Klippel, 1963; 1mm lateral to the midline, 2.5mm wide cut), and CCK-like immunoreactivity disappeared from the ipsilateral VMN following ipsilateral transection at the same level. In addition, immunoreactive material accumulated in many of cells in the dorsal parabrachial nucleus. The location of these cells corresponded to the location of labeled cells following iontophoretic injections of HRP (20%, Sigma VI) into the core of the VMN. CCK also disappeared from the VMN (0.10±0.10 ng/mg) following a long parasagittal cut in the lateral part of the medial forebrain bundle (between A6.2-A3.2; 1.8mm lateral to the midline), whereas only about 60% of the CCK disappeared after a shorter parasagittal cut at the lateral edge of the fornix (A5.9-A5.0; 1mm lateral to the midline). These biochemical findings are in agreement with the topography of the ascending degenerating fibers following the above mentioned brainstem transections. The ascending fiber system forms a rather well contained bundle in the dorsolateral part of the medial forebrain bundle and some fibers join the supraoptic decussation. The system fans out at the rostral half of the VMN and reach the nucleus from the lateral side. Transections of the stria terminalis or the medial corticohypothalamic tract did not change the CCK level of the VMN.

This work was supported in part by NIH grant R NS1774303.

134.4 DO CORTICAL CHOLECYSTOKININ NEURONS HAVE LONG PROJECTIONS? K.B.Seroogy, J.H.Fallon, S. Loughlin, F. Leslie, C. Kodama*, N. Canepa*, and Y.Kim*. Dept's. of Anatomy and Pharmacology, University of California, Irvine, CA 92717

Recent studies suggest that cholecystokinin (CCK)- containing neurons are widely distributed throughout neo-and allocortices, as well as in the substantia nigra-ventral tegmental area of the midbrain. Although it is well-known that dorsal and ventral striatal and limbic structures receive a substantial input from midbrain CCK neurons, it is not clear to what extent cortical CCK neurons innervate cortical, striatal and limbic structures. Moreover, we have obtained quite paradoxical results in studying other long projections of cortical CCK neurons. In this study we present both the positive and negative results related to long CCK pathways in the forebrain.

Eighty adult female albino rats were used. Seventy animals were used for double-labeling experiments and ten were used to plot the distribution of CCK fibers and terminal areas. For double-labeling experiments, animals received .04 - 0.1 µl injections of True Blue/Granular Blue or Propidium Iodide into neocortical, allocortical, limbic, striatal or thalamic structures, followed the next day by multiple 0.2 ul injections of 1% colchicine into cortex, midbrain and ventricles, followed the next day by processing for combined CCK immunofluorescence and retrograde fluorescence tracers.

The following results were obtained: (1) When damaged by colchicine, canullae or injections, nearly all major cortical forebrain tracts (corpus callosum, internal capsule, external capsule, fornix, hippocampal commissure, anterior commissure) contained immunoreactive CCK fibers, (2) Only rarely were cortical CCK neurons double-labeled after injection of tracers into cortex, (3) Numerous frontal cortical CCK neurons were double-labeled after injections in thalamic nuclei, and (4) Midbrain CCK neurons were double-labeled after injections into striatum (especially medially), septum, amygdala, olfactory tubercle and frontal cortex.

Of particular interest in this study is a comparison of results 1 and 2, which are contradictory. Since damaged cortical tracts are CCK-positive, but commissural, associational, and corticostriatal CCK neurons are rarely found, either these neurons are refractory to retrograde labeling, or this CCK immunoreactivity is damage-related with most cortical CCK neurons being local circuit neurons. Because corticothalamic (result 3) and mesocortical (result 4) CCK pathways were easily demonstrated, the difficulty in double-labeling cortical CCK neurons is probably not due to the fact that they are CCK-containing, or that CCK-containing terminals in cortex cannot take up retrograde tracers. Perhaps cortical CCK neurons are primarily local circuit neurons with only minor commissural, associational and striatal projections. One possibility is that the minor collaterals cannot take up enough retrograde label to be visualized in the cell body. Alternately, CCK immunoreactivity in damaged axons may be a temporary axonal response to trauma. (Supported by NIH Grants NS 15321 and 16017.

134.5 LOCALIZATION OF CHOLECYSTOKININ, SOMATOSTATIN AND VASOACTIVE INTESTINAL POLYPEPTIDE IMMUNOREACTIVITY IN RAT HIPPOCAMPUS AND AREA DENTATA. R.S. Sloviter, G. Nilaver and E.A. Zimmerman. Neurology Ctr., Helen Hayes Hospital, W. Haverstraw, N.Y. 10993 and Depts. of Pharmacology and Neurology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Recent studies have shown that cholecystokinin (CCK), somatostatin (ST) and vasoactive intestinal polypeptide (VIP) antigenicity is present in neurons of the rat hippocampal formation. The present study was undertaken to determine the precise hippocampal location of this antigenicity. Alternate 100u-thick vibratome sections of brains from aldehyde-perfused male Sprague-Dawley rats were incubated in primary rabbit antisera to CCK, ST or VIP, then in biotinylated protein A, then avidin/biotin complex followed by reaction with DAB. Staining with CCK antibody revealed the presence of antigen in scattered interneurons of all hippocampal subfields. In CA1, CCK-positive somata were present in st. radiatum with fewer somata in st. pyramidale and st. oriens. In area dentata, CCK-positive somata were primarily those of the pyramidal-shaped "basket" cells of the granule cell layer. A small number of multipolar hilar cells near the granule cell layer were also CCK-positive. In both the granule and pyramidal cell layers, CCK-positive fibers formed a dense axo-somatic plexus thought to originate from the basket cells of each layer. In the hippocampus, VIP staining was seen in interneurons in st. pyramidale and st. radiatum with fewer somata in st. oriens. VIP-positive axons were seen to ramify throughout the strata of the hipocampus but particularly in the pyramidal layer. In area dentata, VIP staining was restricted to scattered interneurons in the molecular, granular and hilar regions. Very few VIP-positive cells had the morphological characteristics of the dentate basket cells stained by CCK antibody. The VIP-positive cells gave rise to axonal plexuses in the molecular and granule cell layers but also throughout the hilus. The pattern of ST-like antigenicity was very different from that of CCK and that of VIP. In CA1, ST-positive somata were mainly in st. oriens with few in st. pyramidale and few or none in st. radiatum. In CA3, ST-positive somata were present in all strata. In area dentata, most cells of the hilus were ST-positive but few, if any, pyramidal-shaped basket cells of the granule cell layer were ST-positive. Little ST-like antigenicity was seen in the dentate granular or molecular layers. These results suggest that CCK, VIP and ST are localized in distinctly different populations of interneurons in the area dentata and hippocampus of the rat and are probably not present in the granule or pyramidal cells of this region.

134.6 OXYTOCIN IN RAT HIPPOCAMPUS: AN IMMUNOCYTOCHEMICAL ANALYSIS G. Nilaver, R. S. Sloviter, and E. A. Zimmerman. Dept. of Neurology, College of Phys. & Surg., Columbia Univ., New York, NY 10032, and Neurology Center, Helen Hayes Hospital, West Haverstraw, NY 10993.

The hormones oxytocin (OT) and vasopressin (VP) have been demonstrated in different brain regions outside the hypothalamus by radioimmunoassay (RIA) and immunocytochemistry. These projections are said to arise from the paraventricular nucleus (PVN) of the hypothalamus, and have been described to be mainly VP-ergic in rostral brain areas, with OT predominating in the more caudal regions. We re-evaluated the relative content of these two peptides in the various extrahypothalamic regions of rat, employing different fixatives and improved immunocytochemical techniques. Long-Evans rat brains perfusion fixed with 1% paraformaldehyde and 0.5% glutaraldehyde containing lysine (0.1M), sodium m-periodate (0.01M) in phosphate buffer were cut at 100 um thickness in the coronal and sagittal planes using a Vibratome. Sections were immunocytochemically labeled for VP and OT by the pre-embedding method of staining employing biotinylated Protein A in the avidin-biotin-peroxidase (ABC) technique. The raabit antisera employed have been previously characterized. Contrary to previous reports, we find more OT- than VP-reactive fibers even in the rostral brain regions, including the cerebral cortex, septum, and entorhinal areas. The OT predominance was particularly evident in the hippocampus. OT fibers were present throughout the septo-temporal axis, in all areas of the hippocampus (stratum oriens, pyramidale, radiatum, and molecular layers). The fibers were particularly numerous in the stratum oriens, with long and varicose densely staining axonal processes showing extensive branching. The OT input to the hippocampus was not organized in a laminar fashion. Relatively few VP fibers could be demonstrated in the hippocampus, despite comparable staining for the two peptides in the hypothalamus. No cell bodies containing either peptide was found in the hippocampus, even following colchicine treatment, suggesting the origin of these fibers outside this brain region. The OT innervation of the hippocampus probably arises from the PVN, since the suprachiasmatic nucleus (the only other source of extrahypothalamic fibers) is exclusively VP-ergic. The precise site of entry and the cells of origin of this hippocampal input however, awaits elucidation. The relative paucity of VP in the hippocampus also needs confirmation by regional RIAs. This demonstration of OT and VP in the hippocampus may form the anatomical basis of their observed electrophysiologic effects on hippocampal neuronal activity, and their effects on memory consolidation. (Supported by NIH Grant HD13147 and a Parkinson's Disease Foundation Grant to Columbia University).

134.7 PEPTIDE INTERACTIONS OF CELLS PROJECTING TO THE CENTRAL NUCLEUS OF THE AMYGDALA. Christine H. Block, Gloria E. Hoffman, Bruce S. Kapp. Dept. of Anatomy, University of Rochester, NY 14642 and Dept. of Psychology, University of Vermont, Burlington, VT 05405.

It is established that the central nucleus of the amygdala (CNA) is involved in autonomic-cardiovascular function and that it receives projections from the parabrachial nuclei (PBN) and paraventricular thalamus (PVT), two areas which, based upon afferent connections from the nucleus of the solitary tract, may also function in autonomic-cardiovascular regulation. Since CNA, PBN and PVT all contain high concentrations of a variety of neuropeptides, in the present study we sought to determine the anatomical relationships of these neuropeptides with these potential autonomic-cardiovascular related projection systems to the CNA.

Twenty to 50 nanoliters of a 5% suspension of true blue was injected into the CNA of adult male rats. After a 10 day survival period, a group of the animals was treated with colchicine administered intracerebroventricularly and allowed to survive an additional 24-48 hours. All of the animals were perfused transcardially with saline and Zamboni's fixative. The brains were cut at 30 um on a vibrating microtome and the tissue was processed for immunocytochemistry. The tissue sections were incubated overnight in primary antiserum generated against neurotensin (NT), substance P (SP), methionine enkephalin (mE), vasoactive intestinal polypeptide (VIP), somatostatin (SS) or cholecystokinin octapeptide (CCK). The unlabeled antibody enzyme or immunofluorescence technique was employed.

Retrogradely labeled neurons were located in the PBN and PVT following microinjection of true blue into the CNA. In animals that were not treated with colchicine, retrogradely labeled perikarya in the PBN were observed to be in contact or close apposition with NT, CCK, SP, VIP or mE immunoreactive fibers. Similarly, true blue labeled neurons in PVT were surrounded and apparently contacted by fibers containing SP, mE, SS.

In the animals treated with colchicine, a small population of NT and SP immunoreactive cells of the PBN were also labeled with true blue. There were no immunoreactive cells in PVT that were double-labeled with true blue and neuropeptides.

In summary, these data reveal that 1) neurons in the PBN and PVT that project to the CNA appear to receive neuropeptidergic input and 2) some NT and SP-containing PBN neurons project to CNA. Thus, these pathways which have been implicated in the control of autonomic-cardiovascular function are, at least in part, peptidergic.

Supported by NIH grant NS 16107.

134.8 THE MEDIAL PREOPTIC NUCLEUS: PEPTIDERGIC COMPONENTS. Robert E. Watson, Jr., Gloria E. Hoffman and Stanley J. Wiegand. Department of Anatomy, University of Rochester, Rochester, NY 14642.

The medial preoptic nucleus (MPN) has recently been shown to exhibit a cytoarchitectonic sexual dimorphism in a number of species, including the rat (Bleier, et al., 1982). Specifically, the MPN, situated immediately posterior to the organum vasculosum of the lamina terminalis (OVLT), is slightly larger and is more densely cellular in the female than in the male. Furthermore, the MPN has been demonstrated to be indispensible for phasic gonadotropin secretion in the female (Wiegand, et al., 1980). Thus, in an effort to begin to characterize this region neurochemically, the present study was conducted in order to identify the peptides localized within the perikarya and fiber components of the MPN. Young adult male and female Sprague-Dawley rats, with or without colchicine pretreatment, were perfused with saline and Zamboni's fixative and brains were processed for immunocytochemistry using the unlabeled antibody enzyme technique. Sections were subsequently counterstained with 1% methyl green to facilitate identification of the MPN. Of the peptides screened, including substance P (SP), neurotensin (NT), cholecystokinin octapeptide (CCK), LHRH, vasoactive intestinal polypeptide (VIP), met-enkephalin (m-ENK), somatostatin (SRIF), vasopressin (VP), oxytocin (OXY) and ACTH, only NT, SP, and CCK showed evidence of immunoreactivity (ir) within the perikarya of the MPN. Numerous NT-ir cells were evident in the MPN and were clearly more densely packed in this region than in surrounding periventricular regions. In contrast, SP-ir perikarya were widely scattered throughout the MPN, with a relatively dense cell population associated with lateral border regions of the nucleus. Lightly staining CCK-ir cells were only widely scattered throughout the bulk of the MPN, with a more discrete magnocellular population evident at the ventrolateral border region of the nucleus. Many LHRH-ir neurons were present anterior to the MPN near the OVLT, but at the level of the MPN, LHRH-ir cells were situated laterally. Regarding the patterns of fiber labeling, two basic trends emerged. The first consisted of relatively dense peptide-ir associated clearly with the MPN proper. Substances for which this pattern was evident included; 1) SP, in which the fiber-ir was most dense within MPN in the immediate sub-ependymal zone; 2) CCK, in which a fine plexus of ir fibers was continuous with the anterior periventricular nucleus more caudally; and 3) NT, for which the density of ir fibers in the MPN was equivalent or only marginally less than that observed in adjacent regions of the preoptic area. The second group consisted of substances for which there was evidence of only limited fiber-ir within the MPN, and included LHRH, VIP, m-ENK, SRIF, VP, OXY and ACTH. Generally, the density of labeling was greater for these substances in regions adjacent to the MPN. No obvious sex differences in peptide distribution have been detected. Supported by NIEHS ES07026 and NS13725.

134.9 DISTRIBUTION OF FMRF-AMIDE IMMUNOREACTIVITY IN THE RAT BRAIN. B.M. Chronwall*, J.A. Olschowka, T.L. O'Donohue (SPON: R.L. Irwin). Experimental Therapeutics Branch, National Institute of Neurological and Communicative Disorders and Stroke, and Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Heath, Bethesda, MD 20205

Molluscan cardioexcitatory peptide or FMRF-amide is a tetrapeptide-amide with the sequence Phe-Met-Arg-Phe-NH_2. Immunoreactive (ir) FMRF-amide is present throughout the invertebrate CNS (see Watson et al., Soc. Neurosci. Abst: 1983) and an ir FMRF-amide-like peptide has been demonstrated in mammalian CNS (Weber et al., Science 214:1248, 1981). In this study, FMRF-amide ir was anatomically mapped in the CNS using antisera generated against FMRF-amide conjugated to succinylated thyroglobulin. Twenty um frozen sections from normal or colchicine pretreated rats perfused with phosphate buffer with 0.5% sodium nitrite followed by phosphate buffered 4% formaldehyde (pH 7.4) were processed for indirect immunohistochemistry. Preabsorption with FMRF-amide totally blocks the staining, whereas preabsorption with FMRF free acid, cholecystokinin, bovine pancreatic peptide, metenkephaline proctolin or neuropeptide Y does not block the staining.

The highest number of FMRF-amide staining cell bodies was found in the nucleus (n) arcuatus. N paraventricularis, n hypothalamus, n ventromedialis and n dorsomedialis also contained high numbers. Along the third ventricle dendrites of bipolar neurons were oriented in a dorso-ventral direction. In the septal complex a distinct group of bipolar and multipolar immunoreative cells was found in the rostral part of the lateral nucleus. Another prominent group of perikarya was found in the n. tractus solitarii.

FMRF-amide positive nerve fibers and terminals were widely distributed. The septal complex contained high densities especially in n interstitialis striae terminalis. The preoptic area contained moderate to high densities and n suprachiasmaticus showed high numbers of fibers. N paraventricularis hypothalami, n paraventricularis, n hypothalamicus, n ventromedials and n dorsomedialis exhibited a high to very high degree of immunoreactivity. In myelencephalon n tractus solitarii had the densest innervation. Spinal cord had fibers primarily in lamina I and II of the dorsal horn.

The results of these studies demonstrate that a FMRF-amide-like peptide has a widespread distribution throughout the mammalian central nervous system. The particularly high densities of FMRF-amide fibers and perikarya in hypothalamic nuclei, n tractus solitarii and the dorsal horn of the spinal cord suggest roles for FMRF-amide in endocrine and autonomic regulation as well as modulation of pain.

134.10 THE EFFECTS OF LESIONING THE TRIGEMINAL, SUPERIOR CERVICAL SYMPATHETIC, C2 AND C3 GANGLIA ON SUBSTANCE-P IN CAT CEREBRAL ARTERIES. T.V. Norregaard*, R.C. Weatherwax* and M.A. Moskowitz (SPON: N.T. Zervas). Depts of Neurosurgery and Neurology, Massachusetts General Hospital, Harvard Med. Sch., Boston, MA 02114

Using horseradish peroxidase as a neuronal tracer, previous studies have demonstrated trigeminal projections to pial and dural blood vessels (Mayberg, et al, Science '81). Substance P (SP), a vasoactive neuropeptide has been measured by radioimmunoassay (RIA) in pia plus arachnoid where it is primarily located within the walls of blood vessels. Levels of iSP in pial arteries are 20-fold higher than in pia arachnoid devoid of large blood vessels (786.3 +/- 286.9 fmoles/mg protein vs 29.3 +/- 4.7 fmoles/mg protein).

Studies were undertaken to determine the source(s) of iSP content in cat cerebral arteries. Changes in iSP content were measured by a highly sensitive and specific RIA after surgical excision of the trigeminal ganglia using an extradural approach and microsurgical techniques. Unilateral trigeminal ganglionectomies caused 40-80% decrease in iSP content in the ipsilateral anterior, middle and posterior cerebral arteries as compared with levels on the unoperated side. Decreases were most marked in middle cerebral and least marked in anterior cerebral arteries. Unilateral excision of the superior cervical sympathetic ganglia (SCG) did not decrease the iSP levels in these arteries. The combination of unilateral trigeminal ganglionectomy and bilateral SCG removal did not further decrease iSP measured after unilateral trigeminal lesions alone. In preliminary studies, iSP levels decreased in superior cerebellar arteries in 3 of 4 cats following unilateral trigeminal lesions although this decrease did not reach statistical significance at $p < 0.05$. Decreases in iSP levels were not found in vertebral or anterior inferior cerebellar arteries. In other preliminary experiments, excision of dorsal root ganglia of C2, C3 bilaterally (n=2) did not appear to decrease the iSP content in the above noted cerebral vessels.

These results indicate that 1) cat trigeminal nerves are responsible for the majority of iSP in supratentorial cerebral vessels located in pia arachnoid 2) sympathetic projections do not contribute to iSP content in cerebral arteries. The fact that SP is both a potent vasodilator and putative transmitter in parts of the nervous system that participate in the transmission of nociceptive information suggests a possible role for this trigeminovascular pathway in the pathogenesis of cerebrovascular pain syndromes.

134.11 EMBRYONIC DEVELOPMENT OF VASOPRESSINERGIC AND OXYTOCINERGIC NEUROSECRETORY CELLS: IMMUNOCYTOCHEMISTRY USING MONOCLONAL ANTIBODIES TO THE NEUROPHYSINS. M.H. Whitnall, J. Ben-Barak*, S. Key*, K. Ozato* and H. Gainer. NICHD, NIH, Bethesda, MD 20205

Previous immunocytochemical (ICC) studies have shown magnocellular neurosecretory cells in the rat hypothalamus containing neurophysin (NP) or vasopressin (VP) starting at embryonic days 16-17 (E16-E17). However, oxytocin (OT) has not been detected by ICC before birth, despite the fact that RIA studies have detected OT in the brain as early as E20-E21. As a result, descriptions of the migration and differentiation of OT cells before birth have been lacking. We have recently generated a set of monoclonal antibodies to OT-associated NP (OT-NP) and VP-NP (Ben-Barak,J., et al., submitted) which have enabled us to study the two subsets of neurosecretory cells independently by ICC during embryonic development. The specificities of the antibodies were demonstrated in adult rats by ELISA and RIA, immunostaining of SDS and IEF blots, and ICC localization to the appropriate sets of hypothalamic neurons on the light microscopic level and to neurosecretory vesicles in the neurohypophysis by postembedding electron microscopic ICC. Embryos and newborn pups (born on E22) from precisely timed pregnant Sprague-Dawley rats were decapitated and their brains fixed by immersion in 4% paraformaldehyde, 0.2% picric acid. 50-µm vibratome sections were immunostained using hybridoma conditioned media and affinity-purified goat anti-mouse IgG conjugated to HRP.

Neurons containing OT-NP-like immunoreactivity (OT-NP-LI) and VP-NP-LI were first observed at E16. OT-NP-LI and VP-NP-LI were located in separate cells with similar distributions in the region of the future supraoptic nucleus (SON). In addition, a few OT-NP-LI cells were located in the region of the future paraventricular nucleus (PVN). Many cells were located in the region between the PVN and the SON and presumably included migrating neurons. By E17, many more cells of both types were located in the PVN and SON regions, and in the internuclear area. At this stage, OT-NP-LI and VP-NP-LI cells were already beginning to assume their characteristic, different distributions in the SON and PVN. However, the cellular differentiation of OT-NP-LI cells was markedly delayed compared to the VP-NP-LI cells in terms of extension of processes; even at 9 days after birth the number of OT-NP-LI fibers emanating from the nuclei and projecting through the median eminence was a small fraction of the number of VP-NP-LI fibers found in these locations.

NP-LI may consist of either NP or precursor proteins containing the sequences for NP and VP or OT. We are currently investigating the possibility of a developmental delay between the synthesis of precursors and conversion to NP, VP and OT.

134.12 ONTOGENY OF SOMATOSTATIN (SOM)* IMMUNOREACTIVE ELEMENTS IN THE RAT SPINAL CORD. R.H. HO. DEPT. OF ANAT., COLL. OF MED., OHIO STATE UNIV., COLUMBUS, OH 43210

Prenatal rats of gestational ages E12-22 were immersion fixed while postnatal rats were perfusion fixed by Zamboni's solution and transverse cryostat sections of their spinal cords processed by Sternberger's PAP technique. SOM was first detected in the spinal cord at E13 when it was observed within presumptive perikarya of the basal plate. In addition, at E13 and 14, SOM was demonstrable in dorsal root ganglia neurons whose SOM immunoreactive central processes approximated the dorsal lateral surface of the spinal cords. At E14, SOM fibers were first detectable in the ventral funiculi. At E15, SOM elements resembling cell bodies first appeared in the superficial laminae of the dorsal horn while SOM fibers were present in the lateral funiculus. By E16, the gray matter contained widely dispersed SOM perikarya of various shapes and sizes, but few SOM varicosities were present. At E18 SOM fibers were present in the dorsal funiculus. By E20, a moderate density of SOM varicosities became obvious in the superficial laminae of the dorsal horn. SOM was widespread in the E22 and neonatal spinal cords being most numerous within the superficial laminae of the dorsal horn. The outer portion of this region contained SOM varicosities whereas the inner portion also contained small perikarya. SOM perikarya of various shapes and sizes were widely dispersed throughout the gray matter ventral to the superficial laminae, with a slightly higher density found in the region ventrolateral to the central canal. A moderate density of SOM fibers was present in the lateral funiculus, whereas only a small number were located in the dorsal and ventral funiculi. Unlike the case in the prenatal spinal cords, the developmental changes in the postnatal spinal cords proceeded gradually. With increasing ages, immunostained cell body profiles were harder to distinguish. On the contary, the distribution of SOM varicosities in the gray matter increased with age. At or within the first postnatal month, SOM varicosities were further found to be present very sparsely throughout most of the gray matter ventral to the dense SOM plexus in the superficial laminae of the dorsal horn. However, lamina X and the intermediolateral cell column exhibited a moderate density of SOM varicosities. This distribution of SOM varicosities persisted to the 1 year old spinal cord. (Supported by NIH NS-17080 and in part NIH NS-10165)

*A substance's immunoreactivity is referred to by its name.

135.1 SYNAPTIC EFFICIENCY AND NONLINEAR INTERACTIONS BETWEEN SYNAPSES: C.Koch* (SPON: European Neuroscience Association) Dept. of Psych. MIT, Cambridge, MASS 02139

Using 1-dimensional cable theory we studied the synaptic efficiency and nonlinear interactions between an excitatory and a shunting inhibitiory synapse in the dendritic tree of a retinal ganglion cell of the cat (a delta cell from Boycott & Wässle 1974) and compared the results against a very simple model of the dendritic tree, consisting of a single infinite cylinder of constant diameter.

Considering the total charge injected at a synapse, we define as synaptic efficiency C the fraction of this charge actually reaching the soma. For the infinite cable C=exp(-l) where l is the electrotonic distance between the position of the synapse and the soma. In the delta-cell which has a profusely branched dendritic tree, C is closely approximated by the infinite cable model.

To study the nonlinear interaction between synapses using conductance changes as inputs, we introduced F as the ratio of the somatic depolarization due to excitation without inhibition to the somatic potential in the presence of inhibition. It is possible to prove (Koch, Poggio & Torre 1982) that in an arbitrary branched dendritic tree the effect of inhibition is strongest when it is placed on the direct path between excitation and soma. An inhibitory synapse behind excitation or situated on a branch off the direct path will drastically reduce the effect of inhibition on the somatic depolarization even for extremly high values of the membrane resistance Rm. Taking the limit Rm to infinity in a single infinite cable (i.e. there are no current losses), leads to F-values independent of the distance between both synapses as long as inhibition is placed behind the excitation. If the inhibition is on the path, F is much higher and depends linear on the distance

We conclude, that the synaptic architecture and dendritic morphology is of vital importance to determine the interaction taking place between different synapses.

135.2 POTENTIATION OF ACETYLCHOLINE RECEPTOR-CHANNEL BLOCKADE BY ATROPINE AND HALLUCINOGENS AFTER IRREVERSIBLE ACETYLCHOLINESTERASE INHIBITION WITH ORGANOPHOSPHATE AGENTS. E.G. Henderson, L.S. Reynolds*, R.L. Volle, S. Moraski Jr.*, and P.M. Epstein*, Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032.

We have previously demonstrated that, in contrast to neostigmine, echothiophate (217MI) and its tertiary amine analogue (217AO) reduce the voltage dependence of endplate current (e.p.c.) decay (α) in frog skeletal muscle (Fed. Proc. 1983, 42:991). In transected frog cutaneous pectoris muscle endplates, 217MI and 217AO (1-25μM) caused a greater than 75% decrease of α at all holding potentials (V_m's). After prolonged washing (2 hours) in Ringer solution there was a slight reversal of the slowing of e.p.c. decay and the reduction of voltage dependence of α by these agents. The addition of atropine (10μM) after this treatment greatly accelerated e.p.c. decay ($\alpha_o = \alpha$ at $V_m = 0 = 0.266$ msec^{-1}, before and 0.652 msec^{-1} after the addition of atropine) and further reduced the voltage dependence of α. In endplates in which the acetylcholinesterase was active this concentration had no effect on α (N. Schmied. Arch. Pharmacol. 1980, 312:117-121). Ketamine (50μM) and phencyclidine (PCP, 25μM) have been shown to increase α approximately two-fold (V_m = -90mV, J. Pharmacol. Exp. Ther. 1982, 221:570-576). After irreversible esterase inhibition the same concentrations of ketamine and PCP increased α 5.6 times and 5 times, respectively. In both cases the voltage dependence of α was interrupted. However, with ketamine, but not PCP, e.p.c.s were biphasic when V_m was positive, with the fast phase of e.p.c. decay faster than control and the slow phase slower.

In order to determine whether irreversible esterase inhibition altered the inhibition of PCP binding by atropine and ketamine to the receptor system, we tested [^{3}H]PCP binding to torpedo microsacs in the presence of 10μM carbachol. The IC_{50} for ketamine was 2 x 10^{-6}M and for atropine was 3 x 10^{-4}M irrespective of whether the esterase was active or inactive. These studies suggest that the increased rate of e.p.c. decay in the presence of these drugs after irreversible esterase inhibition is most likely due to increased frequency of channel opening. The results are consistent with an open channel block of the acetylcholine activated ionic channel by ketamine, PCP and atropine. (Supported by U.S. Army contract DAAG29-81-K-0165.)

135.3 A POSSIBLE COOPERATIVITY BETWEEN SYNAPSES SHARING A COMMON POSTSYNAPTIC LOCUS. H. Korn and D.S. Faber, Laboratoire de Neurobiologie Cellulaire, INSERM U261, Institut Pasteur, Paris, and Div. Neurobiology, SUNY, Buffalo, NY 14214.

The teleost Mauthner (M-) cell is subjected to a now classical collateral inhibition brought about by simultaneous impulses in at least 35 cells, each of which establishes from 6 to 91 morphologically defined synaptic units (m=37.7 ± 29.5, n=12) on this target. About 70% of them are found in a restricted region, the so-called axon cap, where they are commonly grouped in clusters of 2 to 6 terminals (Triller, A. and Korn, H., J. Neurophysiol., 48:708, 1982). This arrangement might be related to evidence presented here that postsynaptic potentials due to activation of the full network cannot be accounted for simply by summation of average conductance changes calculated from individual unitary responses. The latter were obtained following intracellular stimulations of single identified presynaptic inhibitory interneurons. We have consistently found that, after iontophoretic application of strychnine in the axon cap (close to the M-cell soma), the amplitude of the (depolarizing) collateral IPSP was reduced by 26 ± 18% of its initial value (n=20) whereas mean unitary IPSPs fell by 76 ± 21%. As described earlier (Faber, D.S. and Korn, H., J. Neurophysiol., 48:654, 1982), comparison of these two requires calculations of the underlying conductances, g_{ipsp}; this term is given by $V_{ipsp} \cdot G_m / (E - V_{ipsp})$, where V_{ipsp} is response amplitude, G_m the resting input conductance, and E the IPSP driving force (which equals twice the collateral IPSP, or V_{coll} - see ref. above). Solving for any fractional reduction in conductance yields

$$g'_{ipsp}/g_{ipsp} = (V'_{ipsp}/V_{ipsp})(2V_{coll} - V_{ipsp})/(2V_{coll} - V'_{ipsp}),$$

where primed terms are for altered values, e.g. following strychnine. Thus, for a unitary conductance decrease of 77%, that of the full collateral response, which would be the same in the absence of any compensation, was only 40.4% in this series. The site of the facilitatory process responsible for this discrepancy does not appear to be presynaptic since possible mechanisms, such as enhancement of transmitter release by field effects or strychnine, have not been observed with intracellular recordings. On the other hand, a postsynaptic interaction might well account for these data, as well as for the reported observation (Diamond, J., Roper, S. and Yasargil, G.M., J. Physiol., 232:87, 1973) that responses to iontophoretically applied glycine (distributed diffusely over adjacent synaptic regions) are more resistant to strychnine than are synaptic inhibitions (due to high concentrations but localized transmitter output).

135.4 QUANTAL ANALYSIS OF STRYCHNINE ACTION AT A PRESUMED GLYCINERGIC CENTRAL SYNAPSE. D.S. Faber, H. Korn and A. Triller, Div. Neurobiology, Dept Physiology, SUNY, Buffalo, N.Y. 14214 and INSERM U261, Institut Pasteur, France.

Although glycine has been proposed as a putative transmitter at inhibitory synapses onto the teleost Mauthner (M-)cell, this hypothesis has not been tested directly with activation of individual presynaptic neurons. Support for this postulate has now been obtained from the effects of strychnine on i) unitary inhibitory postsynaptic potentials (IPSPs) following impulses in identified causal interneurons and ii) binomial release parameters, n, p and q. The latter, i.e. the quantal size, is consistently reduced, as expected for a postsynaptic receptor antagonism. Two experimental approaches were employed, both involving simultaneous pre- and postsynaptic intracellular recordings, with presynaptic stimulating rates of no more than 2 Hz, and a binomial analysis of the response fluctuations according to methods described previously (Korn, H., Triller, A., Mallet, A. and Faber, D.S., Science, 213: 898, 1981). In the first series (n≈30), control data was obtained prior to applying strychnine iontophoretically from a third microelectrode located extracellularly near the M-cell soma. Unitary IPSPs were abolished in about half the cases and were reduced to 36.5±14.4% of their initial amplitudes in another 15 cells. In 6 of the latter where individual responses were large enough to detect reliably, binomial statistics showed that the major effect was on q, which dropped to 61±4% of the controls. In contrast, n (the number of available units) remained constant, and p (the probability of release) was also unaffected, with the exception of two cases where it was reduced by about 30%. Furthermore, the kinetic parameters of unitary IPSPs were unchanged, which is quite different from the effects of competitive blockers at other junctions. In the second set (n=7) of experiments (in which strychnine was administered intramuscularly) each interneuron was injected with HRP and reconstructed (see ref. above) to determine if, as predicted, the binomial n still equalled the number of presynaptic boutons. Such was the case, for a range of 4 to 31 stained terminals (bin n/hist n=1.04±0.14). Values of p and q were consistent with those above. The specific effect of strychnine on quantal size rather than on the exclusively release parameters n and p is inconsistent with the proposal that this drug acts mostly at a presynaptic level (Diamond et al., J. Physiol., 232: 87, 1973). These data not only i) strengthen the notion that glycine is an inhibitory transmitter at the supraspinal level and ii) extend the validity of the binomial model to situations of reduced postsynaptic receptor density but also provide a means for pharmacological investigations of molecular mechanisms at this central synapse. (Supported in part by NIH grant NS15335).

135.5 A SLOW EXCITATORY POSTSYNAPTIC POTENTIAL MEDIATED BY ACETYLCHOLINE IN HIPPOCAMPAL PYRAMIDAL CELLS. A.E. Cole and R.A. Nicoll. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA 94143.

Considerable biochemical and histochemical evidence indicates that the hippocampus receives cholinergic input from the medial septum. Although exogenously applied acetylcholine (ACh) has been shown to activate muscarinic receptors resulting in a slow depolarization, a synaptic cholinergic potential has not been demonstrated.

Using intracellular recording techniques in the in vitro rat hippocampal slice preparation, we have studied the role of ACh in synaptic transmission in CA1 pyramidal cells. These cells are believed to be innervated by cholinergic fibers from the medial septum. We have found that bath application of ACh has three actions on CA1 neurons: 1) depolarization associated with an increased membrane resistance, 2) blockade of an after-hyperpolarization (AHP) which results primarily from a calcium-activated potassium conductance ($G_{K(Ca)}$) and 3) blockade of accommodation of cell discharge evoked by long (600 ms) depolarizing pulses. All actions were reversed by the muscarinic antagonist, atropine. Electrical stimulation of stratum oriens in the hippocampal slice elicited a series of fast excitatory postsynaptic potentials (EPSPs), followed by an inhibitory postsynaptic potential (IPSP). The IPSP was followed in most cells by a slow EPSP which lasted 20-40 s and was associated with an increase in membrane resistance and cell firing. The size of the slow EPSP was very sensitive to the membrane potential; with membrane potentials more negative than -70mV, little depolarization could be recorded. Electrical stimulation also blocked the $G_{K(Ca)}$ and the accommodation of cell discharge. All responses were enhanced by eserine and antagonized by atropine. In addition, the sites of optimal stimulation closely paralleled the distribution of cholinergic fibers in the hippocampus. The slow depolarization was calcium-dependent since it was eliminated by the calcium antagonist cadmium (100uM). These findings demonstrate that electrical stimulation of sites in the hippocampus known to contain cholinergic fibers evokes a slow muscarinic EPSP in CA1 cells which exactly mimics the postsynaptic effects of exogenously applied ACh. Thus these findings fulfill an important criterion in establishing ACh as a neurotransmitter in the CNS.

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135.6 DIRECT INHIBITORY ACTION OF BACLOFEN ON HIPPOCAMPAL PYRAMIDAL CELLS. N.R. Newberry* and R.A. Nicoll. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA 94143.

The GABA analogue, baclofen, appears to act at a separate site ($GABA_B$) from that mediating the classical bicuculline-sensitive, chloride-dependent GABA response ($GABA_A$) (Bowery, N.G., Trends Pharmacol. Sci. 3: 400, 1982). While early studies reported a postsynaptic inhibitory effect, recent interest has focussed on its presynaptic (depressant) effect on transmitter release.

We have investigated the postsynaptic action of baclofen on rat hippocampal CA1 pyramidal cells using intracellular recording techniques in the slice preparation. The superfusion of (+) baclofen (3×10^{-7} - 10^{-4} M) hyperpolarized the membrane with an associated reduction in the input resistance and silenced those cells which were spontaneously active. The hyperpolarization was primarily dependent on the (-) isomer since it was approximately 100-200 times more potent than the (+) isomer. The response resulted from a direct action on the cell, since it could be recorded after 1 µM tetrodotoxin or 100 µM $CdCl_2$ had blocked synaptic transmission.

The hyperpolarization could be elicited by the local ionophoresis of (+) baclofen (25 mM, pH 3) into stratum radiatum, pyramidale or oriens. The response could be evoked from cells in which the somatic chloride reversal potential had been reversed by intracellular injection of chloride. In paired ionophoresis experiments the baclofen response was compared with the hyperpolarizing somatic GABA response. The baclofen response was slower and associated with a smaller change in input resistance than the GABA response. The reversal potentials for the GABA and baclofen responses were approximately -70 and -85 mV, respectively. While the superfusion of $BaCl_2$ (0.3 - 2 mM) depressed the baclofen response but not the GABA response, the superfusion of bicuculline (10 µM) selectively reduced the GABA response.

These experiments indicate that baclofen hyperpolarizes hippocampal pyramidal cells by a direct postsynaptic action which may involve an increase in potassium conductance. This action appears to involve sites similar to the presynaptic $GABA_B$ sites.

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135.7 **VOLTAGE-DEPENDENCE OF SYNAPTIC TIME COURSE PARTIALLY MODIFIES SYNAPTIC CHARGE TRANSFER.** Daniel Gardner. Dept. of Physiology and Biophysics, Cornell U. Med. Coll., New York, N. Y. 10021.

Postsynaptic currents mediated by inhibitory cholinergic receptors of Aplysia buccal ganglia are choline-sensitive, and can be blocked by the addition of 1 mM choline to the bath. Voltage-clamping cells BL and BR7, in which monosynaptic cholinergic PSPs are diphasic, excitatory-inhibitory, reveals underlying early inward (I_D) and late outward (I_H) currents. Bath choline blocks only I_H, as does eserine and ACh. I_D revealed by choline or ACh block decays exponentially with time constant $\tau_D = 1/\alpha_D$. Decay is voltage-dependent: $\alpha_D = B\, e^{AV}$, with $A = 0.016 \pm 0.001\ mV^{-1}$ and $B = 0.273 \pm 0.025\ ms^{-1}$ (n=13). With eserine used to block I_H and so reveal I_D, decay was longer and voltage-dependence apparently steeper: $A = 0.027\ mV^{-1}$, $B = 0.165\ ms^{-1}$, suggesting an effect of eserine on the voltage-dependent process.

The time course of I_H was approximated by subtraction of I_D recorded in choline from control diphasic currents in sea water. Decay was again exponential, but essentially voltage-independent: $A = -0.003 \pm 0.002\ mV^{-1}$, $B = 0.023 \pm 0.003\ ms^{-1}$, giving $\tau_H = 48 \pm 5$ ms.

In order to independently verify the above, and reveal I_H directly, I attempted to block I_D with hexamethonium (Tauc and Gerschenfeld Nature 192:366-367, 1961). However, the block was insufficiently specific: residual I_D contaminated I_H time course, and partial block of I_H yielded an artificially steepened voltage-dependence of I_D obtained by subtraction.

Peak synaptic current I_{peak} and synaptic charge transfer Q of I_D were computed and plotted vs. V_m, in order to assess the contribution of voltage-dependent time course to synaptic efficacy. The slope of the Q-V curve decreased with depolarization in 10 of 13 cells. The Q/I_{peak} ratio was less voltage-dependent than would be predicted by the behavior of α_D, with $A = 0.009$ for data pooled to eliminate cell-to-cell variations. Significant charge transfer during rise or peak may account for the diminished voltage dependence. Depolarization therefore diminishes the effectiveness of this synaptic component, allowing the inhibitory component to predominate, not only by reducing driving force, but also by reducing the duration of the conductance change. Although this diphasic PSP meets timing criteria of Segev and Parnas (Biophys. J. 41: 41-50, 1983) for maximal inhibition, it uses the additional mechanism of voltage-dependent time course to further reduce the effectiveness of the early EPSP.

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135.8 PRELIMINARY CHARACTERIZATION OF EXCITATORY ACETYLCHOLINE RECEPTORS IN APLYSIA USING THE SINGLE CHANNEL RECORDING TECHNIQUE. L. Simmons. Center for Neurobiology and Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

Using the giga-seal patch-clamp technique of Neher and Sakmann I have made a preliminary characterization of the channels that mediate the excitatory acetylcholine (ACh) response in RB cells in the abdominal ganglion of Aplysia californica. Cells of the RB cluster in the abdominal ganglion exhibit excitation during iontophoretic application of ACh onto their somas. After desheathing the ganglion and treating the cells with proteolytic enzymes the RB cluster still maintains a normal excitatory ACh response. Following enzyme treatment the cells were maintained in 19°C HEPES-buffered artificial seawater (ASW). Single ACh channel openings were recorded with the on-the-cell patch technique, using patch electrodes containing various concentrations of ACh in ASW.

With 1-3 uM ACh in the pipette the inward ACh-induced currents are qualitatively similar to those described in other preparations. At a given agonist concentration, the probability of channel opening measured across various cells varies widely. The elementary current increases linearly with hyperpolarization, and the extrapolated reversal potential is 4.5 mV. The mean conductance of these excitatory channels is 32 ± 3 pS (SD).

The average open time of these channels at resting potential (-45 to -50 mV) is 3.2 msecs. Individual channel openings are often transiently interrupted by brief closings (flickering). Channel lifetime becomes progressively longer when the patch membrane is hyperpolarized. The relationship between lifetime and membrane voltage is roughly linear in the range -40 mV to -95 mV with a slope of 1 msec/10 mV and saturates around -110 to -120 mV.

Previous investigators have described the relatively nonselective behavior of excitatory ACh channels in cells in the pleural ganglion of Aplysia (Ascher et al., 1978; J. Physiol., 278:177). My preliminary results suggest that the excitatory ACh channels in the RB cells also exhibit this weakly selective ionic permeation characteristic. When the ASW in the patch pipette is replaced by a solution containing 1/2 isotonic $MgCl_2$ and 1/2 ASW the ACh-induced current steps are present, though reduced to 1/3 of their normal elementary current amplitude. The average open time of channels at resting potential in this solution is increased by a factor of three.

This work was supported by NIH Grants NS07038 and NS19328.

135.9 COMPARISON OF DECREASED CONDUCTANCE SEROTONERGIC RESPONSES IN INK MOTOR NEURONS AND TAIL SENSORY NEURONS IN APLYSIA. John P. Walsh and John H. Byrne, Dept. of Physiol. and Cell Biology., Univ. Texas Med. Sch. at Houston, Houston TX. 77025

Previous work has demonstrated that serotonin (5-HT) produces a slow decreased conductance depolarization in both the tail sensory neurons and L14 ink motor neurons (Byrne & Walters, 1982; Pollock et al, 1982; Walters et al, 1983; Walsh & Byrne, 1982). The present study was undertaken to analyze further the ionic and cellular mechanisms underlying the 5-HT responses in these cells using voltage clamp and microejection techniques.

Differences between L14 and the sensory neurons were found in the regional sensitivity to 5-HT and in the linearity of the voltage-dependence of the 5-HT response. Somatic application of 5-HT to L14 was relatively ineffective, while application to the neuropil underneath the soma generated a current which was linear with respect to voltage reversing at approximately -78 mV. This response was associated with a 12% decrease in input conductance. In contrast, the cell body of sensory cells was responsive to a much lower concentration of 5-HT than that used to elicit a response in L14. 5-HT produced a large decrease in a voltage dependent conductance in the sensory neurons. The response was most pronounced at depolarized levels and absent at hyperpolarized levels. The 5-HT responses in both L14 and the tail sensory neurons were sensitive to changes in extracellular K^+. Addition of 30mM K^+ -ASW caused a depolarizing shift in the reversal potential of the 5-HT response in L14 to a level in good agreement with the predicted K^+ Nernst equilibrium potential. Addition of high-K^+ ASW also shifted the IV relation of the sensory neurons and produced a reversal of the 5-HT response which was not observed in normal K^+. The 5-HT responses seen in both L14 and the sensory cells were not secondary to activation of interneurons or dependent primarily upon extracellular Ca^{++} since comparable responses were obtained in ASW containing 30mM Co^{++}.

To examine the role of cAMP in mediating the 5-HT responses IBMX, db cAMP, and an adenylate cyclase activator (forskolin) were added to the bath. Each compound mimicked the 5-HT response producing an inward current with a decrease in input conductance. During application of forskolin subsequent 5-HT responses were blocked or attenuated. These studies demonstrate that 5-HT modulates two types of K^+ conductances (differing in their voltage dependence and spatial distribution) in L14 and the sensory neurons, and that the changes in K^+ conductance are mediated by cAMP. These results are consistent with observations that 5-HT increases cAMP levels in the tail sensory neurons (Pollock et al, 1982; Ocorr et al, this volume).

135.10 NEUROTRANSMITTER RECEPTOR-MEDIATED CYCLIC GMP FORMATION: A NEW HYPOTHESIS IMPLICATING THE INVOLVEMENT OF PHOSPHOLIPASE A_2 AND ARACHIDONIC ACID METABOLITES. Elliott Richelson, R. Michael Snider, Michael McKinney* and Carlos Forray*. Dept. of Psychiatry and Pharmacology, Mayo Clinic & Fdn., Rochester, MN 55905.

Activation of muscarinic or histamine H_1 receptors on murine neuroblastoma clone N1E-115 cells results in a marked increase in cGMP formation. The mechanism for this response has been thought to be activation of receptor-operated calcium channels resulting in a rise in the intracellular free [Ca^{++}] which stimulates guanylate cyclase. Our recent data do not support this hypothesis. Neuroblastoma cells loaded with the photoprotein aequorin (an indicator of intracellular free [Ca^{++}]) responded to challenge with a calcium ionophore (X537A) or melittin by a marked and immediate increase in light production, but carbachol or histamine had no effect. Moreover, the cGMP accumulation time-course was much more rapid for receptor agonists than for X537A or melittin, thus clearly discriminating between the intracellular [Ca^{++}] and cGMP stimulation.

In the search for an alternate mechanism of receptor-mediated cGMP formation, we found that inhibition of either phospholipase A_2 (PLA_2) or lipoxygenase (LPO) metabolism of arachidonic acid (AA) attenuated the receptor or ionophore-mediated cGMP response. Quinacrine, a PLA_2 inhibitor, blocked the response with an IC50 of about 100μM. Inhibition of the LPO pathway of AA metabolism with eicosatetraynoic acid or nordihydroguaiaretic acid inhibited cGMP formation with IC50 values of 10 and 30μM, respectively. If PLA_2 is involved in neurotransmitter action, then activation of this enzyme should stimulate cGMP accumulation. Thrombin (1-10 nM), a serine protease involved in hemostasis and well known activator of PLA_2 in platelets, stimulated cGMP formation by neuroblastoma cells with a time course and maximum response similar to neurotransmitter agonists. (Snider and Richelson, Science, in press). Additionally, the binding of [^{125}I]thrombin to intact cells revealed a high-affinity site with a K_D correlating with thrombin's ED50. Melittin is known to activate markedly endogenous PLA_2 in fibroblasts (Shier, P.N.A.S. 76, 195, 1979) and it produced a marked and prolonged cGMP response in neuroblastoma cells. Finally, we have evidence that muscarinic or histaminic receptor activation stimulates the release of [^{3}H]AA from prelabeled cells and this effect is blocked by the respective receptor antagonists, atropine or pyrilamine.

Our current hypothesis is that receptor activation in neuroblastoma cells leads to an increase PLA_2 activity and release of AA which is metabolized via the LPO pathway and stimulates guanylate cyclase. (Support: Mayo Foundation and Grants MH27692, MH08823, AM 07147 and HL12186)

135.11 CYTOCHEMICAL DISTRIBUTION OF CALCIUM IN THE DENTATE MOLECULAR LAYER. E. Fifková, K. Cullen-Dockstader,* J.A. Markham and R.J. Delay.* Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

In search for the underlying mechanism of synaptic plasticity induced in dendritic spines of the dentate fascia by tetanic stimulation, a study of the spine internal structure was indicated. It was assumed that physicochemical properties of the spine cytoplasm , like ion composition and pH, are changed by the tetanic stimulus. It was further assumed that such a change could affect the conformational state of cytoplasmic proteins and thus change the viscosity of the neuroplasm. Under such conditions, actin filaments which are present in dendrites and dendritic spines (Fifková and Delay, J. Cell Biol., 95:345) could contract in association with cytoplasmic myosins and regulatory proteins similarly to other nonmuscle cells. Such an activity could induce the morphometric changes observed in the spines during long-term potentiation. The anticipated contraction of actin filaments requires a transient elevation of free Ca^{2+}. This could be provided by the calcium current during depolarization when voltage dependent Ca^{2+} sensitive channels open and/or by internal sources of calcium. Therefore, we have attempted to identify cytochemically the localization sites of Ca^{2+}. Using three calcium precipitation methods, we have demonstrated discrete deposits in synaptic vesicles and multivesicular bodies. Larger precipitates were regularly observed in mitochondria, astrocytic processes, and in the smooth endoplasmic reticulum of axons and axon terminals. In these locations, calcium has been previously reported in the literature. Our novel contribution is the observation of Ca^{2+} deposits in dendrites and dendritic spines in the smooth endoplasmic reticulum and the spine apparatus, respectively. The presence of Ca^{2+} deposits in the spine apparatus indicates an affinity of this organelle for, and a capacity to, sequester Ca^{2+}. Thus, the spine apparatus could regulate regional concentrations of Ca^{2+} during synaptic activity and keep it relatively high in the spine, likewise the smooth endoplasmic reticulum does in the periphery of the light stimulated visual receptors. The spine apparatus may also serve as a storage site from which Ca^{2+} could be released upon stimulation in a fashion similar to that of the muscle sarcoplasmic reticulum. A Ca^{2+} concentration gradient may build up between the spine and dendrite which would, within the spine stalk, allow for an orderly organization of actin filaments that is required for a contraction to take place.

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135.12 FREE INTRACELLULAR SODIUM AND POTASSIUM IN MAMMALIAN SYMPATHETIC NEURONS DURING NEUROTRANSMITTER APPLICATION. K. Ballanyi*, P. Grafe* and G. ten Bruggencate. Department of Physiology, University of München, 8 München 2, Federal Republic of Germany.

It is well known that carbachol and GABA depolarize mammalian sympathetic neurons. The question was, to what extent changes in intracellular free concentrations of Na or K ($[Na]_i$,$[K]_i$) occured during such cholinergic, and amino acid, agonist actions.

Experiments were performed on isolated, desheathed superior cervical ganglia of rats, maintained at 30°C in Krebs solution. Double barreled ion selective microelectrodes were used to record the membrane potential and the $[Na]_i$ or $[K]_i$. The ion barrel was filled with Corning 477317 ($[K]_i$) or a neutral carrier exchanger (ETH 227, $[Na]_i$), respectively (overall tip size 0.3 μm).

Steady state values obtained were: $[K]_i$ 121.7 + 9.7 mmol/l (mean ± SD, n = 30, resting potential -45.3 ± 5.4 mV, action potential amplitude 70.7 ± 13.9 mV). $[Na]_i$ was 10.0 ± 4.3 mmol/l (n = 21, resting potential -43.8 ± 6.9 mV, action potential amplitude 69.5 ± 12.7 mV). Repetitive stimulation (30 Hz for 20s) of preganglionic fibers induced small increases in $[Na]_i$ (about 3 mmol/l). Application of 50 μmol/l carbachol in Krebs superfusion fluid resulted in a membrane depolarization exceeding 20 mV that was accompanied by an increase in$[Na]_i$ between 5 and 16 mmol/l, and a simultaneous decrease in$[K]_i$ between 8 and 20 mmol. The ion changes reached their maxima during the early phase of the repolarization of the membrane; recovery to baseline levels after the end of the carbachol superfusion took several minutes and usually corresponded to the time course of the extracellular K-undershoot. No recovery was observed in the presence of 300 μmol/l ouabain. GABA (100 μmol/l) led to a smaller decrease in $[K]_i$ as compared to carbachol, and $[Na]_i$ did not change at all. Thus, an increase in$[Na]_i$ does not seem to be involved in the reuptake of potassium that takes place after GABA-application.

In preliminary experiments, norepinephrine (100 μmol/l) did not change $[Na]_i$ nor $[K]_i$ despite a membrane hyperpolarization of about 5 mV. These data do not support the notion that norepinephrine may activate the sodium pump in rat sympathetic ganglia. On the other hand, our data on carbachol application show that the sodium pump is the main factor involved in the homeostasis of carbachol-induced ion changes.

135.13 PLASTICITY IN NEURONAL EXCITABILITY IN THE INFERIOR MESENTERIC GANGLION OF GUINEA PIG. B.F. King* and J.H. Szurszewski (SPON: J.P. Whisnant). Department of Physiology, Mayo Medical School, Rochester, MN 55905.

Principal sympathetic neurons in mammalian prevertebral ganglia express variation in their active electrical properties. For instance, action potentials elicited by these cells are followed by an afterspike hyperpolarization (ASH) which may assume one of three voltage trajectories, in terms of its rate-of-decay. Also, these cells show two patterns of discharge in response to constant depolarization; they show phasic and tonic firing patterns. In addition, a small population of these principal ganglion cells display spontaneously rhythmic firing, an oscillatory behavior which has been associated with pacemaking of other ganglion cells. In view of recent knowledge of novel potassium currents which control excitability in other systems, we have come to suspect that variation in these electrical properties is due to subtle expression of these ionic currents. From intracellular recordings from sympathetic neurons in the inferior mesenteric ganglion of the guinea pig, and by manipulation of potassium currents either pharmacologically or by ionic addition and withdrawal, plasticity in neuronal excitability can be induced. From our observations on firing patterns and the voltage trajectory of the ASH, we have reason to identify at least two potassium currents. One is susceptible to 4-aminopyridine and to barium; another is susceptible to extracellular calcium levels. Whether or not these ionic currents are under transmitter/ humoral control remains equivocal. Supported by NIH Grant AM 17632.

REGULATION OF PITUITARY FUNCTION II

136.1 THE ROLE OF CORTICOMEDIAL AMYGDALA (CMA) AND MEDIAL PREOPTIC HYPOTHALAMIC AREA (MPO) IN THE ESTROGEN-INDUCED PROLACTIN (PRL) AFTERNOON SURGE. Jenn-Tser Pan* and R.R. Gala. Dept. of Physiol., Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Earlier studies from this lab have shown that local implantation of estradiol-17β(E_2) in the MPO but not in the cerebral cortex or ventromedial hypothalamic nuclei (VMH) can induce a significant PRL afternoon surge (Fed. Proc. 42: 575, 1983). The CMA has also been reported to have some effects on PRL secretion. Both lesion and implantation experiments were performed in this study to determine the role of CMA and MPO in the estrogen-induced PRL surge.

Adult female Sprague-Dawley rats weighing approximately 250 gm were used in all studies. Animals were kept 2/cage in a light (14L:10D, with light on at 0600 h) and temperature (24°C) controlled room. Radiofrequency was used in all lesion studies using either 56°C or 60°C for 1 min. Twenty-eight gauge cannulae containing E_2 diluted 1:100 with cholesterol were used in all implantation studies. Intra-atrial catheters were placed through the jugular vein for serial blood sampling which was initiated on day 2 through day 5. Blood samples (0.3 ml) were obtained at 1300, 1700 and 1900 h on each day.

In normal cycling rats, MPO lesions on diestrus I eliminated the expected proestrous PRL afternoon surge seen in normal cycling rats. MPO lesions in long-term ovariectomized (OVX for 2 wks) polyestradiol phosphate (PEP)-treated rats also blocked the PRL surge completely. Some MPO lesions included the suprachiasmatic nuclei, while others did not. However, the results were the same. Acutely CMA lesioned (2 days) OVX + PEP-treated rats exhibited normal onset and magnitude of PRL surge.

High concentration of E_2(1:2 or 1:4) implanted in the CMA induced a significant PRL surge, but the systemic effects of E_2 were apparent as evidenced by augmented uterine weight and the appearance of vaginal cornification. Using 1:100 diluted E_2 implants, no systemic effect was observed and no PRL surge was induced. Similar results were observed previously when 1:100 diluted E_2 implants were placed in either the cerebral cortex or the VMH (Fed. Proc. 42: 575, 1983). Simultaneous implantation of E_2(1:100) in the MPO and CMA did not show an amplification effect on the PRL surge over that observed for MPO implants alone. It is concluded that the MPO is by far the most important area in the action of E_2 in inducing the PRL surge, and the CMA is not necessary for the generation of the surge. However, under certain physiological conditions (e.g., pseudopregnancy) the CMA may have a modulatory role. (Supported in part by NIH Research Grant #HD 14671.

136.2 PROLACTIN SECRETION IN RESPONSE TO A NONSTEROID ESTROGENIC MYCOTOXIN, ZEARALONE G. E. Resch* (SPON: James L. Voogt) Dept. of Biol. and Schl. of Med., Univ. of Mo.-K.C. Kansas City, Mo. 64110

The mycotoxin Zearalenone (F2) has been implicated in problems of reproduction and growth, that have been shown to be responsive to estrogenic stimuli. Some of these effects may be attributed to the fact that F2 acts at estradiol (E2) receptors and has been demonstrated to interfere with estrogenic feedback control of LH in some species. Previous reports by Hobson et. al. (1969) that F2 elicited increases in LH and FSH secretion in the monkey supported the notion that F2 might also mimic E2 stimulation of Prolactin (Prl) secretion. The probability that F2 could also effect E2 control of Prl seemed very high; therefore Prl secretion following F2 treatment was studied in an in vitro continuous flow dispersed pituitary cell preparation by a modification of the method of Lowery and Jay (1974). Rat pituitaries were excised, minced, and trypsinized to obtain dispersed cells. The cells were washed in Earl's BSS containing lima bean trypsin inhibitor and mixed with a polyacrilamide gel bead support matrix. The gel-cell mixture was poured into a column and perfused with medium 199 by a proportioning pump at 1 ml/min. The concentration of Prl and F2 was measured in column effluent at timed intervals after F2 injection onto the column. The Prl response to F2 was first tested by administration of a 10 mg bolus of F2 directly on top of the cell layer. Prl secretion increased over 5-fold from dopamine (DA) inhibited baseline values of 5.24 ng/ml to 32.4 ng/ml following administration of F2 bolus. Response latency, time to peak value, and duration of the Prl response were studied in relation to different F2 vehicle solvents. It was concluded that F2 elicits an increased Prl secretion from isolated dispersed pituitary cells. The latency of the Prl response is less than 3 min and the duration is vehicle dependent. Preliminary dose response data suggest that a maximum response occurs to about 40 ug doses of F2 with lower responses occuring at both 4 and 400 ug amounts. Comparison of F2 and E2 elicited increases in Prl secretion in early experiments indicates that F2 is 2 to 3 times potent than E2.

136.3 GONADOTROPIN-RELEASING HORMONE (GnRH) RECEPTORS IN MALE HAMSTERS: EFFECT OF CASTRATION AND PHOTOPERIOD. David R. Pieper, University of Detroit, Department of Biology and Health Sciences, Detroit MI 48221

Previous studies in male rats have indicated that the number of pituitary GnRH receptors (GnRH-R) is related to GnRH secretion. For instance, the number of GnRH-R is increased by castration or by injection of exogenous GnRH into intact or hypothalamic lesioned rats (Endo 110:749, 1982). Other investigators have shown that male Syrian hamsters exposed to a short photoperiod for 10 weeks undergo gonadal regression due to decreased gonadotropin levels. This is thought to be mediated by an inhibitory effect of pineal melatonin on the hypothalamo-hypophyseal axis. The purpose of the present study was to determine the effect of castration and photoperiod on the number of GnRH-R in Syrian hamsters. Hamsters (63 days old) were placed on either an LD 14:10 or LD 6:18 light:dark cycle for 9 weeks. At this time half the animals on each photoperiod were castrated. One week later, all animals were decapitated. The animals on LD 6:18 had regressed testes (2.4 $\pm$ 0.2 mg paired testes wt per 100 g B.W., $\bar{x}\pm$SE) compared to hamsters on LD 14:10 (23.3$\pm$1.0 mg). Serum LH levels were also lower in intact animals on LD 6:18 (29.5$\pm$5 ng/ml) compared to intact hamsters on LD 14:10 (55.7$\pm$5 ng/ml). Animals on both photoperiods responded to castration with increased LH secretion, although the increase in animals on LD 6:18 (104$\pm$20 ng/ml) was less than that of hamsters on LD 14:10 (470$\pm$44 ng/ml). These results show that the animals on short photoperiod underwent gonadal regression and had reduced gonadotropin secretion. The GnRH-R were assessed by the method previously described for rats (Endo 105:1069, 1979) using the D-Ala^6 analog of GnRH. Scatchard analysis of competition curves indicated that the hamster pituitary GnRH receptors have characteristics similar to those previously described for rats (K_a=6.5x10 M^{-1}). Furthermore, the affinity of the receptor was similar in hamsters on long and short photoperiod. The number of GnRH-R was determined by saturation analysis. Castration of animals on LD 14:10 resulted in an increase in the number of GnRH-R from 83$\pm$7 to 143$\pm$9 fmol/pituitary, a response similar to that of rats. Intact hamsters on LD 6:18 had a reduced number of GnRH-R (48.8$\pm$5 fmol/pit) but there was also an increase (100$\pm$8 fmol/pit) following castration in the animals on short photoperiod. In conclusion, the results show that the hamster pituitary GnRH-R have characteristics similar to the rat and respond similarly to castration. The data also suggest that part of the inhibition of reproduction after maintaining hamsters on short photoperiods is due to a decrease in GnRH secretion and a decrease in the number of GnRH receptors on the pituitary gland.

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136.4 ELECTROCHEMICAL MEASUREMENT OF ANTERIOR PITUITARY DOPAMINE IN THE RAT: CONTRIBUTION BY THE POSTERIOR LOBE AND ITS EFFECTS ON PROLACTIN SECRETION. J. Jeffrey Mulchahey* and Jimmy D. Neill* (SPON: J. Brown). Department of Physiology and Biophysics, University of Alabama in Birmingham, Birmingham, AL 35294.

Dopamine (DA), secreted by tuberoinfundibular neurons into the hypophyseal portal circulation, is widely accepted as a physiological prolactin release-inhibiting factor (PIF). While DA levels in hypophyseal portal plasma are sufficient to suppress prolactin (PRL) levels, they are insufficient to account completely for the tonic inhibition of PRL secretion. The present study was designed to ascertain the relative portal and posterior lobe contributions to DA in the anterior pituitary (AP) and their effects on PRL secretion. DA in the AP of urethane anesthetized diestrus I rats was monitored in situ every 10 min by electrochemical (EC) methods using differential pulse voltammetry with a carbon paste electrode which was relatively ascorbate-insensitive. Measurements were taken during control periods, after posterior lobe removal followed by hypophyseal stalk section, or after inhibition of catecholamine synthesis by α methyl-p-tyrosine (AMPT) followed by DA infusion. Following EC stabilization, posterior lobe aspiration resulted in a slight rise in plasma PRL from 14.4$\pm$3.1 ng/ml to 22.8$\pm$1.0 ng/ml (mean$\pm$SEM, n=7, NS) while the DA-EC output fell to 47.8$\pm$6.10% of initial levels ($p<0.01$). Subsequent stalk section elevated PRL to 270.8$\pm$96.4 ng/ml ($p<0.01$) while the DA-EC output fell to 28.8$\pm$6.14% of initial levels ($p<0.01$). In another group of animals, after EC stabilization, PRL had risen from 47.0$\pm$5.84 ng/ml (mean $\pm$ SEM, n=12) to 345.2$\pm$64.9 ng/ml ($p<0.05$) 90 min after AMPT while the DA-EC output fell to 22.9$\pm$3.52% of initial levels. Infusion of DA at a rate of 1 μg/kg$\cdot$min suppressed PRL to 157.8$\pm$31.0 ng/ml ($p<0.05$) and restored the DA-EC output to 52.2$\pm$5.44% of initial. Infusion of DA at a rate of 2 μg/kg$\cdot$min suppressed PRL to 74.7$\pm$16.7 ng/ml (NS) and restored the DA-EC output to 102.7$\pm$7.82% of initial (NS). These data demonstrate a complex, non-linear relationship between PRL secretion and DA in the AP and support the conclusion that the posterior lobe may contribute significant amounts of DA to the AP. Thus, previous measurements of hypophyseal stalk plasma DA may have underestimated the amount of DA reaching the AP. Furthermore, infusions of DA into AMPT treated rats at a rate sufficient to mimic physiological DA-EC outputs suppressed PRL to levels not statistically different from pretreatment levels. This suggests that DA reaching the AP from the portal circulation and the posterior lobe, may be sufficient to account for the tonic hypothalamic inhibition of PRL secretion in the diestrus rat.

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136.5 PROLACTIN (PRL) STIMULATION OF (^{3}H)-DOPAMINE (DA) RELEASE FROM RAT MEDIAN TISSUE: DEPRESSANT EFFECT OF CHRONIC ESTROGEN TREATMENT. P.E. Gottschall,* D.K. Sarkar,* K. Quigley* and J. Meites,* (SPON: R. Bernard). Dept. of Physiology, Michigan State University, E. Lansing, MI 48824.

The effect of PRL on release of (^{3}H) from rat median eminence was studied following pre-incubation of tissue with (^{3}H)-DA. Female Wistar-Furth rats on diestrous days I or II were used at 1100h (lights on 0500h - lights off 1900h). Procedures and kinetic analysis of uptake and release have been described previously (Sarkar et al, Neuroscience, in press). Briefly, median eminence was incubated for 20min in 3.6x10^{-7}M (^{3}H)-DA in an incubation and superfusion Krebs-Henseleit buffer. Two median eminences were used in each superfusion chamber, and (^{3}H) release was stable after 40min. Fractions were collected (0.6/2min) and release of (^{3}H) was expressed as a fractional rate constant (FRC) per minute, calculated by dividing the amount released by double the amount of (^{3}H) content in the tissue at the start of each 2min period (FRC=x10^{-3}/min). PRL-evoked release (PER) was expressed as the FRC in the second tube collected following infusion of PRL (consistently the maximal response).

Infusion of 0.3, 6 and 120ng rat PRL over a 2min period resulted in a PER of (^{3}H) from the tissue which was dose-dependent (PER=12.4$\pm$1.2, 20.0$\pm$2.2, and 26.7$\pm$2.6x10^{-3}min; respectively). Analysis of the dose-response curve ($r=0.87$) showed no deviation from linearity $F(2,13)=3.79$, $p>0.05$, and did not exhibit significant regression $(1,2)=3.35$, $p>0.05$. Twenty minutes following PRL stimulation, 40mM K^+ was infused, resulting in an evoked release similar to 120ng PRL. To test the effects of chronic E treatment and E withdrawal on the ability of PRL to release (^{3}H)-DA in vitro, Fischer 344 rats were ovariectomized 8 wks prior to the experiments, and were divided into 3 groups 1) estradiol 17-B 100 mm silastic implant)-treated animals which received an E containing implant for the last 4 wks, 2) animals which received E only for the first 4 wks, and 3) those which received an empty capsule only for the first 4 wks. The PER in this study was calculated by subtracting the FRC from the three fractions prior to PRL infusion, from the FRC of the three fractions after infusion. The dose of rat PRL was 120ng. In vitro PRL infusion resulted in a PER of 10.4$\pm$1.8x10^{-3}/min in C, and in chronic E animals, PER was similar to C.(12.6$\pm$1.8x10^{-3}/min). However in animals withdrawn from E, PER increased to 18.8$\pm$2.5x10^{-3}/min. Since it is known that tuberoinfundibular DA (TIDA) turnover is increased following short term E treatment, these results suggest 1) chronic E may depress TIDA neuronal activity, even in the presence of hyperprolactinemia, and 2) if E is removed, the increase in dopaminergic activity reappears.

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136.6 DOPAMINE INHIBITS NEUROTENSIN-INDUCED PROLACTIN RELEASE BY INTERACTING WITH CALCIUM CHANNEL RATHER THAN ADENYLATE CYCLASE SYSTEMS. M. Memo, E. Carboni*, M. Trabucchi and P.F. Spano. Institute of Pharmacology and Pharmacognosy, University of Cagliari and Milan, Italy.

Neurotensin (NT) is an endogenous neuropeptide that is active in many preclinical screening tests for neuroleptic drugs. It was previously found that NT stimulated prolactin (PRL) release from incubated rat hemipituitary (Enjalbert A. et al., Neuroendocrinol. 34, 95-98, 1982). Our study was directed to investigate the molecular mechanisms leading to the PRL release. For this porpouse we kept the same experimental conditions as those described to elicit NT-dependent PRL secretions. NT (from 10^{-8}M to 10^{-6}M) was not able to increase cAMP levels in 1h incubated rat hemipituitary as well as to enhance adenylate cyclase activity in homogenates from rat anterior pituitary membranes. Since PRL release appears to be regulated by intracellular free Ca^{++} content the effect of NT on Ca^{++} influx was studied. We found that NT increased intracellular $^{45}Ca^{++}$ concentrations in a dose-dependent manner, reaching the maximal effect at 10^{-6}M (+38%). In addition the phenomenon was time-dependent. 15 min of 10^{-7}M NT pre-incubation produced 15% increase in $^{45}Ca^{++}$ intracellular concentrations and preincubation of 10^{-7}M NT for 30 min further potentiated the effect (+32%). These results suggest that NT may induce PRL release not affecting adenylate cyclase system but opening specific Ca^{++} channels in rat anterior pituitary. Addition of 10^{-7}M DA completely antagonized Ca^{++} influx increase induced by NT. DA was added 30 min before NT, then incubation was continued for 30 min. The inhibitory effect was dose-dependent and specifically antagonized by neuroleptic drugs such as haloperidol and sulpiride. Our results suggest that DA may regulate intracellular Ca^{++} concentrations by interacting with a subtype adenylate cyclase-independent D_2 dopamine receptors.

136.7 THE ROLE OF ENDOGENOUS DOPAMINE UPON THE DEPLETION-TRANSFORMATION PHASE OF PROLACTIN IN THE LACTATING RAT. S.W. Shyr* and C.E. Grosvenor* (SPON: C.M. Blatteis). Dept. Physiol. & Biophysics, Univ. Tenn. Ctr. Hlth. Sci., Memphis, TN 38163.

It is known that suckling induces PRL depletion in rat anterior pituitary (AP) and transformation into a releasable form. Tonically, PRL secretion is inhibited by dopamine (DA). In an attempt to further investigate the role of endogenous DA on PRL secretion, we applied dopaminergic antagonists, haloperidol and domperidone, to measure the changes in the depletion-transformation of PRL. Also using α-methyl-p-tyrosine (250 mg free base/kg body weight), we measured the turnover rate of DA in the median eminence (ME) during different stages of PRL in response to suckling.

Primiparous lactating rats of the Sprague-Dawley strain, each with a litter of 6 pups were housed in individual cages under standard laboratory conditions (23-25°C, 14 hr light: 10 hr dark). All experiments were done on day 13-15 of lactation. PRL was measured in the AP by PAGE (pH 7.2). DA in the ME was measured by HPLC combined with electrochemical detection.

Within 10 min of suckling, the concentration of PRL in the AP rapidly and significantly fell. With continued suckling the PRL concentration eventually commenced to replete rather than continue to fall and by the 90th min of suckling the AP concentration had returned to near the initial presuckled level.

A single injection of 0.2 mg haloperidol after the 4-5 hr non-suckling period within 20 min resulted in a marked fall in the AP concentration of PRL in comparison with that from 20 min of suckling. However, 40 min after the onset of suckling, i.e., during the refractory period, the dose response curve shifted to the right by about 10-fold. Similarly, domperidone caused a progressively greater depletion of PRL in the AP within a dose range of 0.001-0.1 mg after 4-5 hr of non-suckling. The dose-response curve shifted to the right during the refractory period.

In the ME, the concentration of DA declined during the first 30 min in response to suckling following the 4-5 hr non-suckling period where upon it gradually returned to its presuckled level during the subsequent 90 min. Additional 10 min period of suckling did not further decrease DA concentration during the refractory period, instead, the DA level increased. The turnover rate of DA was higher during the initial depletion-transformation than during the subsequent refractory phase.

These findings suggest that: 1) a greater endogenous dopaminergic inhibitory effectiveness occurs in the AP during the refractory period to protect PRL from further depletion, 2) the increased effectiveness does not appear to be due to an increased turnover of DA in the ME. (Supported by HD 04358)

136.8 EFFECT OF ALPHA-2 ADRENERGIC AGONISTS AND ANTAGONISTS ON RAT SERUM PROLACTIN.
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We have previously reported that yohimbine (YOH), an alpha-2 adrenergic antagonist, stimulated rat prolactin (PRL) secretion in male Sprague-Dawley rats in vivo without inhibiting the release of hypothalamic dopamine (DA) or blocking the inhibitory effect of DA on PRL secretion from pituitary glands in vitro (JPET 224, 21, 1983). Clonidine, an alpha-2 agonist, produced a partial blockade of the in vivo effect of YOH. Phenoxybenzamine and piperoxane, two other alpha-2 antagonists had no effect by themselves on PRL secretion and did not influence the effect of YOH. We have now studied the effect of other alpha-2 adrenergic agonists and antagonists on rat serum PRL secretion. Oxymetazoline (OXY), a mixed alpha-adrenergic agonist and antagonist, ST-91, a mixed alpha-1 and alpha-2 agonist, and an alpha-2 agonist, guanfacin, 2.5 mg/kg, i.p., all produced a 15-20 fold increase in rat serum PRL levels 30 min after injection. Two other alpha-2 adrenergic agonists, ST 600 and ST 608, did not increase rat serum PRL levels nor did CGS 7525A, an alpha-2 adrenergic antagonist. The increases in PRL produced by OXY and ST-91 were completely inhibited by pretreatment with phentolamine, 5 mg/kg, an alpha-2 antagonist and by prazosin, 5 mg/kg, an alpha-1 antagonist. Preliminary data also indicate a partial inhibition of the PRL releasing effect of YOH by prazosin. These findings suggest that both intact alpha-1 and alpha-2 adrenergic receptors are necessary for alpha-2 agonists or antagonists to stimulate PRL secretion.

Pretreatment with reserpine, 5 mg/kg, i.p. or alpha-methyl-paratyrosine, 100 mg/kg i.p., both of which diminish the availability of NE and DA, blocked the PRL-stimulating effect of OXY, suggesting that OXY acts presynaptically and that NE, as well as DA, may be a tonic inhibitor of PRL secretion in vivo. For NE this may be in the hypothalamus rather than at the pituitary. It is also possible that stimulation of postsynaptic alpha-2 receptors by OXY contributes to the release of PRL in rats via release of the as yet unidentified prolactin releasing factor (PRF). The reason why some, but not all alpha-2 adrenergic agonists stimulate PRL secretion and why YOH is the only alpha-2 antagonist we have so far found which stimulates PRL secretion will require further study. Supported, in part, by USPHS MH 30938 and by the State of Illinois Department of Mental Health.

136.9 MODULATION OF LUTEINIZING HORMONE RELEASE AND HYPOTHALAMIC CATECHOLAMINE ACTIVITY BY OPIATES IN THE FEMALE RAT. B.A. Adler* and W.R. Crowley. Dept. of Pharmacol. Univ. of Tennessee Center for the Health Sciences, Memphis, TN 38163.

The stimulation of luteinizing hormone (LH) release by the opiate receptor blocker, naloxone, can be prevented by inhibitors of norepinephrine (NE) and/or epinephrine (EPI) synthesis, suggesting opiate regulation over catecholamine release. The present study tested whether an opiate agonist or antagonist affect the depletion of hypothalamic catecholamines observed after synthesis inhibition, a measure of catecholamine activity, concomitant with changes in LH secretion. In one study, female rats were treated with 50 µg estradiol benzoate and two days later, rats received either saline or naloxone (10mg/kg). Half the animals in each group were also given α-methyl tyrosine (amt, 400 mg/kg) to inhibit catecholamine synthesis, and rats were killed one hour later. LH was measured by radioimmunoassay and catecholamines were determined by radioenzymatic assays. Administration of naloxone to the estradiol primed rats increased LH release, potentiated the depletion of NE in the preoptic-anterior hypothalamus and medial basal hypothalamus and also enhanced the decline of EPI and dopamine in the medial basal hypothalamus after amt, suggesting increased catecholamine activity in these regions. In a second experiment, females were treated with estradiol, followed two days later by 2 mg progesterone. Two hours after progesterone, rats were given injections of either saline vehicle, morphine (10 mg/kg), or morphine plus naloxone. Half in each group also received α-methyl tyrosine simultaneously, and animals were killed one hour later. Administration of the opiate agonist, morphine, decreased LH and decreased the depletion of catecholamines after synthesis inhibition in the above-mentioned areas, suggesting reduced activity. In most cases, naloxone antagonized the inhibitory effect of morphine.

These findings indicate that 1) naloxone may stimulate LH release by enhancing hypothalamic catecholamine turnover and 2) that this may occur by removing the inhibitory influence of an endogenous opioid peptide that stimulates receptors on catecholamine nerve terminals controlling LH-releasing horone neurosecretion.

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136.10 ENDOGENOUS OPIATES MODULATE ESTRADIOL FEEDBACK ON PROLACTIN SECRETION AND SEROTONIN TURNOVER. M.D. Johnson and W.R Crowley. Dept. of Pharmacol. Univ. of Tennessee Center for the Health Sciences, Memphis, TN 38163.

Previously, we reported that estradiol benzoate (EB) acutely activates medial preoptic serotonergic projections stimulatory to prolactin (PRL) secretion. Because opiate agonists also stimulate both PRL release and serotonin (5-HT) turnover, the present experiments tested whether endogenous opiate neurons mediate estrogen feedback effects. In experiment 1, ovariectomized rats received either 50 µg EB or oil vehicle, and either saline or a long acting opiate receptor blocker, nalmetrene (10 mg/kg), simultaneously, 3 h prior to decapitation. The pargyline method was used to determine 5-HT turnover; hence members of each treatment group received either saline or pargyline (75 mg/kg ip) 30 min prior to decaptiation. Plasma PRL and LH concentrations were determined by radioimmunoassays, and serotonin concentrations from microdissected, individual brain nuclei were measured by liquid chromatography with electrochemical detection. Nalmetrene blocked both the acute elevation of PRL and the increase in 5-HT turnover in the medial preoptic nucleus and ventromedial nucleus. Nalmetrene alone enhanced LH release and 5-HT turnover in the bed nucleus of the stria terminalis, and these effects were prevented by estradiol.

A second study tested whether the opiate agonist, morphine, mimics estrogen effects on PRL and preoptic 5-HT turnover. Animals received saline, morphine (10 mg/kg) and/or naloxone (5 mg/ng) ip 30 min prior to decapitation. Simultaneously, half in each group received pargyline as above. Morphine stimulated both PRL release and medial preoptic 5-HT turnover, and naloxone prevented both effects. In addition, morphine enhanced 5-HT turnover in the central amygdala, and naloxone increased 5-HT turnover in the bed nucleus of the stria terminalis, and these changes appeared to be related to opiate-induced alterations in LH.

These results suggest 1) that estradiol may acutely stimulate PRL secretion by actions on endogenous opiate peptide systems that increase activity in serotonergic projections to the medial preoptic nucleus and 2) that opiate 5-HT interactions may also regulate LH secretion.

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136.11 AFFERENT PATHWAY INVOLVED IN THE REFLEX RELEASE OF OXYTOCIN IN THE LACTATING RAT. M. Dubois-Dauphin,* W. E. Armstrong, E. Tribollet,* and J. J. Dreifuss. Departement de Physiologie, Centre Medical Universitaire, 1, rue Michel-Servet, 1211 Geneve (Switzerland).

In the lactating rat, suckling induces an intermittent release of oxytocin from the neurohypophysial terminals of neurons located in the paraventricular and supraoptic nuclei of the hypothalamus, causing intermittent ejection of milk. We have studied the afferent pathway of this reflex. Under pentobarbital and xylasine anesthesia, bilateral electrolytic lesions and/or unilateral injections of 10% true blue or 40% horseradish peroxidase (HRP) solutions were made separately or in tandem, the injections occasionally following the lesions in the same rats using a modified syringe. The main galactophore of one or two mammary glands was cannulated for the continuous measurement of intramammary pressure and suckling tests were performed with ten pups attached to the nipples. Bilateral lesions placed in the lateral tegmentum near the inferior colliculus nucleus reduced the frequency of milk ejections. Lesions of the posterior lateral tegmentum encroaching on the intercollicular nucleus abolished the milk ejections altogether. Retrogradely labelled cells were notably found in relatively large numbers within the contralateral cuneatus and gracilis nuclei as well as in the contralateral lateral cervical nucleus of the spinal cord. Consequently we have lesioned those structures. Bilateral lesioning of the lateral cervical nucleus (level C2) abolished the milk ejection reflex. Unilateral injections of HRP in the lateral cervical nucleus produced contralateral, anterograde labelling of axon terminals in the intercollicular nucleus. Ascending fibers of this system travel in the posterior lateral tegmentum. A similar terminal labelling was observed after large unilateral injections of HRP in the cuneate and gracile nuclei, but bilateral lesions of the cuneate and gracile fasciculi were completely ineffective in interfering with milk ejection. These results suggest that the intercollicular projection of the lateral cervical nucleus is part of the afferent pathway involved in the reflex milk ejection. (Supported by Swiss NSF, Grant 3.875-081).

136.12 FURTHER CORRELATION OF OXYTOCIN AND PROLACTIN RELEASES: LACTATION. W.K. SAMSON, M.D. LUMPKIN AND S.M. MC CANN (SPON: J.G. Parnavelas) Dept. of Physiology, UTHSCD, Dallas, TX 75235.

We have described previously the ability of synthetic oxytocin (OXY) to stimulate significantly and in a dose-related fashion prolactin (PRL) release from cultured, dispersed rat pituitary cells in vitro (Lumpkin et al., Endocrinology 112: 1711, 1983). Additionally, we have demonstrated a temporal correlation between OXY release from neuronal elements and PRL secretion induced by steroid treatment of ovariectomized rats (Samson et al., Soc Neurosci 18, 22, 1982). These data, coupled with the presence of OXY immunoreactivity in the external layer of the rat median eminence, suggest a physiological role for OXY in the hypothalamic control of PRL secretion. Therefore we attempted in this study to correlate OXY and PRL release during a condition associated with release of both, i.e., lactation. Chronic right atrial cannulae were placed in the external jugular vein of lactating Sprague Dawley rats (6-8 pups each). On the next day (day 15 or 16 of lactation) pups were removed and rats left undisturbed for 4 hr, after which time a zero time blood sample (0.8 ml) was withdrawn via an extension cannula attached to the indwelling atrial cannula. Equal volumes of 0.9% NaCl were infused to replace volume loss. Pups were then replaced and additional blood samples taken at 5, 10, 15, 20 and 30 min. Plasma was analyzed for PRL content by RIA. PRL levels began to rise above prepup renin-statement levels (0 time, 20.6 $\pm$ 3.7 ngm/nl, n=8) after 5 (41.3 $\pm$ 13.0) and 10 min (110.2 $\pm$ 40.0) and attained significance at 15 (116.7 $\pm$ 40.0, $p<.05$), 20 (171.9 $\pm$ 53.7, $p<.01$) and 30 min (236.0 $\pm$ 43.1, $p<.001$). In another group of rats pups were similarly removed for 4 hr prior to initiating experimentation. Mothers (n=10 per time point) were sacrificed by decapitation at 0 time and 5, 10, 15, 20 and 30 min after pup reinstatement. Trunk bloods were collected and plasma (1.5 ml) extracted with methanol for subsequent OXY RIA. Details of the extraction procedure and RIA have been presented previously (Soc Neurosci 18.22, 1982). Prior to pup reinstatement plasma OXY levels were 198.7 $\pm$ 21.6 pg/ml. Plasma OXY levels then rose significantly at 5 (349.1 $\pm$ 60.0, $p<.05$) and 10 min (444.5 $\pm$ 63.7, $p<.002$). Peak values were observed at 15 min (474.5 $\pm$ 64.0, $p<.001$) after which plasma OXY levels declined at 20 min (376.1 $\pm$ 68.1, $p<.05$) and approximated prereinstatement values at 30 min (247.0 $\pm$ 48.6). This temporal correlation between OXY release and PRL secretion during suckling further suggests a role for OXY in the hypothalamic control of prolactin secretion. Supported by NIH Grants HD-09988 and AM-10073.

CHEMICAL SENSES: OLFACTION AND TASTE I

137.1 FAST AND LOOSE COVALENT BINDING OF CARBONYL-CONTAINING MOLECULES TO PROTEINS. A ROLE IN VERTEBRATE OLFACTION? Thomas Hellman Morton, Department of Chemistry, University of California, Riverside, CA 92521 and J. Russell Mason*, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104.

Olfactory receptors undoubtedly have fast rates of association with odorant molecules. If the dissociation rate is also fast, then the dissociation constant, K_d, will be comparatively large (micromolar or greater). With such fast and loose binding, a receptor can recover function rapidly. If a loosely bound molecule is intercepted at the binding site in such a way that it becomes irreversibly attached, then the receptor will be blocked. The fraction of receptors modified in this fashion can be related to the initial concentration of substrate and the residence time, t, of substrate molecules in the receptor region. We have derived the pertinent kinetic analysis for the case where an initial pulse of substrate (concentration S_o) is delivered to the receptor, and where irreversible modification of the binding site competes with removal of substrate. If the rate of irreversible modification depends on the concentration, X, of a third agent, let the pseudo-first order rate constant be represented as kX. If reversible association and dissociation are fast on the timescale of t and 1/kX, then the extent of modification can be shown to be equal to

$$1 - \left(\frac{K_d}{K_d + S_o}\right) \underline{t}kX$$

This formula has been tested using, as a model, the Schiff-base forming enzyme AAD. In this case, the substrate was a ketone (cyclohexanone or ethyl acetoacetate). Irreversible modification by treatment of protein-substrate mixtures with borohydride was monitored by incorporation of radiolabel and also by loss of catalytic efficiency. The results are consistent with the derived formula, where attack of unbound substrate by borohydride constitutes the mechanism of substrate removal.

Tiger salamanders (Ambystoma tigrinum) have been trained,by use of avoidance conditioning, to discriminate among reagent grade chemicals as odorant stimuli. Their olfactory epithelia are easily accesible to irrigation with chemical agents designed to derivatize receptor proteins. We have previously reported that lavage with solutions of cyclohexanone or ethyl acetoacetate (10-50 mM in normal saline) induces a selective anosmia to carbonyl-containing odorants (Mason and Morton, Physiol. Behav. 29: 709, 1982). We present here a discussion of results of this behavioral assay in terms of the above chemical model.

137.2 MEMBRANE GLYCOPROTEINS SPECIFIC TO OLFACTORY CILIA. Doron Lancet, Amnon Shapira* and Zehava Chen*. Dept. of Membrane Research, The Weizmann Inst. of Science, Rehovot, Israel.

There is growing evidence that olfactory receptor proteins, which bind odorants and mediate membrane transduction, are localized in the cilia of the sensory neurones of vertebrate olfactory epithelium. Despite considerable effort, these proteins have not yet been isolated, and their molecular characteristics remain unknown. Ligand binding techniques, that served in the purification of many other receptor molecules, have proven less useful in the olfactory system. This is because olfactory receptors (at least in air-breathing vertebrates) may be similar to immunoglobulins, being a large group of molecules with different, partially overlapping ligand specificity spectra. Any given odorant will have measurable affinity only to a small fraction of this diverse repertoire of proteins. Thus, the difficulties encountered in the use of an odorant to isolate olfactory receptor molecules may be compared to those that would arise in an attempt to purify immunoglobulins from non-immune serum using an antigen. To circumvent this problem we adopted an alternative approach, namely the mapping of membrane proteins of olfactory cilia (compared to non-sensory cilia), in search for plausible candidates for olfactory receptors. The validity of this approach is based on the documented simple protein profiles of ciliary membranes, and on the indications that olfactory receptor proteins are present at relatively high concentration in the sensory membrane. We utilized sodium dodecyl sulphate polyacrylamide gel electrophoresis, separating polypeptides according to their molecular weights. Our working hypothesis is that olfactory receptors of different odorant specificities may thus manifest their common molecular properties in the appearance of one or more relatively distinct protein bands, analogous to the light and heavy chain of heterogenous immunoglobulins. Membrane proteins of isolated frog olfactory and respiratory cilia, solubilized by non-ionic detergents, were visualized on the gels by radioiodination, silver staining and concanavalin-A binding. Two major protein polypeptides (of 60K and 90K dalton) and one minor band (of 125K dalton), all glycosylated, were found to be specific to olfactory cilia. Respiratory cilia had no membrane glycoproteins. The reported inhibition of odorant-induced olfactory epithelial potentials by the lectin concanavalin-A (E.Polak, S.Shirley and G.Dodd, Fifth European Chemoreception Research Organization Symposium p.59,1982) is consistent with the hypothesis that at least one of these sensory-specific glycoproteins plays a role in olfactory reception and/or transduction. Monoclonal antibodies, now being raised against the ciliary glycoproteins, will be used to study their physiological role, structure, diversity and tissue distribution.

137.3 ELECTROPHYSIOLOGICAL RESPONSES TO PHEROMONE BLENDS IN SINGLE INSECT OLFACTORY RECEPTOR NEURONS. R.J. O'Connell. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

In an ever increasing number of insect species precise mixtures of chemical compounds (pheromones) have been found which modulate important species specific behaviors such as long distance attraction to potential reproductive partners. The female cabbage looper moth (Trichoplusia ni) synthesizes a two component sex attractant pheromone. The primary component, Z-7,dodecenyl acetate (Z-7, 12:AC), seems to be largely responsible for the initial stages of male activation and upwind flight whereas the minor component dodecyl acetate (12:AC) significantly increases the number and duration of upwind flights and increases the number of attempted copulations with the pheromone dispenser. A third compound, not yet identified in females, Z-7,dodecenol (Z-7,12:OH) inhibits the behavioural response elicited in males by the pheromone blend. The particular behavioural sequence modulated by Z-7,12:OH in order to produce this result has not been identified.

The sensory mechanisms responsible for blend perception are not known. Previous electrophysiological investigations (O'Connell, R.J., et al., Science, 1983, In Press) utilizing purified single compounds, demonstrated two high sensitivity (effective dose, .001 µg) olfactory receptor neurons, one specialized for Z-7,12:AC and a second specialized for Z-7,12:OH. In spite of the obvious behavioural import of small amounts of 12:AC neither of these receptor neurons responded to this substance at intensities normally experienced by the male. However, if stimulus intensity was increased 10,000 fold, responses to 12:AC could be obtained in both types of receptor neurons. This disparity between the behavioural responses to the multicomponent pheromone and the electrical activity produced in olfactory receptor neurons by individual components of the blend suggests that binary mixtures of compounds would be more appropriate stimuli for these neurons. Therefore, I compared the responses to individual compounds with the responses to their binary mixtures for the three behaviourally relevant odorants in the hopes of clarifying the electrophysiological mechanisms which underlie blend perception.

The results of experiments still in progress suggest that mixtures of pheromone elicit responses from individual olfactory receptor neurons that cannot be predicted from a knowledge of their responses to the individual compounds alone. Moreover, elevated doses of Z-7,12:AC such as those which might occur near a calling female (10 µg), cause pronounced long lasting changes in the response properties of the receptor neurons to both single compounds and to their blends.

Supported in part by NSF grant BNS-8016395 and NIH grant NS 14453.

137.4 ISOPROTERENOL-ASSOCIATED GRANULE DEPLETION IN OLFACTORY GLANDS OF THE ADULT SALAMANDER. Thomas V. Getchell and Marilyn L. Getchell.* Department of Anatomy, Wayne State University School of Medicine, Detroit, MI 48201.

The sustentacular cells (SC) in the olfactory epithelium and the multicellular olfactory glands (OG) in the lamina propria are the two major secretory elements in the olfactory mucosa (OM). In the ventral OM, three layers of glands have been described (Getchell and Getchell, AChemS V, 1983 abs.). We employed morphometric analyses to characterize acinar cells of the superficial (sBG) and deep (dG) glands in control and drug-treated animals. Measurements of cell and secretory granule areas and heights were taken from cells which extended from the glandular margin to the lumen and contained a nucleus and secretory granules. A statistically significant difference ($p=0.01$) was found between cell areas of sBG and dG. The mean area of acinar cells in sBG was 291±107 μm^2 ($\bar{x}$ ± S.D.) whereas that of acinar cells in dG was 458 ± 163 μm^2. No significant difference was found in granule areas between cells of sBG and dG. The proportion of the area occupied by the secretory granules was significantly ($p=0.01$) greater in sBG (41±13%) than in dG (26±14%). Cell height was significantly greater ($p=0.05$) in acinar cells of dG (28±6 µm) than in acinar cells of sBG (24 ±6 µm). No significant difference was found in granule heights. Secretory granules occupy a significantly ($p=0.01$) greater proportion of the cell height in sBG (62±17%) than in dG (47±16%). We have examined the effects of the β -adrenergic agonist isoproterenol (IPR) on secretory granule content of the acinar cells. Injection of 30 mg/kg i.p. was associated with a significant ($p=0.01$) reduction of the areas and heights occupied by secretory granules in cells of sBG and dG. In sBG acinar cells, granule area was reduced from 121±59 μm^2 to 42±28 μm^2, a 65% reduction, whereas in cells of the dG, granule area was reduced from 114±82 μm^2 to 22±14 μm^2, an 81% reduction. Granule height in sBG acinar cells was reduced from 15±5 µm to 9±4 µm, a 40% reduction, and in dG acinar cells from 13±5 µm to 6±3 µm, a 54% reduction. The magnitude of the effects of IPR on granule areas and heights in sBG and dG acinar cells are not statistically different; i.e., acinar cells of sBG and dG are affected to the same degree by IPR. The area of the acinar cells of dG is significantly ($p=0.01$) reduced by 28% after IPR exposure but that of sBG is unaffected. Ratios of granule to cell areas and heights were significantly ($p=0.01$) different after IPR injection. Cell heights were unaffected by IPR. These results suggest that granule depletion is either directly or indirectly under β - adrenergic control, suggesting a role for the sympathetic nervous system in the secretory process. NIH-NS-16340.

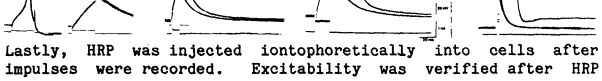
137.5 MUSCARINIC RECEPTOR-OLFACTORY RECEPTOR INTERACTIONS IN THE SALAMANDER OLFACTORY EPITHELIUM. Gordon M. Shepherd and Britta Hedlund (SPON: W. Cain). Section of Neuroanatomy, Yale Sch. of Medicine, New Haven CT 06510

Very little is known about the primary molecular mechanisms underlying sensory transduction. The presence of muscarinic receptors on olfactory receptor cells in mouse olfactory epithelium was suggested by Hirsch and Margolis (1981). We have therefore investigated the possible role of muscarinic receptors in primary olfactory mechanisms as a part of a study correlating biochemistry and physiology of the salamander olfactory receptor cells. Muscarinic cholinergic receptors are present in the olfactory epithelium of the salamander, Ambystoma tigrinum, to an amount of 0.08 pmoles/mg protein, measured via binding of the muscarinic antagonist ^{3}H-3-quinuclidinyl benzilate (^{3}H-3-QNB). Separation of the cilia produced a three- to fivefold enrichment in muscarinic receptors, to 0.35 pmoles ^{3}H-3-QNB specifically bound/mg protein. Seven days after olfactory nerve transection, muscarinic receptors measured via binding of ^{3}H-3-QNB are no longer present in the olfactory epithelium. The results indicate that they are located on the olfactory receptor cell which are known to undergo degeneration after nerve transection. Further investigations have been initiated to analyze the possible functional role of the muscarinic acetylcholine receptors. These have included competition studies and gel electrophoresis. Different odorant molecules were tested for their interaction with ^{3}H-3-QNB binding. It was found that odorant molecules, in contrast to other neurotransmitter molecules, inhibit ^{3}H-3-QNB binding at pharmacologically relevant concentrations (10^{-5} - 10^{-3} M). The results suggest that the muscarinic receptor is closely related to the receptor that binds odor molecules. This suggests the possibility that acetylcholine may act to modulate the sensory transduction of odor molecules, or to affect other aspects of receptor cell function such as cell differentiation and ciliary motility.

137.6 NA AND CA IMPULSES IN TASTE CELLS. S. Roper. Departments of Anatomy and Physiology, University of Colorado Health Sciences Center, Denver, CO 80262.

Cells in the taste buds of Necturus maculosus (mudpuppy) have recently been shown to generate action potentials in response to brief depolarizing current pulses passed through an intracellular microelectrode (Roper, 1983, Science). If chemical stimulation of taste cells produces receptor potentials which reach threshold and generate action potentials having a Ca component, the influx of Ca could trigger transmitter release at synapses between the taste cell and gustatory nerve terminals. Experiments have been conducted to characterize the ionic currents underlying action potentials in mudpuppy taste cells and to identify the cells which generate impulses.

Lingual epithelium from the anterior region of the tongue was removed with blunt dissection and mounted in a shallow chamber containing Ringer solution (op. cit.). Taste cells were identified with the aid of Nomarski optics and were impaled with KCl-filled microelectrodes having resistances of 50-150 megohms. In some experiments, microelectrodes were filled with 4% horseradish peroxidase (HRP) in 0.625 M KCl and 0.05 M Na cacodylate buffer (pH 7). The following experiments indicated that action potentials had a definite sodium component: (1) action potentials could be reversibly blocked by 1 µM tetrodotoxin (TTX), (2) action potentials could be recorded in the absence of Ca, even if 5-10 mM Mn was present. Further, a substantial Ca component to action potentials in taste cells was demonstrated by the following: (3) impulses could be restored in the presence of 1 uM TTX if 5 mM tetraethylammonium was added, (4) impulses could be recorded in isotonic $CaCl_2$.



Lastly, HRP was injected iontophoretically into cells after impulses were recorded. Excitability was verified after HRP injection to confirm that the microelectrode remained in the cell. To date, HRP has been localized in Dark (Type I) taste cells. Characteristics of Light (Type II) taste cells have yet to be determined.

These data indicate that at least Type I taste cells possess action potentials and that these impulses have both Na and Ca components. It is likely that the Ca component plays a pivotal role in chemosensory transduction and synaptic transmission between taste cells and gustatory axons. Supported in part by grants from the Procter & Gamble Company and NIH.

137.7 ELECTROPHYSIOLOGICAL CHANGES IN CELLS OF THE OLFACTORY EPITHELIUM AFTER NERVE TRANSECTION. Britta Hedlund, Leona Masukawa and Gordon Shepherd, Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

The aim of these studies was to determine the normal electrophysiological properties of the cells in the olfactory epithelium and to determine what changes occur in these characteristics after nerve transection.

A unique feature of this sytem is that the receptor cells turn over continuously and therefore represents a good model to study the physiology of regeneration. We used nerve transection to intensify events occurring during regrowth of receptor cells.

Intracellular recordings from the olfactory epithelium of the tiger salamander, Ambystoma tigrinum, were taken from different cell types of the olfactory epithelium using high resistance microelectrodes. Records were from in vitro preparations of control tissue and tissue 1,2, and 4 weeks after olfactory nerve transection.

Three cell types were distinguished electrophysiologically in normal tissue. The first type is the supporting cell which gave no active response to direct current depolarization, but had a high resting membrane potential, about -90 mV, and a low input resistance, 10 Mohms. Another type of cell, the olfactory receptor cell, generated repetitive spike activity to direct current stimulus. Its resting potential was lower than the supporting cells but the resistance was often very high, 150-600 Mohms.

A third type of cell had the electrical properties of receptor cells but did not spike. These are tentatively called immature receptor cells.

In another group of animals the olfactory nerve was transected. We found two changes in the electrical properties of these cells after transection: One, the receptor cells at one and two weeks after transection show an inability to generate repetitive action potentials, and two, an altered form of the supporting cell, which we call type B, has abnormally high input resistance. Our findings indicate that the receptor cells capacity to spike may be correlated to the loss or regeneration of their axon. We tentatively conclude that changes in the electrical properties of the cells in the OE are occurring during cell degeneration and regeneration.

137.8 CULTURE AND IMMUNOCYTOCHEMICAL CHARACTERIZATION OF THE RAT OLFACTORY EPITHELIUM. J.Morgan* and J.Hempstead*.(SPON:A.Blume). Dept.of Physiol. Chem. and Pharmacol., Roche Institute of Molecular Biology, Nutley, N.J. 07110.

The olfactory epithelium contains a population of neuroblasts which have retained the ability to mature into terminally differentiated receptor neurons. Thus this tissue represents a unique model with which to investigate the control of neuronal development in adult mammals. Our major goal has been to develop culture conditions for the olfactory neuroblasts and there-with to define those parameters which are responsible for the regulation of their maturation. This report deals with both the characterization of olfactory epithelium cultures and the production of probes for the identification and purification of cell types from the olfactory neuroepithelium. In terms of cell identification we shall discuss the utility of two monoclonal antibodies,SUS-1 and LUM-1, which react specifically with sustentacular cells and the brush border of the epithelium respectively, as cell-specific markers. These antibodies show that while olfactory neurons degenerate following olfactory bulbectomy the sustentacular cell population is left intact. In addition to monoclonal antibodies specific plant lectins may be used to identify other cell populations both within the epithelium and in dissociated preparations of the tissue. Lectins thus present potential purification probes.

Both dissociated and explant cultures of the rat olfactory epithelium have been produced. These cultures have been examined immunocytochemically with a panel of specific antibodies for various cell types and in addition with a radioimmunoassay for the olfactory marker protein which is only present in the mature olfactory receptor neuron. Using density gradient centrifugation following mild enzymatic digestion of the rat epithelium several subpopulations of cells have been obtained and grown in culture. In conjunction with the administration of tritiated thymidine in vivo it has been possible to determine which of the various cell fractions had been actively synthesizing DNA at the time of their isolation. The ability to proliferate would be one of the predicted properties of the neuroblast.

The most common cell in culture is flat with perinuclear granulation. These cells are capable of division and migration and are also observed in cultures of cerebellum. There is a slower developing population of cells identified as fibrous astrocytes by morphology and immunocytochemical presence of glial fibrillar acidic protein (GFAP). A third class of cells are round and poorly adherent. They proliferate in early stages of culture and may be cloned by limiting dilution. After longer periods in vitro the cells become morphologically stellate astrocytes although they are at best weakly positive for GFAP.

137.9 A HORSERADISH PEROXIDASE STUDY OF OLFACTORY BULB CONNECTIONS IN 12-DAY-OLD RAT PUPS. P.E. Pedersen, P.J. Jastreboff,* and G.M. Shepherd. Sect. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The modified glomerular complex (MGC) is a group of distinct glomeruli situated at the dorsomedial junction of the main and accessory olfactory bulbs in the rat. It exhibits ^{14}C-2-deoxglucose uptake when rat pups 0-21 days of age suckle their dams, and has therefore been implicated in processing the odor cue(s) that mediates suckling (Greer et al., 1982). This functional evidence, in conjunction with preliminary histological evidence that the MGC matures earlier than the glomeruli of the main bulb, has led us to attempt to identify the peripheral and central origins of projections to this region in young pups.

A series of experiments using horseradish peroxidase (HRP) tract-tracing methods in 12-day-old rat pups has been conducted. Pressure injections of .01-.02 uL of 10% HRP (Type VI) were made via a glass micropipette inserted by a micromanipulator into the dorsomedial portion of the caudal aspect of the main olfactory bulb. Twenty-four to forty-eight hours later pups were perfused transcardially with aldehyde fixative. Tissue sections (40um) were analyzed by light microscopy after treatment with tetramethyl benzidine (TMB). Injection sites comprised almost the entire main olfactory bulb as well as the accessory olfactory bulb. Centrally, retrograde labeling occurred in the anterior olfactory nucleus, horizontal limb of the diagonal band, dorsal aspect of the piriform cortex, and corticomedial nucleus of the amygdala. These are well known sources of centrifugal fibers in adult rats. For localized injections, extracellular ionophoretic injections of wheat germ agglutinin HRP (.25%) were made 300u deep into the MGC by stereotaxic placement of a glass micropipette. The nasal cavities were subsequently decalcified for 2 days in .1M EDTA, sectioned, and reacted with TMB. The results have provided the first tentative evidence of retrograde labeling in olfactory epithelium following HRP injections in the olfactory bulb. We are currently analyzing the extent to which label in the epithelium represents input solely to the MGC region. Technical modifications for the use of HRP in neonates will be discussed.

Research is supported by NIH Grants #NS16993 and #NS06978.

137.10 TASTE BUD DEVELOPMENT IN THE HAMSTER. I. J. Miller, Jr. Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

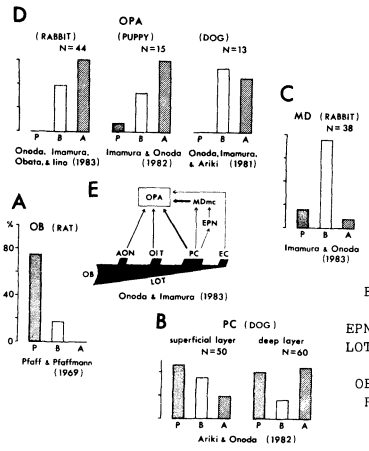
Recent experiments show that taste responses from neonatal rodents differ qualitatively from those of adult animals. The objective of this study was to examine the number of taste buds in specific regions of the oral cavity in the hamster from birth to maturity. The vallate and foliate papillae are the easiest to study because of their high density of taste buds, but other regions are examined. Animals born in the laboratory were sacrificed from the first postnatal day to 150 days of age. Tissue was excised from the region of the vallate papilla, bilateral foliate papillae, soft palate, epiglottis and from the anterior portion of the tongue containing fungiform papillae to be prepared for light microscopy. Complete serial sections were studied for each region, and taste buds were counted by the presence of a taste pore. Preliminary results show no taste buds present on the vallate papilla at birth, and the troughs of the vallate papilla are unopened. Vallate papillae of hamsters aged 10 - 26 days contained from 60 to 106 (N=5) total taste buds with a mean of 78 $\pm$ 4 (SEM). Adult animals 120 - 150 days of age contained a mean total of 168 $\pm$ 7 (N=4) taste buds in the vallate papilla. The foliate papillae consist of 5 or 6 troughs on each lateral side of the tongue. Hamsters 19 to 26 days old displayed a mean total of 71 $\pm$ 4 taste buds in foliate papillae per side. Foliate taste buds in adults aged 120-150 days totaled 115 + 2 (N=6) per side. On soft palates of 4 young hamsters aged 19 - 26 days were found a mean total of 63 $\pm$ 2 taste buds, while two adult animals had a mean of 88 taste buds on the soft palate. The epiglottis contained a mean total of 68 taste buds for 3 animals aged 22 - 26 days, and those of 2 animals aged 120-150 days averaged 74 taste buds. Fungiform papillae contained a mean total of 62 taste buds per side in one adult, while two animals 19-26 days old had an average of 67 taste buds per side. A comparison between taste bud development on the regions innervated by the facial nerve and those innervated by the glossopharyngeal nerve will show whether the regions and nerves develop at similar or different rates. Experiments are in progress with litter mates sacrificed at staged intervals to see if the increase in taste bud totals by region is a function of age for closely related animals.

137.11 GUSTATORY RECEPTOR SUBPOPULATION STIMULATION: RESPONSES OF NEURONS IN THE NUCLEUS OF THE SOLITARY TRACT OF THE RAT. S. Travers, C. Pfaffmann, and R. Norgren. The Rockefeller University, New York, N.Y. 10021.

Although there are at least six gustatory receptor subpopulations, virtually all central taste neurophysiology is based upon sapid stimulation of only one of these receptor groups, the anterior tongue. We have developed a preparation and modified stereotaxic apparatus that permits visualization and independent stimulation of five of the six gustatory receptor subpopulations: the anterior tongue (AT), nasoincisor ducts (NID), soft palate (SP), foliate papillae (FOL), and circumvallate papilla (CV). (Taste buds on the epiglottis are not visible in this preparation.) In our initial experiments, we have assessed the degree of receptor group convergence on and chemical sensitivity of single neurons in the nucleus of the solitary tract (NST) of the rat. Following isolation of a single cell, the AT, NID, FOL and SP were stimulated individually with a mixture of the four basic taste stimuli (1.0M sucrose, 0.3M NaCl, 0.03M HCl and 0.01M QHCl). After determining the gustatory receptive field for the neuron, the receptor groups eliciting responses were tested further with each of the four basic stimuli. Responsiveness to CV stimulation was often tested last since the apparatus used to stimulate those receptors interfered with access to the other parts of the oral cavity. Electrical taste stimulation through small porous-glass tipped probes was sometimes employed to verify the location of the taste receptive field for a neuron. Forty single neurons have been tested for their responsiveness to AT, NID, SP and FOL stimulation. Nineteen of these cells were also tested for their responsiveness to the four basic taste stimuli and 12 were tested for CV responsiveness. With the exception of cells responding to stimulation of the NID, most neurons (n=25) responded to stimulation of a single taste receptor subpopulation, the AT, SP or FOL. Cells that responded to the NID (n=13) also responded to chemicals applied to the AT, but often with a different chemical sensitivity. Preliminary analysis of the data indicates that sucrose is a more effective stimulus for the NID than for the AT. Multi-unit and single-unit responses to stimulation of the AT and NID were recorded anterior to responses to stimulation of the FOL and CV in the NST. Responses to stimulation of the SP were intermediate to the other two groups of responses. Recent anatomical experiments (Hamilton and Norgren, 1983) demonstrate that the chorda tympani and greater superficial petrosal nerves, which innervate the AT and palate, respectively, distribute in the NST anterior to the distribution of the branch of the glossopharyngeal nerve that innervates the CV and FOL. Supported by NIH MH15125, NS07021, NS10150 and NSF BNS8111816.

137.12 SELECTIVE RESPONSES TO ANIMAL-PRODUCT ODORS IN THE NEOCORTICAL NEURONS. N. Onoda* and K. Imamura* (SPON: K. Maekawa). Dept. of Physiol., Sch. of Med., Gunma Univ., Maebashi, Gunma 371, Japan.

Odor responses of neocortical neurons were studied in a restricted area in the lateral half of the prefrontal cortex close to the frontal pole of the hemisphere in rabbits. Odor-sensitive neocortical neurons responded predominantly to biologically derived odors (urine, feces, and dry food for rabbit). Most (90%) of those same neurons failed to respond to pure chemical odors. Even in the absence of the trigeminal and/or vomeronasal inputs, some neurons were observed to respond to animal-product odors. Some odor-sensitive neurons were affected by electrical stimulation of the mediodorsal thalamic nucleus (MD). When HRP was injected into the neocortical olfactory projection area (OPA), HRP-labelled cell bodies were observed in various regions (Fig.E); the medial segment of the MD, the pyriform cortex (PC), the entorhinal cortex (EC), the olfactory tubercle (OlT), and the anterior olfactory nucleus (AON). The OPA was not overlapped by the sensory-motor cortex. When these results are compared with those obtained from other structures (Fig. A-D), the percentage of neurons responded exclusively to animal-product odors (indicated by A in abscissa of each histogram) becomes more prominent in the higher structures. We can safely conclude that selective responses to animal-product odors are to be segregated through the olfactory pathway from the lower to the higher structures.



B: both animal-product & pure chemical odors
EPN: endopyriform nucleus
LOT: lateral olfactory tract
OB: olfactory bulb
P: pure chemical odors

FEEDING AND DRINKING: NEUROPHARMACOLOGY

138.1 NALOXONE ANTAGONISM OF SUCKLING IN GENETICALLY OBESE AND LEAN MOUSE PUPS. H. L. Sinha* and L. M. Wilson, Dept. of Psychology, Univ. of Manitoba, Winnipeg, MB R3T 2N2.

The findings that pituitaries of adult genetically obese mice (C57Bl/6J, ob/ob) contain higher levels of β-endorphin than those of lean mice and hyperphagia in adult ob/ob's can be decreased differentially by naloxone compared to the food intake of lean mice (Margules, D.L., et al., Science, 202:988, 1978) suggests that endorphins play a role in overeating in this strain. Opiate systems are present before birth in rodents, and, if involved in feeding regulation, their contribution to the ob/ob's development of hyperphagia may be reflected in differential suckling behavior in preobese and lean pups. Although hyperphagia in ob/obs does not appear to develop until after weaning (Rath, E.A. & Thenen, S.W., J. Nutr., 109:840, 1979)with increased availability of food, whether hyperphagia antedates weaning when early constraints on feeding are altered remains controversial (Wilson, L.M., et al., Develop. Psychobiol., 14:67, 1981).

In this study the effects of naloxone(donated by Endo Labs: 0 (.15M saline), 0.3, or 1.0 mg/Kg body weight) on suckling behavior were investigated in preobese and lean mouse pups at 6, 15, and 24 days of age. Intact litters (n=36) were tested in either a fed (left with dam until testing) or fasted (20 h with a nonlactating virgin female) condition at each age. Litters of fed and fasted pups were randomly assigned to 1 of 3 injection groups, to which the experimenter was blind for the duration of the study. Before testing, litters were removed from their dams; pups were reflexively micturated and defecated, and weighed. Each pup was given its appropriate body weight dose of saline or naloxone SC. Pups were tested 15 mins. postinjection in pairs or triads on their own anesthetized dams (50 mg/Kg Nembutal IP). Latency to nipple-attach and duration of nipple attachments (secs.) were recorded during 5-min. tests. Pups' phenotypes were identified visually at 35 days.

Obese pups nipple-attached sooner ($p<.03$)than lean pups. Overall obese pups suckled longer than lean pups ($p<.01$), although obese pups suckled longer only at 15 and 24 days. Fasted pups attached sooner and suckled longer than fed pups ($p<.0001$). Although increasing naloxone doses tended to lengthen initial attachment latencies ($p=.08$) and to decrease total suckling time ($p=.057$), it systematically depressed suckling duration and increased attachment times only in the fed condtions (p's<.01). A significant Drug Dose X Age interaction ($p<.0001$) for both attachment latency and suckling duration emphasized that naloxone depressed suckling behavior at 6 days (at both doses) and at 15 days (at 1.0 mg/Kg), but didn't systematically affect pups at 24 days, when suckling no longer constitutes the chief means of ingestion. Age X Drug Dose X Phenotype effects show differences for lean and preobese pups.

138.2 THE EFFECTS OF MORPHINE SULFATE ON THE FREQUENCY, SIZE AND DURATION OF FEEDING AND DRINKING BOUTS IN THE RAT. A. Riley, D. West, A. Sipel*, and S. Woods. Department of Psychology, University of Washington, Seattle, Washington 98195.

Although rats injected with morphine sulfate typically show an increase in feeding and drinking (Morley et al., Neurosci. Biobeh. Rev. in press; Sanger, Appetite, 2, 193-208, 1981), it is unclear how this increase is effected. For example, it is not known whether the opiates increase the frequency, size or duration of feeding and drinking bouts.

To address these questions, in the present experiment food and water consumption of six free-feeding rats was monitored via a microcumputer every 5 sec for 24 hours following the administration of 40 mg/kg morphine sulfate (West et al., Physiol. Behav., in press). Morphine injections were given at 1230 h each day for 14 consecutive days.

Following the first morphine injection, the frequency, size and duration of feeding and drinking bouts were significantly reduced for the entire 24-hour observation period. With repeated daily exposures to morphine, the patterns of food and water consumption changed dramatically. For example, by Day 14 all rats significantly increased the size and duration of feeding bouts in the six hours following morphine injection. The frequency of feeding bouts, however, was not affected in this interval, although over the remainder of the 24-hour observation period, the frequency of these bouts was reduced to approximately 50% of the preinjection baseline. By Day 14, all rats significantly increased the frequency of drinking bouts in the six hours following drug exposure, although the size and duration of these bouts were unaffected. Over the remainder of the 24-hour observation period, all rats decreased the frequency of drinking bouts to approximately 20% of their preinjection baseline. Although these patterns of feeding and drinking resulted in significant increases in food and water intake for the first six hours following morphine administration, the 24-hour food and water intake either remained below or was similar to baseline levels.

These data extend the earlier work demonstrating that opiates increase food and water intake and further indicate that the manner in which this increase occurs is different for food and water, i.e., morphine increases bout size and duration for feeding and bout frequency for drinking.

138.3 CHOLECYSTOKININ (CCK) RECEPTOR BINDING IN SHEEP BRAIN.
M. A. Della-Fera, R. N. Solomons* and C. A. Baile.
Univ. Pennsylvania Sch. Vet. Med., Kennett Square, PA 19348.

CCK peptides in brain have been implicated in several CNS-controlled functions, eg., control of feed intake, gastrointestinal function, metabolism (through control of secretion of such hormones as insulin and adrenal cortical steroids), thermoregulation and nociception. CCK is localized in discrete areas of the brain, particularly in periventricular areas and cortex. Specific high affinity binding sites for CCK appear to follow the same pattern of localization as CCK-containing neurons. Because of the evidence supporting a physiological role for brain CCK in satiety in sheep, it was of interest to determine 1) the pattern of localization of CCK peptides and binding sites in sheep brain, and 2) whether changes in either CCK concentration or binding affinity occur in particular brain areas during feeding and fasting. In the first experiment 10 sheep were killed within two hrs of a meal, and their brains were removed and dissected immediately. Tissue was removed bilaterally from 18 brain sites, including specific cortical, hypothalamic, and brain stem areas. CCK peptides were extracted from tissues of one side of the brain, while tissues from the other side were used for CCK binding assays. In the second study groups of 12 sheep each were killed either immediately after a meal or after a 4- or 24- hr fast. Brain tissues were treated as above. Results from receptor binding studies showed regional differences in distribution of CCK receptor numbers. Frontal cortex, olfactory bulb and ventromedial hypothalamus (hth) showed greatest binding, while anterior preoptic area had moderate levels of binding, and lateral and posterior hth had very low levels of binding. There were no differences for any area in CCK receptor number or affinity between fed and fasted states. CCK content in specific brain areas and the effect of feeding and fasting on CCK content is presently being determined. On the basis of the heterogeneity in the regional distribution of CCK receptors, these results support a role for CCK as a neurotransmitter or neurohormone in sheep brain. The data do not indicate, however, that alteration in CCK receptor binding occurs in response to feeding and fasting. Supported by NIH NS17670 and Sloan Fnd. Fellowship BR2190.

138.4 AMYGDALIN PREVENTS ALLOXAN-INDUCED LOSS OF GLUCOPRIVIC FEEDING IN RATS. J.M. Murnane and S. Ritter, College of Veterinary Medicine, Washington State University, Pullman, WA 99160-6520.

Alloxan is a toxic substance which destroys pancreatic beta (B) cells. It is now known that intracerebroventricular alloxan also damages brain cells involved in glucoprivic feeding in rats. Although the mechanism of alloxan's cytotoxicity has not been established either for brain cells or B cells, a variety of substances have been identified which protect B cells against alloxan-induced damage. Previously we reported that D-glucose, which blocks the effects of alloxan on the B cell, also prevents impairment of glucoprivic feeding by alloxan. In this experiment, we tested 3 additional substances for their ability to protect the glucoprivic feeding response from alloxan-induced damage. Evaluation of the protective effects of these substances on this centrally-mediated response should indicate whether the mechanism of alloxan's action on brain glucoreceptor cells is similar to its action on B-cells. L-glutamine (15 mM) and amygdalin (15 mM), which protect the B cell against alloxan, and L-glucose (3 M) which does not, were injected intraventricularly to identify direct effects of these agents on feeding or blood glucose. Subsequently, they were co-administered into the IV ventricle with alloxan (200 ug) in 5 ul of saline. After recovery, feeding and blood glucose responses were measured after 2-deoxy-D-glucose (2DG, 250 mg/kg,s.c.), 5-thioglucose (5TG, 120ug/3ul, IV ventricle) and saline.

When administered alone, amygdalin raised blood glucose 35 mg% above baseline ($p < .01$). When coadministered with alloxan only amygdalin protected the glucoprivic feeding response from alloxan-induced damage. L-glucose and L-glutamine did not attenuate alloxan-induced deficits. Alloxan-treated rats ate 35% and 30% of control levels after 2DG and 5TG, respectively ($p < .01$). Alloxan/L-glucose rates ate 25% and 11% and alloxan/L-glutamine rats ate 27% and 21% of control intake after 2DG and 5TG, respectively ($p < .01$). Blood glucose responses to 2DG and 5TG were not impaired by any treatment.

The effects of L-glucose and amygdalin on alloxan-induced impairment of glucoprivic feeding are consistent with results obtained in studies of the B cell. The fact that amygdalin, a potent free radical scavenger, protects against B cell damage has been interpreted to suggest that alloxan's cytotoxicity is due to free radical peroxidation. In our experiment, however, amygdalin's protective action could have been mediated indirectly by elevation of blood glucose. Furthermore, L-glutamine, which antagonizes alloxan's action on the B cell, failed to protect glucoprivic feeding even though L-glutamine increases the generation of reducing equivalents. Therefore, the cytotoxic action of alloxan which impairs glucoprivic feeding may not be identical to its cytotoxic mechanism in the B cell.

138.5 ESTROGEN-GLUCOSE SYNERGISM IN THE CONTROL OF LONG TERM BODY WEIGHT IN RATS
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Several reports have described alteration in the catecholamine levels in anterior hypothalamus when estrogen level is altered. In a previous report we demonstrated that catecholamine elicited eating from an anterior hypothalamic site was inhibited at increasing estrogen concentration. The effects however were specific to short-term feeding episodes. Catecholamine injections did not alter 24 hour food intakes or body weights. Estrogen injections above a threshold dose of 1 ug/day did significantly decrease 24 hour food intakes and 24 hour body weights. In this series of experiments we investigated the hypothesis that EB may interact with other putative satiety factors at estrogen-sensitive brain sites to mediate body weight loss. Ovariectomized rats were implanted with a unilateral cannula in LPO, VMH or Stria medullaris sites in different groups of animals. The groups were maintained and tested on ad. lib. food and water conditions. In an initial dose-response experiment it was determined that 1 ug EB/day represented the threshold dose to effect 24 hour body weight. Animals were injected peripherally with 1ug/day EB or oil vehicle and centrally with glucose 20%/ul, CCK 1ug/ul, or GABA 1ug/ul or 10 ug/ul all in a 1ul volume in separate experiments. The combination of 1 ug/day EB and a 1 ul injection of glucose in all of the estrogen sensitive site resulted in significant changes in 24 hour food intakes. The VMH group ate ($p < .05$), the LPO group ate significantly less ($p < .05$) and the SM group also ate significantly less ($P = .02$) in the 24 hours following the dual injections. Central injections of a 20% glucose solution in the same site without simultaneous EB injections did not result in any significant changes in the 24 food intakes for any of the three groups. Comparison of the change in 24 hour body weights following EB and glucose compared to EB and vehicle injections revealed a significant ($p < .01$) reduction of body weight in the LPO injected group. In addition to long term food intakes some feeding at other time intervals was also altered by these injections. In the LPO group EB and glucose injections significantly reduced 1 hour ($p = .05$), 3 hour ($p. < .05$) and 6 hr. ($p < .01$) food intakes. Short term food intakes 1,3 and 6 hrs. were not altered by this injection series in either the SM or the VMH groups. The other satiety factors CCK and GABA produced a different pattern of results when combined with peripheral EB injections. These injections resulted in decreases in short-term intakes only in the treatment group. These data imply that the combination of a threshold dose of EB, i.e., 1ug/day can synergize at estrogen sensitive brain sites, with a variety of putative satiety signals to alter both short-term and long-term feeding mechanisms.

138.6 EFFECTS OF INTRAVENOUS GLUCOSE AND MANNITOL ON CATECHOLAMINE LEVELS OF THE RAT. E. C. Lotter and R. G. Campbell*.
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Administration of an intravenous (IV) glucose pulse is known to increase plasma concentrations of total catecholamines. Moreover, we have previously reported that an IV infusion of glucose resulted in a significant increase of plasma glucose and insulin and no change of plasma glucagon. The present study examines the effects of IV glucose and that of mannitol, a non-absorptive sugar, on plasma glucose, epinephrine and norepinephrine levels.

Five-hr-food-deprived male Wistar rats (n=8) with an indwelling jugular catheter were randomly injected with glucose or mannitol (20%/.5 ml) over a three-minute period and blood samples were collected over 60-minutes. Water but not food was available throughout the experiment. Glucose injections resulted in an immediate increase of plasma glucose and norepinephrine with no significant change of epinephrine. The administration of mannitol resulted in an immediate and significant decrease of plasma glucose and a small non-significant increase of plasma norepinephrine. No change in epinephrine levels was observed. In both the glucose and mannitol group, plasma glucose and epinephrine returned to baseline values at the end of 60-minutes. In the glucose-infused-group, norepinephrine returned to baseline levels 5-minutes after the injection, whereafter, a second peak was observed at the end of 60-minutes. In the mannitol group, a small bi-phasic increase of norepinephrine was observed within the first 15-minutes. At 60-minutes, norepinephrine levels were below baseline values. The bi-phasic increase of norepinephrine is similar to the bi-phasic release of insulin observed when rats eat a carbohydrate meal.

These findings are of interest in view of the inhibiting effects of catecholamines on insulin release and suggest an acute nutritional shift (i.e., increased norepinephrine turnover) and/or a fluid shift.

138.7 ALPHA-NORADRENERGIC PROJECTION BLOCKADE: EFFECTS ON DRINKING BEHAVIOR AND CARDIOVASCULAR RESPONSES TO ANGIOTENSIN II IN THE CHRONIC AWAKE RAT. D. L. Jones. Depts. Physiology and Medicine, University of Western Ontario, London, Ontario, Canada, N6A 5C1.

Catecholaminergic projections to the forebrain have been implicated in the control of pressor and drinking responses. Previous studies implicated the dopaminergic projections to the striatal complex in drinking responses (Camacho & Phillips, 1981, Am. J. Physiol. 240:R106; Jones & Mogenson, 1982, Can. J. Physiol. 60: 720). The noradrenergic projections to the hypothalamus have been implicated in the pressor responses to central angiotensin II administration (Camacho & Phillips, 1981, ibid). Injections of Angiotensin II have also been shown to increase the synthesis (Alpers et al., 1982, Neurosc. Abstr. 8:421) and turnover (Sumners & Phillips, 1983, Am. J. Physiol. 244:R257) of hypothalamic noradrenaline. These studies could not distinguish responses associated solely with pressor responses from those involved in the drinking responses. Recently Bellin et al., (1982, Neurosc. Abstr. 8:226) found that relatively selective 6-OHDA lesions which reduced hypothalamic noradrenaline content by 70-90% blocked both drinking and pressor responses to injections of angiotensin II. A recent study (Jones, 1983, Fed. Proc. 42:1126) also found that blockade of noradrenergic projections to the rostral hypothalamus attenuated angiotensin II induced drinking in awake animals and reduced pressor responses in anaesthetized animals. These experiments extend the preliminary studies to investigate in the chronic awake animal the effects of pretreating the rostral hypothalamus with phentolamine on the drinking and pressor responses induced by central angiotensin II administration.

Male Wistar rats were prepared with a chronic indwelling femoral arterial cannula and guide cannulae implanted bilaterally above the lateral ventricle and rostral hypothalamus. Animals were tested on separate days for responses to saline pretreatment of the hypothalamus and one dose of phentolamine (2.5, 12.5, 25.0 or 125.0 µg) followed immediately by ventricular administration of angiotensin II (25 pM). Pretreatment of the rostral hypothalamus with phentolamine attentuated the pressor and drinking responses to central angiotensin II in a dose dependent manner. Sites lateral or posterior by 1-2 mm had greatly diminished blockade. Some animals exhibited increased activity and chewing behavior associated with elevated blood pressures and heart rates but did not drink. These results sugguest that α-noradrenergic projections to the rostral hypothalamus subserve both the drinking and pressor responses elicited by central angiotensin II administration. Further, they suggest that the region involved in the pressor response may have a more restricted distribution in the rostral hypothalamus.

(Supported by the Medical Research Council of Canada).

138.8 DORSAL PERIVENTRICULAR FIBER SYSTEM DESCENDING FROM PARAVENTRICULAR NUCLEUS MEDIATES FEEDING INDUCED BY NOREPINEPHRINE AND CLONIDINE. G. F. Weiss* and S. F. Leibowitz. Lab. of Neuropsychopharmacology, The Rockefeller Univ., New York, NY 10021.

Hypothalamic injection of norepinephrine (NE) is known to induce feeding in the satiated rat. This effect is found to be strongest in the paraventricular nucleus (PVN). Clonidine also elicits a feeding response when injected into the PVN, and is believed to be acting through the PVN noradrenergic feeding system. In the present study, the objective was to map out the course taken by the PVN efferent fibers that mediate NE and clonidine feeding. This was accomplished by investigating a variety of bilateral coronal knife cuts, in various forebrain and midbrain regions, and determining their impact on PVN noradrenergic-induced eating.

Male albino Sprague-Dawley rats (n=80) with chronic brain cannulas received, on separate days, PVN injections of saline, NE (40 nmoles), and clonidine (30 nmoles. Baseline responsiveness to these drugs, relative to saline control scores, was determined prior to administering the coronal knife cut. Animals were tested 3 weeks before and 6 weeks after knife cut. Sham-operated rats showed stable drug responses throughout the testing periods. Large cuts 1 mm rostral to the PVN, extending from midline to 1.1 mm lateral, had no impact on the animals' responsiveness to NE and clonidine. Large coronal knife cuts severing most of the midbrain tegmentum, with the exception of the central grey region, also produced no significant change.

In contrast, knife cuts located in the periventricular region of the thalamus and in the midbrain central grey were both successful in significantly attenuating the NE and clonidine eating responses, by 70% and 60%, respectively.

Based on these findings, it is suggested that the PVN efferent fibers mediating NE- and clonidine-induced eating course dorsally into the thalamic periventricular area and caudally through the midbrain central grey. Additional evidence suggests that within the pons, the fibers then radiate ventrolaterally as they course toward the dorsal medulla.

(The research was supported by grant MH 22879 and by funds from the Whitehall Foundation.)

138.9 α_2-ADRENERGIC RECEPTORS IN THE PARAVENTRICULAR NUCLEUS MEDIATE FEEDING INDUCED BY NOREPINEPHRINE AND CLONIDINE. L.A.Marino*, M.D.DeBellis*, and S. F. Leibowitz (SPON: D. Micco). Lab. of Neuropsychopharmacology, The Rockefeller Univ., New York, N.Y. 10021.

The paraventricular nucleus (PVN) of the medial hypothalamus is the most effective site for feeding induced by central norepinephrine (NE) injection. The PVN is also found to respond to the α-adrenergic agonist clonidine (CLON). The present study utilized a variety of α-adrenergic antagonists to determine the specific α-adrenergic receptor subtype mediating this phenomenon.

Using the brain-cannula technique in satiated male albino rats, the specific α_1-adrenergic antagonists corynanthine and prazosin, the specific α_2-adrenergic antagonists yohimbine and rauwolscine, and the general α-adrenergic antagonist phentolamine were administered into the PVN 30-45 minutes prior to PVN injection of either NE (40 nmoles) or CLON (20 nmoles). Blocker doses of 12.5 to 100 nmoles were tested.

Consistent with previous findings, the general α-adrenergic antagonist phentolamine was found to essentially abolish (-82%) the NE eating response. Clonidine-induced feeding was also significantly attenuated by phentolamine. Tests with the specific α_1 antagonists, corynanthine and prazosin, failed to reveal any blocker-induced attenuation of NE and CLON feeding and, in fact, showed some enhancement of NE and CLON eating with corynanthine. In contrast, the α_2-adrenergic antagonists yohimbine and rauwolscine significantly attenuated, by 50-75%, both NE- and CLON-induced feeding. In a separate experiment, it was shown that PVN injection of the α_2 antagonist yohimbine (25-100 nmoles) significantly suppressed the effects of systemically administered CLON (25 µg/kg) by 40-80%.

The present study demonstrates that: 1) PVN injections of NE and CLON induce feeding via α_2-adrenergic receptors located in this nucleus, and 2) feeding induced by systemically administered CLON is also mediated, at least in part, by these PVN α_2-adrenergic receptors.

(This research was supported by grant MH 22879 and by funds from the Whitehall Foundation.)

138.10 CLONIDINE EFFECT ON HYPOTHALAMIC NORADRENERGIC MECHANIAMS: INVESTIGATIONS OF CATECHOLAMINE TURNOVER AND EATING BEHAVIOR. M. Jhanwar-Uniyal*; J. McCabe; B.E. Levin and S.F. Leibowitz (SPON: N.E. Miller). The Rockefeller Univ., New York 10021 and Dept. of Neurosci., N.J. Med. School, Newark, N.J. 07103.

A noradrenergic mechanism in the hypothalamic paraventricular nucleus (PVN) is believed to be involved in the stimulation of eating behavior. Investigations in this laboratory with the a_2-adrenergic agonist, clonidine (CLON) indicate that this compound is a potent stimulator of eating and that it may be acting via the PVN noradrenergic system.

Peripheral injection of CLON produces a reliable and dose-dependent eating response in the rat, at doses ranging from 12.5 to 50 µg/kg. This stimulatory effect of peripheral CLON, like the eating response induced by central norepinephrine (NE), is found to be abolished by discrete electrolytic lesions in the PVN. Lesions dorsal to the PVN, in the dorsomedial nucleus (DMN), or the perifornical lateral hypothalamus (PFH), produce no change in CLON's action. CLON injected directly in the PVN is also effective in stimulating eating. This PVN CLON eating response, like that induced by PVN NE injection and by peripheral CLON, is associated with a preferential increase in carbohydrate ingestion.

Using the radioenzymatic assay as described by Levin et al. (1980), the effect of peripheral CLON adminstration (50 µg/kg) on the levels and turnover of NE, epinephrine (EPI), and dopamine (DA), in five hypothalamic areas (PVN, DMN, ventromedial hypothalamus, medial preoptic nucleus, PFH) and four extrahypothalamic areas (frontal cortex, locus coeruleus, cerebellum, hippocampus), were examined. The turnover was estimated in rats pretreated with alpha-methyl-p-tyrosine. CLON affected NE levels in only one brain area, namely, the PVN, where endogenous NE was decreased by 63%. EPI levels were increased by 46% in the PFH. The turnover of NE was significantly decreased in all areas examined, with the exception of PVN and locus coeruleus, where no change was seen. In the PFH, as opposed to the other brain areas, turnover of EPI and DA, as well as NE, was reliably reduced.

These data suggest that the well-established inhibitory effect of CLON on brain NE turnover does not occur in the PVN, and therefore may not be involved in CLON's stimulatory action on feeding. One possibility is that CLON, like NE, potentiates food intake by acting upon postsynaptic a_2-adrenergic receptors in the PVN. (This research was supported by grant MH22879 and by funds from the Whitehall Foundation).

138.11 THE ROLE OF ADRENERGIC AND DOPAMINERGIC SYSTEMS IN THE PERIFORNICAL HYPOTHALAMUS IN THE DEVELOPMENT OF APPARENT ANOREXIC TOLERANCE TO D-AMPHETAMINE IN RATS. P. Bhakthavatsalam*, A. Pourany* and M.N. Ghosh* (SPON: David E. Levy). Depts. of Pharmacology and Anatomy, JIPMER, Pondicherry, 605006, India.

The central mechanism of the apparent anorexic tolerance to amphetamine was studied in rats by testing beta-adrenergic and dopaminergic (DA) receptor sensitivity in the perifornical lateral hypothalamus (PFH), which is reported to be the most sensitive site for catecholamine (CA) and amphetamine-induced anorexia (S.F. Leibowitz, 1975, 1978). In addition, certain presynaptic manipulations, such as CA synthesis blockade and neurotoxin lesions, have been conducted to investigate the differential influence of the dopaminergic and adrenergic mechanisms.

The tolerance pattern to PFH amphetamine injection was essentially the same as that reported earlier with s.c. amphetamine injection, i.e., a persistent anorexia with no evidence of tolerance in the first 2h of the test, whereas the second 2h exhibited overeating which increased progressively on subsequent treatments. Thus, the two 2h periods combined and plotted as a 4h measurement showed an apparent tolerance development.

Isoprenaline, adrenaline and noradrenaline (NA), which produce anorexia in the PFH via beta receptor stimulation, exhibited steady anorexic responses on all days of chronic injection. This contrasts with PFH injection of DA, which gradually lost its effectiveness. These results show a subsensitivity of anorexia-mediating DA receptors, rather than of beta-adrenergic receptors. Selective bilateral depletion of DA in the PFH, with 6-OHDA injection, resulted in a significant reduction of anorexia induced by peripheral amphetamine administration and markedly delayed the time of onset and completion of the apparent tolerance. Catecholamine synthesis blockade studies with PFH injection of alpha-methyl-p-tyrosine and Fla-63, showed that both NA and DA are necessary for the anorexia and that these two systems in some manner interact to cause the development of the observed tolerance.

This study suggests that both NA and DA systems in PFH mediate amphetamine anorexia; however, it is the DA system which appears to be predominantly responsible for the observed pattern of anorexic tolerance to amphetamine.

138.12 DOPAMINERGIC MODULATION OF CONSUMMATORY BEHAVIOR IN HUNGRY AND SATED RATS. H.R. Friedman* and E.E. Coons* (SPON: C. Bruce).

Intracranial injection of dopamine (DA) were administered to sated and 22-hour food-deprived rats to study the influence of DA upon consummatory behavior. In hungry rats, DA produced a long-lasting potentiation of food intake. Feeding was elevated on the 1-hour test with food which followed the DA injection and on post-drug tests (occuring at 4 day intervals) conducted as long as 16 days after the drug injection. The longevity of this effect appeared to be temporally determined because potentiated food intake was not recorded when post-drug testing was postponed beyond 16 days. Potentiated feeding after DA was specific to the testing situation and was antagonized by DA receptor blockade.

The brain sites at which DA produced this facilitatory effect coincided with the trajectory of the medial forebrain bundle and other basal forebrain zones through which DA-containing and hedonic-afferent fibers course. Injections at other posterior diencephalic sites produced a decrease in food intake on the DA injection test date. However, on post-drug tests, food intake was somewhat elevated suggesting that brain site determined the polarity of the immediate effect of the drug whereas the longevity of this effect was, more generally, facilitatory to feeding.

In contrast, DA injections at brain zones associated with facilitated feeding in hungry rats, attenuated spontaneous food intake on drug and post-drug tests when injected to sated rats. Again, this suppressive effect of the drug in sated rats was specific to the testing situation because later, hunger-motivated food intake was not affected. However, when DA was administered to rats at the conclusion of a test-meal after a period of food-deprivation, hunger-motivated food intake on the next post-drug test was attenuated. Additional studies showed that the magnitude and polarity of the immediate effect of DA was more dependent upon the temporal contiguity of injection and testing conditions than the longevity of the influence of DA upon feeding.

These data show that intracranial injections of DA at circumscribed brain regions potentiated the expression of the consummatory responses which were motivationally prepotent at the time of the DA injection. It is suggested that brain DA activity normally may modulate the hedonic responsiveness of the rat to consummatory stimuli.

REFLEX FUNCTION I

139.1 DYNAMICS OF THE TONIC NECK REFLEX. V.J. Wilson and K. Ezure.* Rockefeller University, New York, N.Y. 10021

We studied the dynamics of the tonic neck reflex of decerebrate cats by rotating the head sinusoidally in roll and recording EMG from the medial and long heads of triceps bilaterally. The reflex, reciprocal in the two limbs, was sufficiently linear for a sinusoidal analysis. One series of experiments was performed on acutely labyrinthectomized cats (frequency range studied 0.01 - 4 Hz, maximal stimulus amplitude 40°). There was a reflex in 6/11 preparations. At 0.1 Hz and below, peak modulation was approximately in phase with position and gain (% EMG modulation per degree of head rotation) was flat; gain was typically 1% per degree, or less. As frequency increased, gain increased with a slope of about 10 dB per decade and phase advanced slightly in some cats but not others: there was some sensitivity to stimulus velocity. These dynamics are different from those of reflexes evoked by the same stimulus in neck extensor muscles, either because the receptors are different or because of a difference in central processing. Comparison of the properties of reflexes evoked by head rotation in triceps with those of vestibular reflexes evoked by roll tilt of the whole animal (Schor and Miller, J. Neurophysiol. 1981, 46: 167) suggests that up to 0.2 Hz the two reflexes should cancel (von Holst and Mittelstaedt, Naturwissenschaften 1950, 37: 464; Lindsay et al., J. Physiol. 1976, 261: 583). We tested this in another series of experiments on cats with intact labyrinths: neck or vestibular reflexes could be evoked separately or in combination at frequencies up to 0.5 Hz. Neck reflexes were present in 6/6 preparations. Dynamics were similar to those of the reflex in labyrinthectomized cats, but absolute gain was somewhat higher. Neck and vestibular reflexes often had comparable gain and in such cases, as predicted, combined stimulation did not produce significant EMG modulation. Results with labyrinthectomized cats suggest that there will not be such effective cancellation at higher frequencies. Supported by NIH grants NS02619 and RR07065 and NASA grant NSG2380.

139.2 RESPONSE OF SPINAL INTERNEURONS TO NATURAL STIMULATION OF NECK RECEPTORS. K. Ezure*, R.H. Schor, and V.J. Wilson (SPON: A.D. Miller). Rockefeller University, New York, N.Y. 10021

We recorded extracellularly from spinal interneurons whose activity was modulated by stimuli that evoke a tonic neck reflex. Decerebrate cats were used. Some were acutely labyrinthectomized, and the stimulus was sinusoidal head rotation in roll. In others the labyrinths were intact: in these we evoked a neck reflex (body rotation in roll with respect to a fixed head), a vestibular reflex (roll tilt of the whole body) or both together (rotation of the head alone). Up to 3 spinal segments were exposed by dorsal laminectomy. Cats were first tested for the presence of a tonic neck reflex with EMG recording, then paralyzed and artifically respirated. Neuron sampling was from C4 to C8, and cells whose activity was modulated by stimulation of neck receptors were usually in laminae 7-8.

45 neurons were studied in labyrinthectomized cats at frequencies of 0.05-4Hz. 26 were excited by rotation of the chin to the ipsilateral side and inhibited by contralateral rotation (Type 1 neurons). 19 behaved in the opposite manner (Type 2 neurons). 28/45 neurons could be studied with at least 3 frequencies; of these, 22 behaved in a consistent manner. As is the case with modulation of muscle EMG, gain increased with frequency and phase was approximately flat. Phase was close to that of muscle measured before paralysis, but was often somewhat more advanced. In cats with intact labyrinths, 31 neurons could be tested for response to both neck and vestibular reflexes, at frequencies of 0.05 to 0.5Hz. Over this range the dynamics of the response to stimulation of neck receptors were the same as in labyrinthectomized cats. The firing of 25/31 neurons was modulated by both neck and vestibular stimulation. In every instance the responses were complementary. That is, Type 1 neurons were also excited by whole body tilt to the ipsilateral side (ipsilateral ear down) and Type 2 neurons by contralateral body tilt. As with muscle, head rotation alone often evoked no response. When it did, the response could be predicted from vectorial addition of neck and vestibular responses.

Some of the neurons we studied could be in the reflex pathway to motoneurons, but further experiments are required to test their projections. Supported by NIH grants NS 02619 and RR07065 and NASA grant NSG 2380.

139.3 DYNAMIC MODELING OF THE VISUAL-VESTIBULAR INTERACTION IN CHAMELEON HEAD MOVEMENTS. Martha Flanders* (SPON: James L. Zacks). Dept. of Zoology, Michigan State Univ., E. Lansing, MI 48824

In vertebrates, head movements can be caused by visual cues, vestibular reflexes, or proprioceptive input, and are limited by the mechanical properties of the head-neck system. Lizard head movements, made during visual and/or vestibular stimulation, were examined in order to generate a comprehensive model to predict head position in the intact animal.

Chameleo sp. was used because of the binocular head tracking exhibited by this lizard during its normal feeding behavior. Pursuit head movements are easily elicited in the chameleon by moving a cricket a few inches in front of it. The chameleon fixes on its prey with both eyes and its head follows the target to keep its tongue in line with the prey, ready to strike. Moving the lizard's body also triggers a head movement processed through the vestibular system (the vestibulo-collic reflex, VCR) which keeps the head stable in space. An apparatus moved the cricket and/or the animal's body with horizontal, angular, sinusoidal motions, and the resulting head movements were filmed from above. The two types of movement were examined separately by varying the frequency of stimulation, and compared to movements made during combined visual and vestibular stimulation.

Gain and phase data from film analysis show a non-linear interaction between the stabilization and pursuit systems such that the gain of the VCR is reduced during high frequency pursuit stimulation. During equal amplitude, combined stimulation, peak acceleration, not peak velocity, controls the movement of the cricket relative to the chameleon's line of sight (the perceptable motion). Weight of the head is used to quantify forces during the various movements. The chameleon head is an ideal preparation for studying electromyographic activity of antagonistic neck muscles during movements that are kinematically identical, but processed through different neural pathways.

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139.4 EFFECTS OF MILD MECHANICAL CUTANEOUS STIMULATION ON THE STRETCH REFLEX. J. J. Seguin and J. D. Cooke. Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

The effects of cutaneous stimulation on tonic and phasic EMG responses to stretch of the triceps surae were studied in the unanesthetized decerebrate cat. Stretch was applied directly to the isolated tendon at the calcaneus and cutaneous stimulation was applied to the paw. This allowed the stretch of the muscle and cutaneous stimulation to be applied independently and at various intervals. EMGs were recorded from the gastrocnemius and soleus muscles through fine insulated wires or a concentric needle electrode. After amplification and full wave rectification, 20 to 40 responses were averaged and recorded on an X-Y plotter. A tonic response was produced by maintained stretch of the triceps surae to 80-85% of its maximum physiological length. Mild brief mechanical stimulation (a tap) applied to the metatarsal pad of the hind foot caused a sequence of excitation-inhibition-excitation beginning 11 to 17 msecs after onset of stimulation.

Phasic responses were elicited by a brief ramp stretch of 1-2 mm superimposed on the maintained stretch. A brief tap, applied 0.01 to 5 msecs before the start of the ramp stretch caused a decrease in the size of the early response to the stretch and converted it to a biphasic response. Electrical stimuli (0.1 to 1 ms, 0.1 msec duration) applied to the pad produced similar results.

Ancillary experiments in man showed that a light tap on the palm of the hand also caused a sequence of excitatory and inhibitory response in the averaged EMG of the biceps muscles during a voluntary maintained contraction at a latency of about 40 msecs. In addition, a short burst (50 msec, 80 Hz) of vibration applied to the palm 35 to 50 msecs before perturbation of the biceps caused a depression of the averaged EMG responses to the perturbation.

It is suggested that innocuous mechanoreceptor stimulation may be responsible for variations in EMG activity seen during perturbation applied to the intact limb in man and animals.

(Supported by the Medical Research Council of Canada, MT-6699).

139.5 FREE NERVE ENDINGS IN MUSCLE MEDIATE THE CLASP-KNIFE REFLEX IN THE CAT. Corey Cleland and W.Zev Rymer, Neuroscience Program and Department of Physiology, Northwestern University Medical School, Chicago, IL, 60611

The clasp-knife reflex, first described in spastic human patients, also occurs in decerebrated cats that have had their dorsolateral spinal funiculi sectioned. The reflex consists of powerful autogenetic inhibition which occurs when an extensor muscle is stretched beyond a certain length. Contrary to earlier views, we believe that muscular free nerve endings(FNEs) are responsible.

We recorded from single muscle afferents in the SOL-LG and MG muscle nerves or dorsal roots of 17 anesthetized cats. Twenty-three group II and III muscle afferents were found that did not originate from muscle spindles, Golgi tendon organs or paciniform corpuscles. Therefore, they presumably arose from FNEs in muscle. Many were located in the achilles tendon or aponeurosis and were extremely sensitive to light pressure. Six had irregular, spontaneous discharge at long muscle lengths. Thermal or chemosensory properties were not investigated.

Most FNEs responded to both ramp stretch and isometric force tetanus. Their response to stretch was primarily dynamic with some maintained static response during the hold phase of stretch. All substantially adapted to repeated stretches. FNEs responded both dynamically and statically to force tetani. Several were also excited by ramp shortening or the falling phase of tetanus or twitch. The dynamics of FNE activity, dependence on both length and force, and their threshold to stretch match the analogous properties of clasp-knife inhibition. In contrast, the input-output properties of secondary spindle afferents and Golgi tendon organs are quite different from those of clasp-knife inhibition.

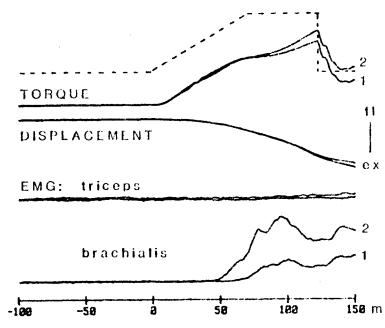
The reflex effects of FNEs were studied in 8 decerebrated and dorsal spinal hemisectioned cats which showed a prominent clasp-knife reflex. Selective stimulation of FNEs by squeezing the distal portion of the achilles tendon with a pair of fine forceps powerfully inhibited extensor muscles and excited flexor muscles throughout the hindlimb. This stimulus was shown in anesthetized cats to potently excite many FNEs but not affect other muscle sensory receptors. The spatial divergence of the reflex effects also matches the spatial divergence of clasp-knife inhibition and excitation.

Since FNEs have the appropriate input-output properties and potently inhibit extensor and excite flexor muscles, they must mediate at least part of the clasp-knife inhibition.

Supported by NIH 14959 and NIMH 5F31MH08593.

139.6 THE STIFFNESS INCREMENT PRODUCED BY THE LATE STRETCH REFLEX DURING VOLUNTARY ELBOW MOVEMENTS IN MAN. W.A. MacKay, D.J. Crammond* and J.T. Murphy. Dept. of Physiology, Univ. of Toronto, Toronto, Canada M5S 1A8.

The late stretch reflex has a significant influence on muscle stiffness and is strongly modulated during voluntary movements. The purpose of this study was to demonstrate that the mechanical output of the reflex also varies during voluntary elbow movements. A trapezoidal torque pulse was delivered to the forearm via a DC motor-manipulandum assembly. Following the onset of the late stretch reflex in elbow muscles, the monitored torque at the manipulandum showed a steady increase directly proportional to continuing elbow angular displacement, although no additional torque was being externally applied. The magnitude of this elastic restoring torque was highly correlated to the size of the late stretch reflex. Borrowing from the "stiffness servo" hypothesis, the late reflex output may be postulated to compensate for yield to stretch beyond the limit of short-range stiffness. The ratio of muscle stiffness observable with relatively large displacements eliciting a reflex to that with "short-range" displacements (eliciting virtually no reflex), was defined as the stiffness compensation factor of the reflex. A factor of 1 indicated 100% compensation by the reflex for muscle yield. During an elbow movement, the compensation factor altered markedly, usually exceeding 1 only at the onset of muscle contraction. In subjects with a strong reflex, compensation rose as high as 2, but for those with a weak reflex the factor did not attain 1. Muscle stiffness was not maintained at a constant level during voluntary movements. We conclude that the late reflex does compensate for muscle yield at times when loads are predictably encountered, but to a degree which varies considerably among individuals. Supported by MRC of Canada.



Torque pulse opposing forearm flexion applied 500 ms (1) and 100 ms (2) before onset of voluntary elbow flexion. Broken line indicates form of torque motor input signal.

139.7 A METHOD FOR MEASURING MUSCLE STIFFNESS IN UNRESTRAINED CATS. J.A. Hoffer, T.R. Leonard* and N.L. Spence*. Dept. of Clinical Neurosciences, U. Calgary Fac. Med., Calgary Alberta T2N 4N1.

The original postulate that muscle stiffness is the regulated property of the stretch reflex was based on experiments involving decerebrate cats. In interpreting how these findings apply to intact animals, an important concern has been the extent to which decerebration may alter reflex gain at the segmental level. In particular, several lines of evidence have recently indicated that, in decerebrate cats, the Ib feedback pathway from Golgi tendon organs to lumbar motoneurons is virtually non-functional. Whether, and how, the gain along the Ib pathway may differ in the normal and decerebrate cases, is the ultimate focus of this study.

We have used chronically implanted transducers and electrodes (described by Hoffer & Loeb, Ann. Biomed. Engng. 8:351, 1980) to measure incremental stiffness of the ankle extensor muscles in intact, unrestrained cats, against a range of background forces. Cats were surgically fitted with the following devices: a force transducer implanted on the Achilles tendon; a length transducer spanning from origin to insertion of the gastrocnemii; bipolar EMG electrodes in the lateral gastrocnemius and anterior tibial muscles; and three nerve cuffs on the common peroneal nerve. The most distally located cuff contained stimulating electrodes. It was used to synchronously activate the ankle flexor muscles, thereby stretching incrementally the ankle extensors. The middle cuff was connected to a catheter, through which a 2% solution of xylocaine was infused to block conduction of the evoked flexor nerve volleys toward the spinal cord. The proximal cuff contained a tripolar set of recording electrodes. It was used to monitor the absence of a nerve compound action potential upon stimulation and thus verify the effectiveness of the flexor nerve blockade. Upon delivery of a stimulus, the amplitude of the stretch imposed on the ankle extensors was recorded by the length gauge, and the amplitude of the combined response (due to the elastic properties of muscle, plus reflex effects) was recorded by the force gauge. The ratio of these two quantities gave the incremental stiffness.

The test perturbations provided by this method are of the "torque pulse" type. As predicted from muscle length-tension properties, the amplitude of stretch (and, to some extent, its duration and shape) depend markedly on the operating length of the ankle flexors, as well as on the operating force of the ankle extensors. We found that the length of the test stretch and the magnitude of the response are quite reproducible when measured at matched operating points. In intact cats, the stiffness of the ankle extensors increases monotonically for forces from 0 to 7Kg. We are now using this method to measure stiffness and obtain a direct comparison of reflex gain before and after decerebration.

(Funded by the Alberta Heritage Foundation for Medical Research.)

139.8 ANALYSIS OF JAW JERK VARIABILITY. B.Bishop, R.S. Hickenbottom* and T.M. Moriarty*. Dept. of Physiol. and Dept. of Physical Therapy, State Univ. of NY at Buffalo, Buffalo, NY 14214.

The purpose of this study was to identify and assess the relative contributions of various extrinsic and intrinsic neural factors on the jaw jerk reflex (JR). Thirty five healthy adults with no oro-facial pathology were studied. Each subject (S) sat in a dental chair with the head securely stabilized with a metal halo and chin rest. Chin taps were delivered by way of a solenoid-driven plunger mounted 1 cm from the site where a tap with a reflex hammer evoked the "best" response. Tap force was measured with a piezo-transducer in series with the plunger. Response amplitude was taken as the negative phase of the compound action potential recorded from surface electrodes placed over the right masseter muscle with an earclip electrode as ground. Phases of respiration were signalled by a nasal thermistor.

Five of the 35 Ss had no JR when relaxed, but a JR appeared when the S voluntarily contracted the platysma muscle and thereby stretched the masseter muscle or facilitated the motoneuron pool in some other way. The JRs in the other Ss varied from trial to trial despite a constant current activating the plunger. This variability was partially due to minute changes in tap force regardless of head and chin stabilization suggesting either a shift in jaw posture or a change in facial expression. In the majority of cases the mean amplitudes of the JR increased with these very small increases in tap force, but the standard deviations of the JRs to taps of constant force were large indicating intrinsic fluctuations in motoneuron excitability. Much of this variability in the responses to taps of constant force was respiratory related. JRs evoked in late expiration and early inspiration were significantly larger than those evoked during early expiration. When the head was stabilized in 15 degrees extension, every JR response was larger than any with the head upright. It could not be ascertained whether this facilitation was neurally or mechanically mediated.

In conclusion, the masseter monosynaptic reflex is sufficiently variable within any given S or among Ss to make the response a poor index of masseteric motoneuron excitability. It appears that branchial motoneurons innervating the masticatory muscles receive far more diverse and fluctuating inputs than do somatic motoneurons innervating the limb muscles.

(Partially supported by USPH-BRSG H079.)

139.9 Prior Instruction Affects Single Motor Unit Discharge Rate During the Stretch Reflex. B. Calancie and P. Bawa. Dept. of Kinesiology, S.F.U., Burnaby, B.C. V5A 1S6. CANADA.

A sudden stretch of forelimb muscles in human subjects results in two separate reflex peaks in the EMG activity of those muscles (Bawa & McKenzie, 1981). These reflex peaks, the short latency (SL) at 25-50 msec and the long latency (LL) at 50-90 msec, precede the earliest voluntary response to stretch. Hammond (1954, J. Physiol. 127: 23-25) reported that subjects were able to modify the EMG amplitude of the LL component of the stretch reflex, depending on what voluntary movement they produced in response to the stretch. When told to react against the stretch (compensate), a subject's LL reflex amplitude was much larger than when the subject 'let go' (no voluntary interference) in response to the stretch. The SL reflex activity was relatively unaffected by the subject's response to stretch. We have investigated in 2 adult subjects the firing pattern of single motor units (SMU) during both phases of the stretch reflex, and in particular the influence of prior instruction on this firing pattern.

Step load perturbations extending the wrist joint were produced by a torque motor. Surface EMG of wrist flexor muscles and single motor unit activity of flexor carpi radialis (FCR) were recorded simultaneously. Subjects were asked to either 'let go' to the step load, or to 'compensate' for the displacements as quickly as possible. Data was used only where identification of a given unit remained certain during the large muscle movements.

In most cases the unit studied was made to fire tonically, and changes in firing probability within the SL and LL periods were thus superimposed on this background firing. For each of 10 tonically firing units studied to date, the probability of firing during the LL period following muscle stretch was significantly higher during the 'compensate' task than during the 'let go' task. That is, when the subject expected to activate his wrist flexors upon perceiving the stretch, a unit was much more likely to fire during the LL period. There were no consistent changes in either background or SL firing probabilities when comparing instruction effects.

This work was supported by grants from NSERC and BCHCRF. B. Calancie is supported by NSERC studentship.

139.10 INCREASED STRETCH RESPONSIVENESS IN HUMANS DURING HYPERBARIC EXPOSURE. James L. Parmentier and David J. Harris*, Depts. of Anesthesiology, Univ. South Alabama, Mobile, Ala. 36617 and Duke Medical Center, Durham, N.C. 27710.

Hyperbaric hyperreflexia has been shown to occur in man during and after breathing a mixture of compressed helium and oxygen at 25 bars or more. This pattern may be part of the High Pressure Nervous Syndrome (HPNS) which characterizes a multiplicity of neuromuscular, EEG, and psychological disturbances associated with exposure to hyperbaric gas mixtures and appears to result from upper CNS dysfunction rather than an alteration of any peripheral mechanism. We have studied tendon-strike and Hoffmann reflexes of the soleus muscle in three subjects breathing 5% TRIMIX at various pressures to 650 bar during the experimental simulated saturation dive Atlantis IV.

We found that the peak force of contractions resulting from both the electrically stimulated Hoffmann reflex and the spindle activated Tendon strike reflex increased as much as 130% during compression and recovered to control levels when pressure was released. During compression the ratio of the EMG amplitudes of Hmax to Mmax, which is a measure of the excitability of the monosynaptic spinal reflex arc of the posterior tibialis nerve, was not altered. However, the amplitude of the tendon-strike EMG was increased to levels comparable to the force of contraction. The intramuscular hyperexcitability was still above control levels by the fourth day of the post-dive period.

It is not possible from these non-invasive experiments to determine if the hyperflexia seen during Atlantis IV is due to increased muscle spindle sensitivity or to direct effects on muscle properties which control e-c coupling or the mechanochemistry of force production. While gamma-loop resetting cannot be ruled out these effects of pressure appear to be peripheral in origin and were not ameliorated by the presence of 5% nitrogen in the breathing mixture, a level sufficient to control HPNS in two of the three diver subjects. In order to determine the specific cellular mechanisms underlying hyperbaric hyperreflexia these experiments must be repeated on excised preparations.

139.11 A FICTIVE TAIL FLICK REFLEX IN THE RAT. C.L. Cargill*, J.L. Steinman* and W.D. Willis (SPON: F.H. Rudenberg). Marine Biomed. Inst. & Depts. of Physiol. & Biophys. & Anatomy, Univ. TX Med. Branch, Galveston, TX 77550 U.S.A.

Many tests of analgesia in the rat have been based upon the tail flick reflex (TFR). Attempts to elucidate the neural mechanisms mediating the TFR have only recently been undertaken. The purpose of the present experiments was to develop a model of the TFR in an anesthetized, paralyzed preparation. Hence, the response was termed a fictive TFR (i.e., motoneuron activity without the corresponding muscle movement).

A TFR was demonstrated in rats anesthetized with Chloropent (0.23 ml/100 g body weight). Muscles participating in the tail flick included the medial longissimus (ML) (also known as the extensor caudae lateralis), extensor caudae medialis (ECM), and the abductor caudae dorsalis (ACD) muscles. Filaments of small peripheral nerves innervating the ML were separated from the surrounding muscle and cut distally. Single and multiple motor unit discharges were recorded, simultaneously with an electromyogram (EMG) from ML. The tail flick reflex was evoked by either heating the tail with a 500 watt quartz light bulb or by a noxious pinch to the tail. The reflex (TFR) was defined as movement of the tail in any direction away from the source of noxious input. The average TFR latency was 2.5 seconds.

During a TFR, there was a dramatic increase in firing of the motor units and an EMG response. The animal was then respired (60-80 cycles/min) via a tracheal cannula and given intraperitoneal injections of gallamine triethiodide (Flaxedil, 2.5 mg/kg body weight). As the cumulative amount of Flaxedil was increased to 40 mg/kg, there was a gradual decrease in the movement of the tail with a corresponding decrease in the EMG response until both disappeared completely. The motor units continued to fire following exposure of the tail to noxious heat or pinch. We regard this activity to represent a fictive tail flick reflex.

In order to determine the relevance and applicability of this model, we tested the effects of vaginal stimulation (VS) on motoneuron responses to noxious heat and pinch. VS has previously been shown to inhibit TFR or leg withdrawal in intact and spinalized rats. When applied for 5 sec prior to the onset of radiant heat or pinch, VS (200 g force) completely attenuated motor unit firing during noxious stimulation. However, spontaneous activity was not diminished, indicating that the noxious input was selectively inhibited. Future experiments will include a systematic examination of the neural basis of the TFR in rats. (Supported by grants NS 07022 (JLS), NS 09743 and NS 11255 from NIH, and a grant from the Moody Foundation.)

SPINAL CORD: SOMATOSENSORY PHYSIOLOGY AND BEHAVIOR

140.1 SOME ASPECTS OF THE DEVELOPMENT OF CATECHOLAMINERGIC NERVES IN THE SPINAL CORD OF THE RAT. J.W. Commissiong. Dept. of Physiol., McGill University, Montreal, Quebec, Canada H3G 1Y6.

The development of noradrenergic and dopaminergic nerves in the whole spinal cord of the rat using fluorescence microscopical and neurochemical techniques was presented previously (Commissiong, J. Brain Res. 264, 197-208, 1983). In the present study the development of these catecholaminergic nerves in five regions of the developing rat cord from fetal day (FD) 16 to the young adult stage is presented. In terms of the normal synthesis of norepinephrine (NE), the release and metabolism of NE, the capacity to synthesize NE from exogenous L-DOPA, and the ability to metabolize the newly synthesized NE, the noradrenergic nerves in the ventral horn (VH) of the cervical and lumbar regions are quite well developed at 12 hrs after birth. An equivalent stage of development is achieved in the thoracic zona intermedia (T-ZI) and the dorsal horn (DH) several days later. In all regions, NE concentration peaked in the latter half of neonatal life and then declined to that found in the young adult. At neonatal day (ND) 15, there was a concentration of fluorescent varicosities in a narrow rostrocaudal band in the ventral horn, perhaps among the apical dendritic trees of motoneurons. There was no consistent pattern in the development of spinal dopaminergic nerves except in the dorsal horn of the cervical region (C-DH). However, in all regions, at ND 20, there was a peak of DA. The capacity of the developing cord to synthesize DA from exogenous L-DOPA is already well established as early as FD 16, and peaked in all regions at ND 4. Only 10% of DA synthesized in the cord from injected L-DOPA occurs in monoaminergic nerves. Norepinephrine is synthesized exclusively in noradrenergic nerves. At ND 18, large numbers of small cells with large nuclei appear transiently in the thoracolumbar region of the cord and are capable of synthesizing catecholamines (probably mainly DA) from injected L-DOPA. During postnatal development, and probably in the adult as well, the network of catecholaminergic nerves actually present, is probably much more extensive than that usually visualized during routine fluorescence microscopy. The results will be discussed in the context of current attempts to understand the functional importance of catecholaminergic nerves in the mammalian spinal cord.

140.2 INFLUENCE OF RAT STRAIN ON CAPSAICIN-INDUCED ANTINOCICEPTION AND DEPLETION OF SUBSTANCE P. T. R. LaHann* and M. A. Bittner* (SPON: A. E. Snow) Miami Valley Laboratories, The Procter & Gamble Company, Cincinnati, Ohio 45247

In recent years, many laboratories have reported on the antinociceptive actions of capsaicin (C). There is general agreement that C possesses antinociceptive activity, but there is disagreement about its relative efficacy against various forms of noxious stimuli. This disagreement may be due, in part, to species or strain differences. The object of our experiments was to determine if C-induced antinociception and substance P (SP) depletion is influenced by the strain of the animals. Adult male rats of six different strains (CD [SD], CDH [SD], CDF [F-344], WI, SHR/N and WKY/N) were injected sc with synthetic C or vehicle alone (10% ethanol, 10% Tween 80, 80% saline). Antinociceptive activity was assessed using noxious mechanical compression, chemical and thermal stimuli. Activity against noxious heat was assessed in the hot plate (55.0 $\pm$ 0.5^{o}C, endpoints: paw fanning and licking) and the tail flick (response time of naive rats: 3 sec) tests. Nociceptive pressure thresholds were determined using an Ugo Basile analgesymeter (endpoint: concerted effort to withdraw the paw). Rats were tested for thermal and pressure antinociception 1, 3, 5, 24, and 48 hrs. after C. Antinociceptive activity against noxious chemical stimuli was measured 6 hrs after C by instilling 50 µl of 0.15 N NH_4OH into one eye and counting the number of blinks occurring over the next 60 seconds. Drug solutions and/or animals were coded to eliminate investigator bias. SP determinations were made by extracting the lumbar portion of the spinal cords with 2 M acetic acid. The SP content was determined by RIA. All C-treated rats showed a decreased sensitivity to chemical pain. Similarly all rats treated with 100 mg/kg C showed a decreased sensitivity to thermal pain in both the hot plate and tail flick tests. WKY/N rats were the most responsive strain. C-induced antinociception to pressure pain was marked in WI and WKY/N at 100 mg/kg C, moderate in CDF at 400 mg/kg C and minimal or unchanged in CDH, CD (SD) or SHR/N rats at 400 mg/kg C. Spinal cord SP levels were measured in all strains except WI. No significant strain-related differences in C-induced SP depletion were seen 3, 24 or 96 hrs. after C. We conclude that the strain of rat alters the expression of C-induced antinociception and that some of the reported literature differences result from the use of different strains. By contrast, the strain of rat does not influence C-induced depletion of spinal cord SP so this depletion cannot account for C's effect on pressure pain. The strain differences should prove to be a useful means of investigating the pathways that mediate chemical, thermal and pressure pain.

140.3 RESPONSE DIFFERENCES OF LAMINA-3,4 NEURONS TO INPUT FROM SA1 AND G1 AFFERENT FIBERS, D. N. Tapper and J. Aldridge*. Dept/Sect. of Physiology, N.Y. State Coll. Vet. Med., Ithaca, NY 14853.

Output neurons of the [L3,4:SA1,X] network often make monosynaptic contact with other primary afferent fiber classes, such as, Guard hair fibers (G1) in addition to Type 1 slowly adapting fibers (SA1). Using graded mechanical pulses to move individual Guard hairs, activation of single G1 fibers can be controlled at the level of the single nerve impulse, similar to the control we have with SA1 input. In the decerebrate-low spinal preparation in cat, the output cells of the [L3,4:SA1,G1] network respond to single impulses in single afferent fibers, either SA1 or G1, in a qualitatively similar manner. The response to 100 impulses delivered one per 3 sec consists of an early time-locked discharge followed by a more dispersed late discharge which is often terminated by an inhibition of ongoing activity. With SA1 input the early and late discharges last for 5 and 29 ms, respectively. In contrast, with G1 input they last for 2 and 9 ms. This quantitative difference to the same input, a single impulse, indicates that the identity of the class of input is maintained in the organization of the network. The faster time constant for central analysis of G1 input is indicative of such organization. (Supported by USPH Grant NS-07505).

140.4 ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE POSTSYNAPTIC DORSAL COLUMN (PSDC) PROJECTION IN THE RAT. G.J. Giesler, Jr., R.L. Nahin and A.M. Madsen*. Dept. of Anatomy, Univ. of Minnesota, Minneapolis, MN 55455.

As one of a series of studies of the ascending spinal cord pathways thought to be involved in nociception in the rat, we have examined the projection from dorsal horn neurons to the dorsal column nuclei using both the retrograde transport of horseradish peroxidase (HRP) and single cell recording techniques. Small iontophoretic HRP injections confined to the gracile nucleus (GN) labeled, in alternate sections, more than 200 neurons within a narrow band extending across the ipsilateral dorsal horn immediately subjacent to substantia gelatinosa. Fewer than 10 labeled cells were found within the gray matter of the cervical enlargement following such injections. Small injections of HRP into the cuneate nucleus (CN) labeled more than 350 neurons in the ipsilateral nucleus proprius within segments C6-8. Fewer than 20 neurons were labeled within the lumbar enlargement by injections into CN. Labeling in lumbar neurons was totally prevented following GN injections by transection of the dorsal columns at either T10, T8 or C2. Thus, the PSDC projection to GN appears to ascend entirely within the dorsal columns. Lesions of the dorsal columns in segment C2 consistently reduced the number of labeled neurons in the cervical cord following CN injections by 90%. Combined lesions of the dorsal columns and ipsilateral dorsal lateral funiculus (DLF) reduced the number of cells labeled in the cervical enlargement by more than 95%. Thus a small component of the cervical projection to CN appears to ascend within the DLF. To compare the relative sizes of the projections to the dorsal column nuclei from PSDC neurons and the central processes of primary afferent fibers, labeled cells were counted in the gray matter of the cervical and lumbar enlargements and their corresponding dorsal root ganglia. In the four animals so examined, over 38% of the neurons labeled by CN injections and approximately 30% of the cells labeled by GN injections were located within the dorsal horn. The PSDC pathway is therefore capable of providing a major input to the dorsal column nuclei. We have also used antidromic activation to identify individual PSDC cells in the lumbar enlargement of unanesthetized, decerebrated animals in which the lateral funiculi were transected at C1. To date we have recorded from 16 identified PSDC neurons. Eight PSDC neurons were driven only by innocuous stimuli delivered to the ipsilateral hind paw. Seven PSDC neurons appeared to respond differently to noxious pinch. With a single exception however, these cells could not be driven by noxious thermal stimuli, even following application of repeated sustained noxious heat. Supported by NIH grant NS 17540.

140.5 CAT SPINAL DORSAL HORN NEURONS WITH BRANCHED AXONS ASCENDING BOTH THE DORSAL AND DORSOLATERAL FUNICULI. G.J. Bennett, G.-W. Lu*, N. Nishikawa* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

The axons of dorsal column postsynaptic (DCPS) neurons ascend in the dorsal columns (DC) while those of spinocervical tract (SCT) neurons ascend in the dorsolateral funiculus (DLF). In other respects, however, DCPS and SCT neurons are very similar. Retrograde labeling shows that both DCPS and SCT neurons are concentrated in laminae III-IV. Intracellular staining shows that their dendritic arbors are rostrocaudally elongated, mediolaterally narrow, and concentrated in laminae III-IV. Moreover, both are known to issue axon collaterals that arborize in the vicinity of their perikarya. These similarities suggest that a single neuron might give rise to both DCPS and SCT axons. In order to examine this possibility, we delivered antidromic search stimuli to cervical (C_2-C_4) DC and DLF that were dissected apart from one another and apart from the rest of the spinal cord. Fifty-six antidromically driven neurons were found in the lumbar dorsal horn; 23 of these were driven from both the DC and the ipsilateral DLF. Intracellular records showed that both the DC- and DLF-evoked spikes were typical antidromic potentials. The antidromic conduction latencies obtained from the DC and DLF branches of any given neuron were nearly the same ($\bar{x}$ difference = 0.26 msec), indicating either that the branches' diameters were nearly equal or that the branch point was close to the caudal margin of the dissection. Antidromic conduction velocities averaged 50 m/sec (range, 17-88 m/sec). The cells with branched axons were found at depths corresponding to laminae III-IV. About half of the cells responded only to innocuous, tactile stimuli while the remainder responded to both innocuous and noxious stimuli. These results suggest that the DCPS and SCT projections are not completely independent since at least some of their cells of origin may contribute to both projections.

140.6 TACTILE DISCRIMINATION IN CATS AFTER LESIONS IN DORSAL SPINAL CORD. G. P. Frommer (SPON: S. Curtis). Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

Sagittal lesions transecting the decussation of the spinocervicothalamic system disrupt roughness discrimination much more than do lesions in dorsal columns (DC) (Kitai & Weinberg, Exp. Br. Res., 1968, 6, 234). Nearly complete DC lesions are also effective (Dobry & Casey, Br. Res., 1972, 44, 385). The present study compares effects on roughness discriminations of lesions in DC, DLC (dorsal lateral columns, which carry the spinocervical tract) and DC+DLC at the C3-C4 level.

Cats were trained 12 trials/day with correction in a darkened double alleyway (Grice box) with movable floors bearing different texture pairs. The first discrimination, between textured and smooth metal, started as a combined visual+tactile task. After initial mastery (usually 90% correct for 3 consecutive sessions), visual cues were faded by dimming ambient illumination to zero. The next 5 tasks were between progressively finer sandpapers and smooth Masonite. The remaining tasks were between the first 4 sandpapers and a very fine sandpaper. All cats failed the last task. Lesions were made under deep barbiturate anesthesia. Anatomical verifications available from 3 cats show complete or nearly complete destruction of DC and/or of the dorsal 1/3 of DLC.

Six cats were trained preoperatively. After DC lesions 1 cat showed no deficit, and 1 cat showed negative savings on 3 of the more difficult tasks. After DLC lesions 2 cats showed no or mild deficits. After DC+DLC lesions 2 cats showed large negative savings on the first and easiest sandpaper-Masonite discrimination, rapid relearning of subsequent easier tasks, and negative savings on the more difficult tasks. This is suggestive of acquiring a new strategy postoperatively.

Four cats were trained after lesions were made. One cat with DC lesions failed to learn even the initial tactile discrimination, even though it had mastered the visual+tactile discrimination. The other failed on an intermediate task. The 2 cats with DLC lesions were slow to learn the initial discrimination, but with 1 exception their later performance was within normal limits.

These data are consistent with Dobry and Casey's data. They fail to confirm Kitai and Weinberg's. Differences between the present experiment and Kitai and Weinberg's include locus of lesion (DLC vs. cervicothalamic tract decussation) and procedures in the present intended to maximize cats' performance (e.g. correction, visual fading). Supported by USPHS Grants MH29204 and S07 RR07031.

140.7 SPINAL CORD UNIT ACTIVITY IN THE AWAKE CAT. L. S. Sorkin,* T.J. Morrow and K.L. Casey. Department of Physiology, Univ. of Michigan and Neurology Research Laboratories, VAMC, Ann Arbor, MI 48105.

The response properties of somatosensory spinal cord cells have not yet been described in the intact unanesthetized animal. This study examines the responses of spinal cord cells to natural and electrical somatic stimuli in the awake, partially restrained cat.

Cats had a chronic microelectrode recording device attached to the lower lumbar vertebral column. This implant also immobilized three segments (L3-L5) of the column. Various mechanical and electrical stimuli applied to the skin were used to characterize the units. Neurons that: 1) failed to respond to each suprathreshold driving stimulus delivered at 1-100 Hz., 2) showed a latency shift as the stimulation frequency increased or 3) displayed a biphasic or triphasic waveform, were classified as postsynaptic elements. All other neurons were considered presynaptic.

Receptive field and adequate stimuli were determined for 84 isolated cutaneously driven neurons. Twenty-nine cells were presynaptic, 24 were postsynaptic, and 31 were not classified because 100 Hz. stimuli sometimes elicited struggling. In addition, 28 units were excited by joint position or movement and could not be electrically driven.

Convergence of cutaneous and proprioceptive input was never seen. Cutaneously driven units did not fire spontaneously. In contrast, proprioceptive units discharged spontaneously at rates of 5-40 Hz. Only 5 (21%) postsynaptic cells showed evidence of input from more than one type of cutaneous receptor. Four postsynaptic and 1 unidentified unit showed behaviorally related modulation of responsiveness as previously reported (Sorkin et al, IASP Abstracts, 1981).

The lack of spontaneous activity in cutaneous units, the relatively infrequent afferent convergence and the behaviorally related response modulation found in our experiments suggest that both tonic and phasic supraspinal descending inhibitory influences are active in the intact unanesthetized cat.

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140.8 EFFECTS OF ATTENTION ON DETECTION OF NOXIOUS AND INNOCUOUS THERMAL STIMULI. M.C. Bushnell, R.L. Jones*, G.H. Duncan* and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, Maryland 20205.

Responses of some thermally sensitive medullary dorsal horn neurons are greater when a monkey is required to attend to the stimulus than when he is not (Hayes et al., J. Neurophysiol., 46: 428-443, 1981). This enhancement in neuronal activity may reflect differences in sensory-discriminative aspects of a sensation or may be involved in affective or reflexive responses to the stimulus. The current study evaluates the effects of attention on the discriminative aspects of sensations evoked by innocuous and noxious thermal stimuli applied to the face of humans and monkeys.

Eight human subjects detected temperature perturbations of $< 1^{o}C$ from baselines of $39^{o}C$ and $45^{o}C$. Two contact thermodes were positioned bilaterally above the upper lip. Upon trial initiation both thermodes heated to $39^{o}C$ in some sessions and $45^{o}C$ in others and remained at that temperature for 3-10 sec before one thermode heated an additional step of $< 1^{o}C$. Subjects responded to the second temperature change by releasing a key. On 50% of the trials a signal light occurred on the left or right side of the response panel, indicating the side of the next temperature perturbation. Subjects were instructed to attend to the signaled thermode. For most trials the signal was accurate, but for 10% of the trials the signal was incorrect. From both innocuous ($39^{o}C$) and noxious ($45^{o}C$) baselines, detection latencies were shortest when the subject attended to the relevant thermode (correct signal condition) and longest when he attended to the irrelevant thermode (incorrect signal condition) (Friedman test, $p < .05$). When there was no location signaled, latencies were intermediate. In addition, the percentage of perturbations not detected was less for the correct than for the incorrect signal condition (Friedman test, $p < .05$). The shorter latencies and greater accuracy do not reflect a shift in response criterion, as percentage of responses occurring before the temperature shift (false alarms) was not different among stimulus conditions at either baseline.

One monkey was trained to detect small temperature changes from a $45^{o}C$ baseline or to detect the onset of a dim white light. For 50% of the trials stimulus modality was signaled by one of two red lights. The monkey detected more of the temperature shifts and produced shorter detection latencies for the signaled than for the unsignaled condition (sign rank test, $p < .05$). There was no difference in the percentage of false alarms.

These data show that detectability of thermal stimuli is influenced by attentional factors, both within and between stimulus modalities. This alteration in sensory detection could reflect the behavioral modulation observed in the medullary dorsal horn, an early stage of central nervous system processing.

140.9 SKIN NEAR THE DORSAL AND VENTRAL MIDLINES IS REPRESENTED BILATERALLY IN THE SPINAL CORD OF THE RAT. C.G.Smith* (spon: C.B. Gundersen). Sobell Department, Institute of Neurology, Queen Square, London WC1, England.

The traditional view is that the two sides of the body are represented separately in the spinal cord. This view does not take into account the finding that some cutaneous afferents terminate bilaterally. Electrophysiological studies have shown that afferents projecting bilaterally to the thoracic spinal cord in the rat innervate tactile domes near the dorsal and ventral midlines (Smith, C.G., J.Physiol., 334: 72P 1983). A population of dorsal horn neurons with bilateral receptive fields has now been identified.

Experiments were done on rats under urethane anesthesia. Recordings were made from single units in the thoracic spinal cord with a tungsten microelectrode. Activity in peripheral nerves was monitored with wire electrodes. The skin was divided along the midlines so that afferents supplying the two sides could be activated independently by mechanical stimulation.

Units with receptive fields extending across the dorsal midline were found in the lateral dorsal horn and units with receptive fields extending across the ventral midline were found in the medial part. The contralateral part of the receptive field was restricted to skin within 5-10 mm of the midlines. The ipsilateral part was usually much larger. The units were excited by displacing hairs in tactile domes and, in some cases, also by displacing other hairs.

Field potentials evoked by impulses in single cutaneous afferents could be detected in the dorsal horn, even without averaging. Impulses in dorsal horn neurons sometimes occurred during the field potentials suggesting that they were monosynaptically evoked. Some units appeared to receive monosynaptic connections from cutaneous afferents on both sides of the body.

Most bilaterally projecting afferents encountered in this study were slowly adapting units supplying tactile domes, in confirmation of my previous findings. However, two were rapidly adapting units. One was a typical hair follicle afferent supplying a group of hairs. The second was only excited by displacing one hair.

These findings show that the region of skin represented in each dorsal horn extends across the dorsal and ventral midlines and that inputs from skin on the two sides of the midlines are integrated within the spinal cord. This leads to the expectation that the skin near the midlines may also be represented bilaterally at higher levels of the somatosensory system by pathways independent of commissural connections in the brain.

140.10 SOMATOTOPIC ORGANIZATION OF CAT SPINAL CORD SEGMENTS WITH FUSED DORSAL HORNS. L. A. Ritz, J. L. Culberson* and P. B. Brown. Depts. of Physiology and Anatomy, West Virginia University Medical Center, Morgantown, W. V. 26506, U.S.A.

Segments T_4-T_{13} and S_3-Ca_8 of the cat spinal cord have fused dorsal horns; i.e., laminae I-V are continuous across the midline. We used these segments to test predictions about dorsal horn somatotopy, based on previous studies of hindlimb and forelimb regions. We predicted that dorsal skin projects laterally and ventral skin projects medially in the dorsal horn. We also predicted that the dorsal root dermatomes are tilted dorsorostral and ventrocaudal relative to segmental representations, to account for the rostrolateral/caudomedial orientation of dorsal root termination zones in the dorsal horn. To test these predictions we examined: 1) somatotopy of single units in the dorsal horn; 2) dorsal root dermatomes; 3) dorsal root projections in the dorsal horn. We hypothesize that 1) and 2) are determined by 3) (Brown and Fuchs, 1975).

As recording sites within the thoracic dorsal horn are shifted from the left edge to the right edge of the fused dorsal horns, single unit receptive fields (RFs) shift in overlapping fashion from the dorsal left trunk, ventrally across the midline, to the dorsal right trunk. Although no RFs spanned the dorsal midline, the RFs of units near the midline of the fused dorsal horns spanned the ventral midline of the trunk. Caudal and thoracic dermatomes reach, but do not cross, the dorsal and ventral midlines. The rostrocaudal dermatomal trajectory is proximodistal on the tail, with each dermatome overlapping adjacent ones but rarely reaching the edge of the second dermatome in either direction. They are not tilted relative to segmental representations, suggesting that (a) rostrolateral/caudomedial orientations of dorsal root projections do not exist in these segments, or (b) such orientations exist but are not related to somatotopy. S_3 is the most rostral dermatome entirely on the tail. Ca_5 through Ca_8 dermatomes all reach the tip of the tail; the rostral borders of these dermatomes continue the rostrocaudal dermatomal trajectory. Our degeneration results suggest an anatomical substrate for ventral midline continuity of thoracic dorsal horn RFs: dorsal root projections cross the midline of the fused regions. The lack of dorsal midline continuity is consistent with the minimal observed projections to contralateral lateral dorsal horn.

(Supported by USPHS grants 2R01 NS12061 and RR00374-13.)

140.11 CUTANEOUS INNERVATION OF THE HIND LEG OF THE RAT AND ITS SOMATOTOPIC ORGANIZATION IN LAMINAE I AND II OF THE LUMBAR SPINAL CORD. J.E. Swett and C.J. Woolf*. Cerebral Functions Group, Dept. of Anatomy, Univ. Coll. London, London WC1E 6BT, U.K.

From electrophysiological and anatomical experiments performed on cutaneous afferent fibers in subdivisions of the femoral and sciatic nerves of the rat we have compared the relative sizes and positions of their skin territories with their terminal fields in the superficial laminae of the dorsal horn. The subdivisions were the saphenous (SA), sural (C), lateral sural (LS), superficial peroneal (SP), tibial (T), and posterior cutaneous nerve of the thigh (PC).

Skin fields were charted in anaesthetized preparations by recording multiunit action potentials in each nerve evoked by probing the skin with non-noxious mechanical stimuli. Each nerve supplied a region separate and distinct from adjacent territories innervated by other nerves.

The terminal distributions of afferent fibers in the dorsal horn were determined for each nerve by reconstructing the sites of transganglionically transported wheat-germ agglutining conjugated horseradish peroxidase (WGA-HRP). Each nerve was exposed under anesthesia, cut, and the proximal stump isolated from surrounding tissues with low melting-point wax. Distilled water was applied to nerve end for 10 min followed by a 30% solution of WGA-HRP in water for 3-4 hours. The nerve was rinsed in saline and the wound closed. After survival periods of 48 to 72 hours the rats were sacrificed and the tissues processed according to the TMB procedure of Mesulam (1978). The distribution of label in Lamina I and substantia gelatinosa (SG) was reconstructed from coronal 40μm frozen sections from the L1 to S1 spinal segments. The rostral limit of the label did not extend beyond mid-L2 and the label occupied the medial portions of SG. In L3 and L4 the label broadened to occupy the medial 3/4 of SG. The caudal limit extended medially to mid or caudal L5. The longitudinal extent of the label was 7.5 to 8.4 mm: its maximum width 0.6 - 0.7 mm. In this zone each nerve labelled a separate and distinct longitudinal strip of lamina I and SG with the most dense label in the latter. The entire medial portion bordering on the dorsal columns received afferents from T. In L2 and L3 the SA label lay lateral to T. The SP label lay lateral to SA in L3 and T in L4. The label for S and LS was more lateral and extended between rostral L5 to the L2 - L3 junction. Finally PC occupied the most lateral margin of the zone in L4 and L5.

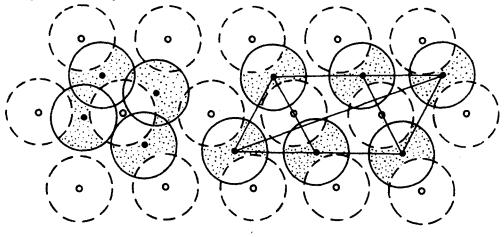
The principal observation was that each nerve occupied discrete part of SG so that together they formed a distorted but coherent impage of the skin surface of the hind limb in a two-dimensional plane. The evidence suggests that afferent inputs to SG are highly somatotopically organized.

(Supported by NINCDS Grant 17630 and the MRC).

VISUAL CORTEX: INTRINSIC ORGANIZATION I

141.1 EXPLANATION OF ORIENTATION COLUMNS IN TERMS OF A HOMOGENEOUS NETWORK OF NEURONS IN THE VISUAL CORTEX, V. Braitenberg. Max-Planck-Institut für Biologische Kybernetik, Tübingen, W. Germany.

We had suggested (Biol. Cybern. 33, 179, 1979) an arrangement of oriented line detectors around centers situated 0.5 mm from each other as being compatible with the available data. Singularities with that separation were indeed found later, the cytochrome oxidase blobs, and their location appeared to be related to orientation columns (Horton and Hubel, 1980). The blobs are due to the staining of mitochondria predominantly inside stellate cells (Carrol and Wong-Riley, 1982). The supposition that the blobs mark the site of inhibitory neurons leads to the following model, presented as a view of the cortex from the top.



The inhibitors (small circles) have dendritic fields (dashed circles) smaller than their separation in a hexagonal array. Each inhibits the pyramidal cells (black dots) surrounding its dendritic field. The input excites both kinds of neurons. A pyramidal cell is activated only if it is hit by the input within its own dendritic field (large circles) and if the dendritic fields of the nearby inhibitors are not hit. This leaves mostly elongated areas (stippled) with long axes oriented along circles surrounding the inhibitors.

These microfields measure only a few minutes of arc (in the fovea). The large receptive fields observed in single neuron recording must be compounded out of many microfields of the same orientation within a certain neighbourhood presumably by the formation of a Hebbian cell assembly. All the neurons of one assembly would have the same receptive field, and the scatter of receptive field centers relative to the recording site would naturally ensue. Since the connections subserving the assemblies are random, the formation of large and small, simple and complex receptive fields is left to chance. Movement sensitivity and directionality may depend on the relative distance of a neuron from the inhibitors on either side.

141.2 RETINOTOPY AND ORIENTATION COLUMNS IN THE MONKEY: A NEW MODEL. B.M. Dow and R. Bauer*, Neurobiology Division, Physiology Department, School of Medicine, SUNY, Buffalo, NY 14226.

The units of visuotopic localization in the retina are most likely single cones. The units of retinotopic localization in striate cortex are at present unknown. Cone separation projected onto the cortical surface achieves values close to 300μ in the central foveal region (Dow et al., Exp. Brain Res. 44:213, 1981). This is only slightly smaller than the distance between cytochrome oxidase patches (Hendrickson et al., Nature 292:605, 1981; Horton and Hubel, Nature 292:762, 1981), suggesting that cytochrome oxidase patches might serve as cortical retinotopic points. Based on this idea, a model is presented in which striate cortical orientation columns are generated directly out of retinotopy.

On the opercular surface except in center fovea retinotopic horizontal lines follow ocular dominance stripes, and retinotopic vertical lines cross ocular dominance stripes at right angles. Cytochrome oxidase patches are located in the middle of ocular dominance stripes (Hendrickson et al., 1981; Horton and Hubel, 1981). The model proposes that iso-orientation lines are arrayed radially around cytochrome oxidase patches as nodes. The nodes form a rectangular matrix superimposed upon the map of ocular dominance stripes. Horizontal iso-orientation lines follow retinotopic horizontal lines, running down the centers of ocular dominance stripes. Vertical iso-orientation lines follow retinotopic vertical lines, crossing ocular dominance stripes at right angles.

Preferred orientations in the infragranular layers are reversed with respect to the supragranular layers (Bauer et al., Exp. Brain Res. 50:133, 1983). New data indicate that the shift in orientation between upper and lower layers is minimal in zones of high monocularity, presumed to be cytochrome oxidase patches. The model proposes that inhibition radiating laterally into the supragranular layers from alternate cytochrome oxidase patches is responsible for the generation of diagonal (45° and 135°) orientation preferences. Diagonal orientations are consequently represented as alternating iso-orientation zones at the centers of the interstices in the matrix (internodal centers). There is, in fact, a predominance of diagonal orientations in the uppermost layers of striate cortex at eccentricities greater than 30 min (Bauer et al., Exp. Brain Res. 41:54, 1980). Inhibition radiating into the infragranular layers from the other set of alternate cytochrome oxidase patches is proposed as a mechanism for the orientation shift between upper and lower layers.

The model may help to explain some previously puzzling features of the relationship between ocular dominance columns, orientation columns, and retinotopy. It is highly specific and testable.

(Supported by NIH grant EY02349)

141.3 STRUCTURAL BASIS OF ORIENTATION SENSITIVITY IN CAT VISUAL SYSTEM J.D. Schall* and A.G. Leventhal. Dept. Anat., Univ. of Utah, Sch. Med., Salt Lake City, Utah 84132

Pronounced orientation sensitivity is a distinctive characteristic of neurons in cat visual cortex. This property, however, does not appear to be unique to cortical cells. It has been reported recently that most cat retinal ganglion cells (Levick & Thibos, 1982, J. Physiol. (Lond.), 329:243) and most relay cells in the cat's dorsal lateral geniculate nucleus (LGNd) (Vidyasagar & Urbas, 1982, Exp. Brain Res., 46:157) are sensitive to stimulus orientation. The orientation preferences of retinal ganglion cells are related systematically to their positions. Outside of the area centralis most retinal ganglion cells respond best to stimuli oriented radially, i.e., oriented parallel to the line connecting their receptive fields to the area centralis.

We investigated the structural basis of this orientation sensitivity. The dendritic fields of 840 retinal ganglion cells labeled by injections of horseradish peroxidase into the LGNd or optic tracts of normal cats, Siamese cats and cats deprived of patterned visual experience from birth by monocular lid-suture (MD) were studied. Retrogradely labeled cells were first drawn under the microscope using a 40x or 100x oil immersion objective and camera lucida. Cells were sampled along the horizontal, vertical and oblique meridians of the retina. Drawings of all cells were then traced onto a digitizing tablet interfaced to the laboratory's PDP 11/23 computer system. The Cartesian coordinates comprising the drawing were stored and the dendritic fields of all cells analyzed quantitatively. Mathematical techniques designed to analyze direction were used to find the dendritic field orientation of each cell. Statistical techniques designed for angular data were used to determine the relationship between dendritic field orientation and angular position on the retina (polar angle). Our results indicate that 88 percent of retinal ganglion cells have oriented dendritic fields and that dendritic field orientation is related systematically to retinal position. In all regions of retina more than 0.5 mm from the area centralis the dendritic fields of retinal ganglion cells are oriented radially, i.e., like the spokes of a wheel having the area centralis at its hub. This relationship was present in all animals and cell types studied and was strongest for cells located close to the horizontal meridian (visual streak) of the retina. Retinal ganglion cells appear to be sensitive to stimulus orientation because they have oriented dendritic fields.

141.4 SYSTEMATIC RELATIONSHIP BETWEEN PREFERRED ORIENTATION AND RECEPTIVE FIELD POSITION OF NEURONS IN CAT STRIATE CORTEX A.G. Leventhal. Dept. Anat., Univ. of Utah, Sch. Med., Salt Lake City, Utah 84132

It has been known for two decades that neurons in mammalian visual cortex respond selectively to stimuli falling on the retina at a particular angular orientation. Recent evidence suggests that most cat retinal ganglion cells (Levick & Thibos, 1982, J. Physiol. (Lond.), 329:243) and relay cells (Vidyasagar & Urbas, 1982, Exp. Brain Res., 46:157) in the cat's dorsal lateral geniculate nucleus are also orientation selective. In the retina there is a systematic relationship between receptive field position (polar angle) and preferred orientation. Outside of the area centralis, most retinal ganglion cells have oriented dendritic fields and respond best to stimuli oriented radially, i.e., oriented parallel to the line connecting their receptive fields to the area centralis (Levick & Thibos, 1982, J. Physiol. (Lond.), 329:243). This relationship is strongest close to the horizontal meridian (the visual streak) of the retina.

To determine if a relationship between preferred orientation and polar angle exists in visual cortex, the preferred orientations and receptive field positions of 768 striate cortical neurons were studied. As in the retina a systematic relationship exists between preferred orientation and visual field position in area 17. In parts of striate cortex 15 to 80 degrees from the area centralis projection there is a strong tendency for cells to respond best to lines oriented radially. In regions 4 to 15 degrees from the area centralis projection this relationship is somewhat weaker. In regions subserving the central 4 degrees of visual angle no such relationship exists. Throughout area 17 the relationship between preferred orientation and polar angle is strongest in regions subserving the horizontal meridian.

It is suggested that the systematic relationship between preferred orientation and polar angle which begins in the retina provides an intrinsic framework for the organized arrangement of orientation "columns" in visual cortex. This relationship may also be responsible for the "oblique effect" as well as the visual system's preferential response to radially oriented gratings (Rovamo et al., 1982, Invest. Ophthal. Vis. Sci., 23:666).

141.5 SURFACE ORGANISATION OF FUNCTIONAL AND TOPOGRAPHIC MAPS IN CAT VISUAL CORTEX. M.S.Cynader, J.Matsubara and N.V.Swindale*. Depts. of Psychology and Physiology, Dalhousie Univ., Halifax,N.S.,Canada, B3H 4J1.

We have examined the topographic organisation of functional neuronal properties in cat area 18 by making a large number of penetrations (often over 100) into the cortex and examining neuronal responses. Penetrations were spaced about 300 um apart in a regular grid pattern on the cortical surface; the depth of recording was about 400 um. At each recording site we examined 1) receptive field location, 2) ocular dominance and 3) orientation selectivity.

The results indicate that the retinotopic map is markedly anisotropic in area 18, with the magnification factor for vertical being at least twice that for horizontal. This anisotropy makes it clear that the mapping between the retina and the cortex is not conformal: right angles in the visual world are not, in general, mapped onto right angles on the cortical surface. The exception occurs with the cortical representation for horizontal and vertical axes. The cortical representation of retinal iso-elevation and iso-azimuthal contours is a set of orthogonal contours on the cortical surface. This implies a special significance for these two directions in cortical processing.

A cortical point spread function was defined as that region of cortex likely to contain cells with receptive field centers in common: this turned out to be about 1.2 mm wide and 1.8 mm long, the elongation being in the direction in which magnification factor is a maximum.

Neurons with similar functional properties occur in patches within the cortex. The fine grain map reveals patches of cells dominated by one eye or the other. Likewise, cells responding to a given orientation were located near other cells preferring the same orientation. Yet the cortical representations of different response features varied in their orderliness, periodicity, inter-animal consistency and direction of elongation. Units preferring the same orientation were arranged in branching stripes running posterior-medial to antero-lateral across the cortical surface. The period of these bands measured by spectral analysis was 1.26±0.03 mm. By contrast, the organisation of units according to eye dominance was much less regular and the periodicity less well defined, being in the range 1.2 - 1.8 mm.

The iso-orientation domains tend to run in the same direction as the cortical representation of the iso-elevation contours of visual space. The anisotropy in the magnification factor and the elongation of the point spread function has the effect of allowing a complete set of orientation columns to fit economically into a cortical territory which represents a roughly circular region of visual space.

141.6 THE ROLE OF ORIENTATION TUNING ON THE SPECIFICITY OF LOCAL INTRACORTICAL CONNECTIONS IN CAT VISUAL CORTEX: AN ANATOMICAL AND PHYSIOLOGICAL STUDY. J. Matsubara & M. Cynader, Depts. of Psychology & Physiology, Dalhousie Univ., Halifax, N.S., Canada.

Clusters, lattices and bands of cells have been observed to participate in intra- and inter-cortical projections. Are physiological response properties of anatomically-connected cells similar or different? We address this question by combining physiological recordings and anatomical tracing techniques in area 18 of cat visual cortex. Following fine-grain mapping (Cynader, Matsubara & Swindale, 1983) neural tracers (WGA-HRP or fluorescent dyes) are injected 700μ below surface into areas of the cortex chosen on the basis of their response properties. After perfusion, the brain is blocked and horizontal sections cut parallel to the cortical surface. Comparisons of the charts of labelled cells with the physiological data allow us to characterize response properties of projection neurons.

Within area 18 our results indicate the following: (1) Orientation selectivity plays a dominant role in local cortical connections. Injections of WGA-HRP into iso-orientation domains result in a patchy distribution of retrogradely-filled neighbouring cells. Like the cells of the injection site, these projection cells are also within iso-orientation domains. Furthermore, the best orientation of the projection cells is orthogonal to the best orientation of the injection site cells: e.g. cells preferring vertically-oriented stimuli project to cells preferring horizontally-oriented stimuli. We speculate that this projection is inhibitory and that it is necessary for enhancement of the orientation selectivity of cortical cells. (2) The area over which labelled cells are found extends further in the anterior-posterior rather than the medial-lateral direction. Thus the population of projection cells covers more cortical surface in the A-P direction (i.e. vertical in visual space). This was observed for every injection confined to an iso-orientation domain regardless of the best orientation of the cells within the injection site. Due to the anisotropy in cortical magnification factor found for the representation of vertical and horizontal visual space, the accumulated receptive field of the population of labelled cells corresponds to a roughly circular area in visual space. (3) An injection into one iso-orientation domain typically yields 3-5 patches of projection cells. Mean distance between neighbouring patches is 1.12±0.2 mm(corrected for shrinkage of tissue). (4) Local connections do not appear to display any obvious relationship to ocular dominance properties.

141.7 INTRINSIC CONNECTIONS OF MACAQUE MONKEY STRIATE CORTEX. J.S. Lund, D. Fitzpatrick*, G.G. Blasdel. (SPON: C.J. Wallis) Lab. of Ophthalmology Research, Medical University of South Carolina, Charleston, S.C. 29425.

Orthograde and retrograde transport of horseradish peroxidase (HRP) was used to trace the inter- and intralaminar projections of small populations of neurons following small iontophoretic injections of HRP into individual laminae of striate cortex. Uptake by fibers of passage or apical dendrites did not appear to present problems in the interpretation of the results. We have been particularly interested in the relative degree of lateral spread of inter- and intralaminar intrinsic projections since they may be reflected in particular physiological properties.

The neurons of the magnocellular recipient zone ($4C\alpha$) project in a diffuse and laterally spreading fashion upon 4B in contrast to the dense vertically organized projection of $4C\beta$ neurons upon 4A-3B. The lateral spread of connectivity is emphasized even further in 4B itself where injections demonstrate widespread intralaminar connections with a patchy distribution of terminal fields (which include upper $4C\alpha$). The neurons of laminar 6 project heavily with little lateral spread upon $4C\alpha$ and $4C\beta$ and can be distinguished from thalamic axons by their fine calibre.

The projection zone of upper $4C\beta$ neurons inpinges upon 4A-3B which receives direct geniculate input and also a strong vertically organized input from lamina 6 neurons. 4A-3B projects in a diffuse spreading fashion to lamina 2-3A where a spreading projection is further emphasized by intralaminar connections with a broad and patchy distribution. This region also project's heavily to lamina 5B with an emphasis on vertical connections. Lamina 2-3A receives additional input from 4B and 5B in both a prominent, vertical fashion and a more diffuse lateral input.

Laminae 5 and 6 do not show prominent lateral intralaminar connections using the present technique and in this respect differ from lamina 4B and 2-3A.

These results indicate striking differences in the patterns of connectivity for each of the laminae in monkey striate cortex. Supported by EY03321.

141.8 ORGANIZATION AND INTRACORTICAL CONNECTIVITY OF LAYER 4 IN MACAQUE STRIATE CORTEX. Gary G. Blasdel, David Fitzpatrick*, and Jennifer S. Lund. Departments of Anatomy and Ophthalmology, Medical University of South Carolina, Charleston, South Carolina, 29425.

From previous studies it is known that most geniculate afferents terminate in layers 4A and 4C of macaque striate cortex. It is also known that striate efferents originate from neurons located outside these laminae. We have used micro-iontophoretic injections of horseradish peroxidase (HRP), in conjunction with electrophysiological recordings, to study the organization of the intervening connections. On the basis of these studies we find evidence for the subdivision of layer 4C into 4 zones. In upper 4C-alpha we find label in multiple lateral patches (that extend into 4B) after a single injection of HRP into layers 4B and 4C-alpha. Electrophysiologically we find that upper 4C-alpha may contain orientationally selective Simple and Complex cells as well. Lower 4C-alpha, by contrast, does not contain laterally displaced patches of label (following HRP injections into 4B) and is observed to be composed primarily of units with small receptive fields that lack orientation selectivity. The results of both orthogradely and retrogradely transported HRP indicate that upper 4C-beta has a precise, point to point, projection onto layers 4A and lower 3. Lower 4C-beta, by contrast, appears not to project to this zone since its cell bodies remain unlabeled following microinjections of HRP into 4A and lower lamina 3. The units recorded in both upper and lower 4C-beta have extremely small receptive fields and respond to all orientations of visual stimulation.

For lower 4C-alpha as well as upper and lower 4C-beta it is possible to observe precisely ordered micromaps within the dimensions of single ocular dominance columns. The progression in receptive field position that occurs with each advancement of a tangentially directed electrode is small in comparison with the minimum response fields of the recorded units; accordingly it is best characterized as one of partially shifted overlap. From the local magnification factor it is possible to calculate the horizontal distance in striate cortex that separates regions of layer 4C that deal with separate and non-overlapping parts of visual space. In lower 4C-alpha this distance is about 660 micra and in 4C-beta it is about 290 micra. These distances correspond, within a factor of 2, to the known axonal arborizations of single geniculate afferents within each of these sublaminae. Supported by EY03321.

141.9 HORIZONTAL CONNECTIONS IN STRIATE CORTEX AS REVEALED BY CROSS-CORRELATION ANALYSIS. D. Ts'o, C. Gilbert, and T.N. Wiesel. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

Anatomical and physiological methods have been used to demonstrate the columnar organization of neurons and neuronal circuitry in the visual cortex. Intracellular filling of cells and other methods of labeling have also revealed extensive horizontal connections in the visual cortex which seem to be clustered in nature[1,2]. The functional significance of these horizontal connections and their contribution to the features of cortical receptive fields is largely unknown. The present experiments investigate the relationship between the physiological connectivity of pairs of cells as shown by cross-correlation methods[3] and the cells' receptive field properties.

Recordings in striate cortex from two independently manipulated extracellular electrodes were processed by window discriminators and fed into a digital computer programmed to perform real-time auto- and cross-correlations with high temporal resolution. This arrangement allowed rapid assessment of the physiological connectivity of cell pairs as the electrodes were advanced from cell to cell and receptive fields were characterized. We began our experiments in the monkey striate cortex by electrophysiologically mapping the orientation columns in a limited region of cortex. Using one roving electrode and one fixed electrode, this map provided a systematic means for studying connectivity between spatially separate regions of various orientation specificities.

Preliminary results in both the monkey and the cat showed that units separated by .2mm or more with overlapping receptive fields but differing in receptive field properties such as orientation or directionality had responses which tended not to be correlated. At these distances, pockets of units exhibiting correlated firing had receptive field properties that matched. However, not all regions of units with matching receptive field properties showed correlated responses. The correlograms obtained suggest that horizontal interactions at these distances are excitatory. These findings provide physiological evidence for a functional contribution of horizontal connections and are consistant with anatomical demonstrations of the clustered nature of horizontal connectivity in the visual cortex. (Supported by grants EY00606, NS16189, and EY070402.)

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141.10 LATTICE-LIKE INTRINSIC NEURAL CONNECTIONS IN MACAQUE PRESTRIATE VISUAL CORTEX. K.S. Rockland. Southard Lab of Neuropathology, E.K. Shriver Center, Waltham, Mass. 02154

Earlier work (Rockland and Lund, '83) described a lattice-like pattern of intrinsic connections in striate cortex (area 17) of macaque and squirrel monkeys. The lattice walls, visualized by injections of horseradish peroxidase (HRP) into area 17, contain retrogradely filled neurons and orthogradely filled axon terminals, as well as labeled fibers. The lattice interstices are crossed by labeled fibers, but are not otherwise labeled. Injections of HRP in several prestriate areas in macaque reveal complexly patterned intrinsic connections in these areas also. In area V2 (injections placed in the lunate sulcus, annectent gyrus, and ventral to the calcarine sulcus) retrogradely labeled neurons occur in layers III and V. These neurons are in register with terminal arborizations, which are labeled in layers I-VI, but are particularly dense in layers III and V. In coronal sections, these connections appear organized in radial columns, measuring about 400u across and visualized for 2.0 -3.0mm from the injection site. Injections in area V4 (on the prelunate gyrus) similarly reveal labeled neurons in layers III and V, in register with labeled terminations. In area V4, these connections measure about 500-750u across and can be followed for 3.5 -4.0mm from an injection. Intrinsic connections in both areas V2 and V4 can also be demonstrated by injections of 3H-amino acids. When examined in the tangential plane of section, these connections, like those in area 17, are seen to have a reticular or lattice-like configuration. In squirrel monkey, similarly organized periodic intrinsic connections occur in several prestriate areas (V2 and MT).

In both macaque and squirrel monkeys, cytochrome oxidase preparations reveal regular inhomogeneities in area V2 that are organized in a stripelike array, running orthogonal to the border with area 17. These cytochrome oxidase stripes occur in layers III and V (measuring 1000-1500u across in macaque, 750-1000u in squirrel monkey). Preliminary data suggest that the cytochrome stripes (corresponding to pulvinocortical terminations, Livingstone and Hubel,'82) do not overlie the intrinsic connectional lattice in prestriate cortex. Although the functional role of neither the cytochrome nor the intrinsic connectional pattern in prestriate cortex is clear, the large tangential spread of the intrinsic lateral connections (over 3.0-4.0mm) may imply that they mediate effects larger than one receptive field area. (Supported by EY04946)

141.11 TOPOGRAPHY OF CYTOCHROME OXIDASE PATTERNS IN EXTRASTRIATE CORTEX OF THE OWL MONKEY. Roger B. H. Tootell*, Martin S. Silverman*, and Russell L. DeValois* (spon:D. Albrecht). Dept. of Psychology, University of California, Berkeley, California 94720.

The discovery of spots (blobs, puffs) in V1 and strips in V2 has prompted a number of experiments on the radial architecture of these regions in the primate. In both areas, the periodic radial landmarks allow an analysis of module structure and function, and in V2 the termination of the strips furnishes a clear anatomical marker for defining the anterior boundary of the region.

Presumably, similar anatomical analyses will be possible in any cortical area in which a distinctive topography can be demonstrated. In order to look for such areas, we flat-mounted the entire surface area of the owl monkey cortex, sectioned it tangentially, and processed the tissue for cytochrome oxidase. Alternate sections were stained for myelin or nissl substance.

Distinctive cytochrome oxidase patterns are found in at least five cortical areas in addition to V1 and V2: 1) The middle and upper layers of MT appear as a dark oval region (10 X 5 mm) interspersed with irregular ovoid or annular regions of lighter density, surrounded by a thin, dark, beaded ring along all, or part, of the MT-DL border. In the lower layers this intricate cytochrome oxidase pattern is entirely absent, but it is "replaced" by a topographically identical (but slightly blurred) pattern in adjacent sections stained for myelin. It is unclear whether the outer ring should be considered part of MT or DL. 2) VA/VP is characterized by a set of thin (and sometimes spotty) strips which run parallel to the V2 strips, but can be differentiated from them on topographical grounds. 3) There is also a long thin area (about 2 by 7 mm) near the border of DM and M which is quite variable, but generally contains one or two darker stripes with spots all around. 4) Auditory cortex (tentatively areas A1 and/or R) appears as a very dark, homogenous, irregularly-shaped patch about 8 X 5 mm with fuzzy boundaries, which is visible in all cortical layers but most obvious in the middle and deeper layers. 5) Beyond the sylvian fissure, there is a very long (about 4 cm), thin (2-4 mm) ⌋ -shaped structure which is restricted to a middle cortical layer. The base of the "⌋" appears to correspond to area 3b of somatasensory cortex; the longer "side" of the structure runs through ventral cortical regions which have not been well-mapped by electrophysiological means. The boundaries of this long bent strip are sharp, irregularly wide, and blotchy. Supported by EY00014 and BNS 78-06171. Special thanks are due to John Allman, Michael Merzenick, and Michael Stryker for advice and material assistance during the course of this study.

141.12 INTERACTION DISTANCES FOR SUCCESSIVELY FLASHED STIMULI IN MACAQUE MT. A. Mikami, W.T. Newsome and R.H. Wurtz, Lab. of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.

Sequentially flashed stimuli have been profitably used by several investigators to study mechanisms of direction selectivity, and we have applied this method to the study of direction selectivity in extrastriate area MT of the macaque monkey (Mikami et al., ARVO Absts., 1983). One of the most striking results of our experiments has been the large interflash distances for which directional interactions can be obtained. We now report that this "interaction distance" is highly correlated with the speed for which direction selectivity can be obtained with smoothly moving stimuli.

MT neurons were isolated in awake behaving macaque monkeys. Receptive field boundaries were plotted, and tuning for direction and speed of smoothly moving stimuli were quantitatively determined. We then systematically varied the spatial separation and temporal interval between flashes for sequences in the preferred and null directions to determine the maximum interaction distance and interaction interval, respectively, for each cell. The ratio of response in the preferred direction to response in the opposite direction was arbitrarily required to be 5 to 1 before interactions were considered to be direction selective. By this criterion, the maximum interflash distance which yielded directional interactions in MT neurons ranged from .32° to 6.0° with a geometric mean of 2.1°. In recordings from a few neurons in MST (a motion-related area to which MT projects) interaction distances were as large as 10°. Some of these interaction distances were an order of magnitude larger than those reported in any previously studied visual structure.

For 36 neurons we attempted to correlate the maximum interaction distance with the highest speed for which the neuron was direction selective to smoothly moving stimuli. Natural logs of the two measures were highly correlated ($r=.82$; $p<.001$; $df=34$). The maximum temporal intervals for which directional interactions could be obtained were not correlated with speed for directionality for smoothly moving stimuli. These results show that the capability of an MT neuron to discriminate direction at high stimulus speeds is a function of the spatial extent of directional interactions within the receptive field. Since many of the interaction distances we observed are larger than the average receptive field diameter of striate neurons at equivalent eccentricities, it seems likely that the discrimination of direction at high stimulus speeds is a functional elaboration found primarily in the large receptive fields of extrastriate visual areas.

141.13 INFLUENCE OF STIMULUS VELOCITY ON MONKEY STRIATE NEURONS: CHANGES WITH ECCENTRICITY. G.A. Orban, H. Kennedy* and J. Bullier*. Laboratoire de Neuropsychologie Expérimentale, INSERM-U 94, Bron (France) and Laboratorium voor Neuro-en Psychofysiologie, KUL, Medical School, Leuven (Belgium).

Area 17 neurons were recorded in anesthetized and paralyzed cynomolgus monkeys. The influence of stimulus velocity of an optimal narrow slit was tested with a randomized multihistogram technique (10 to 15 velocities ranging from .2 to 800°/sec). Background illumination was 8 cd/m^2 and stimulus luminance 36 cd/m^2. The neurons recorded in area 17 subserving central vision (0-2° eccentricity, n = 30) were compared with those subserving peripheral vision (eccentricity over 15°, n = 31). Histological verification showed that in both eccentricity classes all layers were sampled. Velocity sensitivity was measured by the half height upper cut-off velocity (Orban et al., J. Neurophysiol., 45, 1043, 1981) i.e. the velocity at which the response falls to half the maximum response and by the response to slow movement, i.e. the response to .2°/sec movement expressed as percent of the maximum response. The velocity sensitivity changed with eccentricity : the half height upper cut-off velocity increased on average from 8.7°/sec to 61°/sec, while the average response to slow movement decreased from 86% to 61%. In area 17 subserving central vision most cells (80%) were velocity low pass (Orban et al., J. Neurophysiol., 45, 1043, 1981) while the majority of the cells in the periphery (68%) were velocity broad band. The proportions of velocity tuned cells and direction selective cells (as defined in Orban et al., 1981) were small in both eccentricity classes. These results show that the dependence of velocity sensitivity and direction selectivity on eccentricity in area 17 of the monkey is similar to the same relationship in area 17 of the cat but differs from the situation in cat area 18 where the direction selectivity changes with eccentricity.

141.14 2-D PATTERNS ARE REPRESENTED IN TEMPORALLY MODULATED ACTIVITY OF STRIATE CORTEX CELLS. B. J. Richmond, L. M. Optican*, and M. Podell*, Laboratory of Neuropsychology, NIMH; Laboratory of Sensorimotor Research, NEI; NIH, Bethesda, MD 20205.

Although the receptive field properties of single neurons in area 17 have been studied extensively, receptive field structure alone can not predict a cell's temporal response to 2-D patterns because it does not describe a spatial-to-temporal transformation. We wished to determine how 2-D pictures were represented in the temporal activity of single units in area 17.

We used 64 black and white stimuli, 3 deg square, that formed a complete, orthogonal basis set (based on Walsh functions) for pattern representation. The monkeys were trained to fixate a spot of light. The fixation light blinked off 300ms before the 400ms stimulus presentation; 400-700ms later the fixation point returned and dimmed. Only successfully completed behavioral trials were analyzed. The receptive fields of the neurons studied were approximately 3 deg contralateral to the center of gaze.

Activity of these single units was found to differentially represent members of the stimulus set. The poststimulus spike train was convolved with a Gaussian pulse to form a probability density function of spike occurrence. An average spike density function was then computed for each of the 64 stimuli. The temporal modulation caused by each pattern was quantified by decomposing the responses into their principal components with a Karhunen-Loeve transform. Two to three components were needed to reconstruct the cell's activity well enough to preserve its ability to differentiate among stimuli. Since the KL transform requires fewer components than any other linear transform (e.g. Fourier) for a given accuracy in representing the spike density functions, no single linear measurement can adequately represent the ability of these cells to discriminate among 2-D patterns.

Some patterns which give the same response when measured with any single parameter, e.g. spike count, are differentiable when the waveform of the activity (as measured with multiple components) is considered. There was very little difference in the cell's ability to differentiate stimuli when the waveforms were analyzed over short (128ms), rather than long (384ms) periods. We conclude that single striate neurons can convey detailed information about 2-D visual features in the temporal modulation of their activity.

We have recently shown temporal modulation by 2-D patterns of cell activity in inferior temporal cortex (ARVO abstracts, 1983). These results in two visual areas suggest that temporal modulation in response to visual features is important for pattern processing in the visual system.

142.1 SEPARATION OF DIRECT AND AROUSAL-RELATED INFLUENCES ON LEARNING AND MEMORY. Rita B. Messing and Sheldon B. Sparber*. Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Many agents may act in learning and memory tests by altering arousal levels. However, little systematic attention has been given to the problem of separating neurohumoral or drug effects on arousal from more direct actions on encoding and retrieval of information. This is most likely because it is thought to be difficult to conveniently manipulate arousal independently of drug doses and unconditioned stimulus parameters.

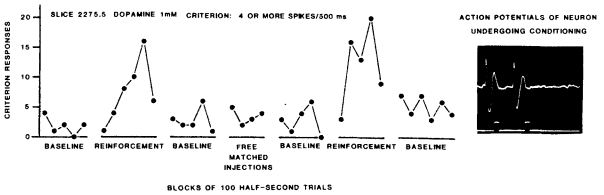
Recently, while investigating drug effects on autoshaped behavior, we predicted that the rate of acquisition could be enhanced by increased arousal produced by injecting rats with saline shortly before testing. Accordingly, male Long Evans rats, maintained at 85% of free-feeding body weights, were trained in a discrete trial, autoshape procedure, in which they were rewarded with 45 mg food pellets for touching a retractable lever presented on a 45 s random time schedule. Retracted lever contacts (nose-pokes) and unconditioned exploratory (rearing) activity were simultaneously monitored. Groups of rats (n=5-6), subjected or not subjected to handling and injection for 10 days, were administered 1 ml/kg of 0.9% NaCl s.c. 5 or 30 min before each of 2 autoshaping sessions, 2 days apart. They received 1 block (A) of 12 trials (lever presentations) in the first session and 3 blocks (B,C,D) in the second. No significant treatment effects on basal (Block A) response rates were found for any behavior. Treatment effects on learning were evaluated by a repeated measures analysis of variance on the change in responding between block A and blocks B and C. The results indicated that handling and injection history had no effect on acquisition, but that the rats administered saline closer to testing acquired the response more rapidly (F=4.78, df=1,17, P=0.04) (see Table).

	Δ Extended Lever Touch Responses (X ± S.E.)	
Inj. Time	Block B - Block A	Block C - Block A
-5 min	4.2 ± 0.9	5.1 ± 1.2
-30 min	0.9 ± 1.0	2.6 ± 1.0

We thus propose that this procedure provides a simple method for manipulating arousal independently of other experimental variables. Based on classical conceptions of arousal effects on learning and performance, substances which act by increasing arousal should facilitate acquisition in animals given saline more than 30 min before training, but should impair it in more highly aroused rats injected with the saline probe 5 min before.
(Partially supported by USPHS Grant T32DA07097.)

142.2 OPERANT CONDITIONING: CELLULAR OR SYSTEMS PROPERTY? James D. Belluzzi and Larry Stein. Department of Pharmacology, College of Medicine, University of California, Irvine, Irvine, CA 92717.

When behavior is followed by reward, the probability of the behavior is increased by a process called positive reinforcement or operant conditioning. A fundamental problem is to identify the functional unit for positive reinforcement in the brain. The simplest possible unit whose behavior may be capable of operant conditioning is the single brain cell. Accordingly, we attempted to demonstrate operant conditioning at the cellular level by a procedure that resembles behavioral operant conditioning. A glass micropipette for simultaneous recording and pressure injection is filled with dopamine (1 mM in 165 mM saline) or other drugs and aimed at spontaneously active cells in whole brain or CA1 cells in hippocampal slices. The neuronal response for reinforcement ("criterion response") is defined as a half-second of relatively fast activity. The requisite number of spikes/500 ms is individually established for each neuron studied so that, prior to operant conditioning, criterion responses occur at a rate of less than 5 per minute. During conditioning, a pump is briefly activated immediately after each criterion response to deliver a 10 μ-diameter droplet of drug or saline to the cell. The FIGURE shows a complete experiment for a CA1 cell reinforced with dopamine. In the first baseline phase, two criterion responses on average are observed in each block of 100 trials. Criterion responses increased sharply following the introduction of dopamine reinforcement. A matched number of noncontingent ("free") dopamine injections had no such effect, suggesting that direct stimulation could not explain dopamine's facilitatory action — indeed, noncontingent dopamine injections generally suppress the activity of CA1 cells. A second successful reinforcement phase suggested that the preparation remained viable throughout. Positive results were obtained in 8 of 16 additional dopamine experiments and 11 of 48 cocaine experiments, whereas saline, GABA, and serotonin gave negative results. These findings suggest that neuronal firing patterns can be individually conditioned by the local application of a reinforcing transmitter or drug. Since it is unlikely that a neuron would display a gratuitous capacity for positive reinforcement, we suggest that operant conditioning is a cellular rather than systems property.
(Supported by NIDA 02725 and AFOSR F49620-81-K-0015).



142.3 THE CHOLINERGIC NERVOUS SYSTEM AND TEMPORAL MEMORY IN RATS. Warren H. Meck* and Russell M. Church. Department of Psychology, Brown University, Providence, RI 02912.

The purpose was to determine if reference memory for reinforced durations could be selectively adjusted by the pharmacological manipulation of central cholinergic neurons. A peak procedure was used to study the scaling of duration by rats (e.g., Roberts, J. Exp. Psychol. Anim. Behav. Processes 7: 242, 1981). In this procedure, after an intertrial interval a signal occurs and, on some trials, food is primed after a fixed duration and the rat's next lever press is reinforced; on other trials no food is available and the trial lasts for a relatively long time after the fixed duration. As a result, the response rate of the rat initially increases as a function of the time since signal onset, as it does during standard fixed-interval training. After the time that food is sometimes available, however, the response rate decreases in a fairly symmetrical fashion when response rate is plotted on a linear time scale. The time the response rate is maximal is called the "peak time." It occurs near the time that food is maximally expected and serves as a measure of the rat's temporal criterion which is a value stored in reference memory. Using this procedure with reinforcement sometimes following the rat's first lever press after a white noise signal had been present for 20 sec, we demonstrated that physostigmine administered i.p. decreased the variability of the temporal discrimination and shifted peak times permanently leftward on the time scale in a dose-dependent fashion (0, .01, .03, & .09 mg/kg). Neostigmine, an anticholinesterase that does not readily cross the blood-brain barrier, did not produce these effects. Atropine administered i.p. increased the variability of the temporal discrimination and shifted peak times permanently rightward on the time scale in a dose-dependent fashion (0, .05, .15, & .45 mg/kg). Methyl-atropine, a cholinergic receptor blocker that does not readily cross the blood-brain barrier, did not produce these effects. Application of a scalar timing model indicated that physostigmine decreased the remembered values for reinforced durations and increased sensitivity to time, while atropine increased the remembered values for reinforced durations and decreased sensitivity to time. These results extend the findings of Meck (J. Exp. Psychol. Anim. Behav. Processes 9: 171, 1983) to a different timing procedure and suggest that the effective level of brain acetylcholine sets memory storage speed for sound durations in the rat. Thus, physostigmine increased memory storage speed and rats remembered clock readings as shorter than they actually were, and atropine decreased memory storage speed and rats remembered clock readings as longer than they actually were.
(Supported by NIMH Grant MH 37049.)

142.4 DISSOCIATION OF CHOLINERGIC DRUG EFFECTS ON SHORT-TERM AND LONG-TERM MEMORY IN MICE.
J.F. Flood, G.E. Smith and Arthur Cherkin. GRECC, VA Medical Center, Sepulveda, CA 91343

Research on drug-induced enhancement or impairment of memory retention is plagued by inconsistent results. Suggested sources of inconsistency include variations in: behavioral paradigms; time intervals between training, drug administration and retention testing; and in drug doses. We present evidence which: (1) emphasizes the importance of these variables; (2) demonstrates their impact with a supra-additive two-drug combination as well as with the single drugs alone; and (3) reveals a dissociation of drug effects upon memory retention measured 3 hours vs 168 hours after training and drug injection.

The drugs, arecoline hydrobromide (ARE) and edrophonium chloride (EDR; Tensilon) were injected subcutaneously immediately after training. The subjects were CD-1 male mice, 8 wk of age. The paradigm was T-maze active avoidance, under two training conditions, TC1 and TC2. TC1 was adjusted so that control mice showed good retention performance 1 hour but not 3 hours after training. Under TC2, controls showed good retention 24 hours but not 168 hours after training. Dose-response was studied under both TC1 and TC2, using ARE, EDR or the ARE + EDR combination at a fixed ratio. The optimal dose (mg/kg) for maximal memory retention scores are presented in the table.

Drug	Dose - TC1 (3-h Test)	Dose - TC2 (168-h Test)	Ratio TC2/TC1
ARE	0.30	1.25	4.17
EDR	1.20	7.00	5.83
ARE + EDR	0.20 + 0.60	0.05 + 0.22	0.25; 0.37

The results indicate that the optimal dose of each single drug for 168-hour retention averages 5 times the optimal dose for 3-hour retention. Such higher doses, when used under TC1, impaired memory retention or impaired sensory and motor performance when the mice were tested 3 hours after injection. If only a single high dose were tested under TC1, it would appear to have no effect on memory.

The results indicate that the two-drug combination permitted a substantial reduction of the dose required for optimal retention under TC2 (96% less ARE and 97% less EDR). The reductions were much less under TC1 (33% less ARE and 50% less EDR). The optimal combination dose for maximal 168-hour retention had no effect upon 3-hour retention. The single drug doses which improved 168-hour retention impaired 3-hour retention or performance.

142.5 ECT-INDUCED ANTEROGRADE AMNESIA-A POSSIBLE CHOLINERGIC MECHANISM. B. Lerer*, M. Stanley*, H. Altman and I. McIntyre*. (SPON: H. Goldman). Lafayette Clinic Wayne State University Sch. of Med., Detroit, MI 48207

Patients treated with electroconvulsive therapy (ECT) manifest an anterograde amnesia characterized by impaired delayed recall of newly learned material. The following studies were undertaken in order to elucidate a possible neurochemical basis for ECT-induced amnesia.

Electroconvulsive shock (ECS) was administered daily for 7 days to rats which were sacrificed 24 hours after the last shock. ECS was found to induce a statistically significant 15% and 13% reduction in ^{3}H-QNB binding to muscarinic cholinergic receptors in rat cerebral cortex and hippocampus respectively. Scatchard analysis of binding data showed a reduction in Bmax without a change in Kd. Rats sacrificed 24 hours after a single ECS did not show a similar reduction in ^{3}H-QNB binding. Concurrent daily ECS was also found to completely block a significant increase in cortical ^{3}H-QNB binding caused by chronic atropine administration (10mg per Kg ip. for 5 days).

In a parallel experment groups of ECS and sham-treated rats underwent passive-avoidance training 24 hours following the last of a series of daily ECS for 7 days (training parameters: 0.5mA for 1 sec. inescapable shock). Retention of the original avoidance habit was measured 7 days later by returning the animals to the lighted side of the shuttle-box; after a brief accommodation period (10 sec.) the door separating the lighted from the dark chamber was opened and the animal's latency to cross (step-through latency) was recorded. Highly significant impairment of delayed recall was demonstrable in the ECS-treated group. Such impairment was not present in rats trained 24 hours following a single ECS.

The parallel time-course of muscarinic receptor subsensitivity and memory impairment induced by repeated ECS suggests a possible causative relationship. This possibility will be discussed in the context of the results of further studies evaluating the effect of cholinergic agonists on the ECS-induced memory impairment.

142.6 ALLEVIATION OF FORGETTING IN RATS BY INTRAVENTRICULAR INJECTION OF APOMORPHINE BEFORE RETENTION TEST. S.J. Sara *, G. Grecksch * and M. Hongenaert * (SPON : D. Quartermain). Dépt de Psychophysiologie CNRS 91190 Gif-sur-Yvette, France and Inst für Pharmakologie und Toxikologie, Med. Akad. Magdeburg, DDR.

Rats which are trained in a maze consisting of 6 left-right choices learn the task in 5 daily trials. When tested 3 weeks later, they exhibit significant retention deficits. We have found that this forgetting can be alleviated by several pretest treatments, the most effective beeing exposure to contextual cues of the experimental environment immediately prior to the retention test (Deweer, B. et al, Anim. Learn. Behav. 8:2, 1980). Systemic injections of small doses of amphetamine also facilitated performance in the same paradigm (Sara, S.J. & Deweer, B., Behav. Neural Biol. 36:146, 1982), leading to the hypothesis that contextual cue reminders facilitate memory retrieval through conditioned release of catecholamines (see also Cassens, G. et al, Science 209:1138, 1980). The present experiments address the question of specificity of site of action (central or peripheral), by using intraventricular injections of amphetamine, which releases both dopamine and noradrenalin, and the more specific agonist, apomorphine, a dopamine receptor stimulator.

The rats were trained with 5 daily trials ; one week later they were implanted with intraventricular cannula ; they were tested for retention 3 weeks after the last training trial. Fifteen minutes prior to retention test they were injected with amphetamine (20 µg or 40 µg) or apomorphine (10 µg or 40 µg) or an equal volume of saline (4 µl). Saline treated rats made significantly more errors at retention test. Amphetamine-treated rats tended to make fewer errors than controls, but the results had a large variance and were nonsignificant ($p < .10$). A second experiment yielded the same pattern of results, despite a larger N, thus leaving open the question of whether the peripheral effects of systemic amphetamine can alone account for its facilitation of memory retrieval. Apomorphine, on the other hand clearly alleviated the forgetting ($p < .001$) at the smaller dose, but had no effect at the larger dose. This suggests that central dopamine systems are involved in regulating processes related to memory retrieval. However, recent discovery of ventral tegmentum dopaminergic projections to locus coeruleus (Milon, H. & Mc Rae-Degueurce, A., Neurosci. Let. 30:297, 1982) leaves open the possibility that noradrenergic regulation is involved. Neurochemical studies of the effect of apomorphine injections on various brain regions are presently underway and the results will be reported at this meeting.

142.7 SEROTONERGIC MEDIATION OF CONDITIONED BRADYCARDIA IN THE ALBINO RABBIT (Oryctolagus cuniculus). Sheryl R. Ginn*, Linda L. Hernandez, and D. A. Powell. Neuroscience Lab., VA Hospital and University of South Carolina, Columbia, S.C.

Research from our laboratory indicates that forebrain serotonin (5-HT) may be involved in mediating Pavlovian cardiovascular (CV) conditioned responses (CRs). For this reason, the effects of 5-HT agonist and antagonist drugs on the cardiac component of the orienting reflex (OR) and on Pavlovian conditioned CV responses were investigated in rabbits. Subcutaneous injections of the central 5-HT antagonist pizotifen (BC-105; 2.5 mg/kg, 5.0 mg/kg and 10.0 mg/kg), the peripheral 5-HT antagonist xylamidine tosylate (10.0 mg/kg), the central 5-HT agonist d-lysergic acid diethylamide (d-LSD; 0.03 mg/kg), d-LSD (0.03 mg/kg) in conjunction with BC-105 (5.0 mg/kg) or saline were administered to rabbits 15 min prior to OR assessment and aversive differential Pavlovian heart rate (HR) conditioning. Four sec, 75 dB tones of 1216 or 304 Hz frequency served as the stimuli during OR assessment and as the CS+ or CS- during conditioning. A 500 msec, 3 mA paraorbital electric shock train served as the US.

Both the HR CR and OR consisted of bradycardia. The central antagonist BC-105 attenuated habituation of the OR; however, the peripheral antagonist xylamidine potentiated OR habituation. d-LSD attenuated the magnitude of the OR compared to saline administration; this attenuation was partially blocked by BC-105. BC-105 produced a dose-related attenuation of the bradycardiac HR CR. The largest HR CRs of all groups tested occurred in those animals receiving 10 mg/kg xylamidine, suggesting that the attenuation by BC-105 of the HR CR is central, rather than peripheral, in origin. d-LSD appeared to have little effect on the CR. BC-105 in conjunction with d-LSD attenuated the deceleration to both the CS+ and CS- as well as attenuating the discrimination between CS+ and CS-. These results suggest that 5-HT neurons in the CNS may modulate the magnitude of bradycardiac responses during orienting and aversive Pavlovian conditioning.

142.8 CENTRAL VERSUS PERIPHERAL ACTIONS OF LEU-ENKEPHALIN ON ACQUISITION OF A ONE-WAY ACTIVE AVOIDANCE RESPONSE, LOCOMOTOR ACTIVITY AND SHOCK SENSITIVITY. J.L. Martinez, Jr., P. Conner*, and R.C. Dana*. Psychobiol. Dept., Univ. of Calif., Irvine, CA 92717.

Previously, Martinez and Rigter (Neurosci. Abstr. 1980, 6, 319) reported that adrenal medullectomy abolished the impairing actions of enkephalins on acquisition of a one-way active avoidance response. The present study was undertaken to investigate further whether Leu-enkephalin (LE) acts through some peripheral mechanism by directly comparing peripheral (i.p.) and intracerbroventricular (i.c.v.) routes of administration.

LE (1.0 or 10.0 µg/kg) or saline was administered to rats (Harlan Sprague Dawley) 5 min before training. Rats were placed in the shock compartment of a two-compartment alley and given 10 sec to shuttle into the safe compartment or after 10 sec a shock (1.0 mA) came on which was terminated when the rats escaped. Eight trials were given; the intertrial interval was 30 sec. As shown by Dunnett's procedure, the rats that received 10 µg/kg of LE made fewer avoidances than the saline group [$t(2,49)=2.19, p < .05$].

The effects of a four log unit dose range of LE (0.00275-2.75 µg/rat) administered i.c.v. 5 min before training on acquisition of the avoidance response was investigated. The highest i.c.v. dose was approximately the same as the 10 µg/kg dose given i.p., since the average weight of the rats was 275 g. No dose of LE given i.c.v. was found to influence acquisition of the response.

The effects of either saline, LE (10 µg/kg) or d-amphetamine (3.0 mg/kg) administered i.p., or Ringer's, LE (0.00275-2.75 µg/rat) or d-amphetamine (300 µg/rat) administered i.c.v. on locomotor activity was investigated. Before being placed in the open field all rats received a 1.0 mA, 1.0 sec footshock. Only d-amphetamine given i.p. significantly enhanced activity [$U(7,12) = 3, p < .002$]. For i.c.v. administration both d-amphetamine [$U(8,9) = 4, p < .002$] and 0.275 µg of LE [$U(5,9) = 7, p < .05$] increased activity.

Finally, the effects of either Ringer's (i.p. and i.c.v.), LE (0.0275 or 0.275 µg/rat) or morphine (6 mg/kg, i.p. or 30 µg/rat) were determined on shock sensitivity. The range of shock intensities was 0.1-1.0 mA. Morphine given i.p. or i.c.v. produced analgesia as did 0.275 µg LE. The lowest dose of LE (0.0275 µg) produced hyperalgesia at the lowest shock intensity.

The results suggest that LE impairs conditioning through a peripheral mechanism of action, since it is ineffective if administered directly into the brain. However, LE given i.c.v. clearly affects other behaviors such as locomotor activity and shock sensitivity. (Supported by ONR contract N00014-82-K-0385 to JLM.)

142.9 THE ROLE OF MONOAMINES IN SUBSTANCE P INDUCED MEMORY ENHANCEMENT. M.A. Pelleymounter* and Kurt Schlesinger* (SPON: H. Alpern). Dept. of Psych. and the Inst. for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Large concentrations of Substance P (SP) have been located in higher central nervous system areas in coexistence with axons and terminals of dopamine (DA) and serotonin (5HT), particularly in substantia nigra and the striatum. Peripheral injection of SP immediately following, but not prior to, passive avoidance training, results in increased retention of the task suggesting that SP may be involved in the consolidation phase of memory processing. This study examined the role of monoamines in a mechanism for SP-induced memory enhancement by measuring changes in nigral and striatal DA, 5HT and norepinephrine (NE) levels during different stages of memory processing in passive avoidance learning. Male HS mice were placed into the clear chamber of a passive avoidance apparatus, and their latency to enter the dark area was measured. Upon entry into the dark chamber, a 0.4 mA, 5 sec footshock was administered. Immediately following the above training session, animals were injected (s.c.) with 1 ng/g SP or with acidified saline. Animals were tested 24 hr later for latency to enter the dark area. Retention was defined as the difference in latencies. Animals were sacrificed either 1 hr after injection and training (1IT), 24 hr after injection and training (24IT), 1 hr after injection and training without shock (1I) or 1 hr after training without injection (1T). Brains were removed, frozen and rapidly dissected into striata and substantia nigra. Sections were homogenized in butanol and analyzed for DA, 5HT and NE levels using the fluorescence technique of Jacobowitz and Richardson. A dose response measure of monoamine levels in these brain areas was also done on another group of mice 1 hr after peripheral injection of acidified saline, 1, 10, 100, 200, or 1,000 ng/g of SP. Biochemical analysis was done in the manner described above. 5HT levels were not different in 1I and 1T groups in either brain area, but were significantly lower in 24IT mice than in 1IT animals; $p<.05$. DA levels were similar in 1I and 1T but were significantly elevated in the 24IT group; $p<.01$. NE levels were higher in 1T mice than in 1I mice but were not changed in other groups. The dose response study showed that 5HT levels in both brain areas decreased in a linear manner with increasing dose of SP. DA levels, however, were increased at the 1, 100, and 1,000 ng/g doses of SP in comparison to the saline group. NE levels did not change with increasing dose of SP in either brain area. These results support the hypothesis that SP may be a cotransmitter in certain monoamine pathways and that monoamines may be involved in the memory enhancing effects of SP. (This work was supported by NIH Contract #0780.4.524 B).

142.10 CALCIUM, LONG-TERM POTENTIATION (LTP) AND DEPRESSION OF HIPPOCAMPAL POPULATION SPIKE. B. R. Sastry, S. S. Chirwa*, J. W. Goh* and H. Maretic*. Neuroscience Research Laboratory, Department of Pharmacology, Faculty of Medicine, The University of British Columbia, Vancouver, B. C., V6T 1W5, Canada.

Whether LTP is presynaptic and/or postsynaptic is still unclear, although there appears to be a general belief that LTP is postsynaptic. The present investigations were conducted on rat hippocampal slices to examine Ca^{++} involvement in LTP and depression of the CA_1 population spike and population "EPSP" produced by Schaffer collateral (SCH) and commissural (COM) stimulation. Exposure of slices to a medium in which Ca^{++} and K^+ concentrations were 4 mM and 3.1 mM, respectively, resulted in an increase in the size of the population spike (10.1 ± 2.1 SEM fold, n = 6), but the response rapidly returned to almost control levels after re-exposure to the control medium (Ca^{++}:2 mM; Mg^{++}:2 mM; K^+:3.1 mM; 30 min: 2.06 ± 0.67 fold, n = 6). In a medium with 4 mM Ca^{++} and 5 mM K^+, the population spike was greatly enhanced (34.44 ± 7.6 fold, n = 7) and the increase was maintained in standard medium (30 min: 9.97 ± 2.08 fold; n = 7). A high frequency tetanus (400 Hz, 200 pulses) of SCH caused LTP of the CA_1 population spike (30 min post-tetanus: 2.43 ± 0.56 fold; n = 7), but had little effect on the COM input-induced population spike. A 20 Hz tetanus (200 pulses) of the SCH masked the above LTP for 5-20 min (% response 5 min post-20 Hz: 52.5 ± 9.7; 20 min post-20 Hz: 93.7 ± 7.2; n = 10) and brought in a depression of the population spike produced by the untetanized COM input (% response 5 min: 59.2 ± 18.1; 20 min: 88.0 ± 13.4; n = 10). A 20 Hz (200 pulses) tetanus of one input produced a temporary masking, but not a permanent reversal of an established LTP of the population spike produced by the other input (n = 5). Verapamil is known to interfere with soma-dendritic Ca^{++}-channels and not to alter transmitter release. This agent (0.33 μM; 3 min) counteracted the 20 Hz tetanus-induced masking of LTP and the depression of the untetanized pathway (n = 6), but LTP could be induced by a 400 Hz (200 pulses) tetanus during the drug application (increase 30 min post-tetanus: 4.94 ± 0.95 fold, n = 5). A 10-min exposure of slices to the medium with 4 mM Mg^{++} and 0 mM Ca^{++} resulted in an increase in the population spike 30 min post-treatment (1.32 ± 0.09 fold, n = 11). Ca^{++} (2-100 nA), applied at the CA_1 cell-body layer with or without K^+ (2-100 nA), depressed the population spike (5/6), as well as the population "EPSP" (7/9) following the cessation of drug application. These results indicate that a Ca^{++}-dependent depression of the population spike exists in CA_1 neurones, that a voltage-dependent Ca^{++}-channel may be involved in the development of LTP, and that LTP may well be presynaptic.

142.11 A SPECIFIC HYPOTHESIS CONCERNING THE BIOCHEMICAL SUBSTRATES OF MEMORY. M. Baudry and G. Lynch. Department of Psychobiology, UC Irvine, Irvine, CA 92717.

Despite extensive theoretical and experimental investigation, specific and testable hypotheses concerning the biochemistry of learning and memory in higher vertebrates have not yet been proposed. Our studies on the regulation of synaptic efficiency and glutamate receptors suggest a new, specific, and seemingly testable hypothesis about the biochemical events that lead to memory storage in mammals. The central points of the hypothesis to be discussed are as follows:

1) intense synaptic activity causes an elevation of intracellular calcium in postsynaptic structures,

2) calcium activates a membrane associated neutral thiol proteinase ("Calpain I") that degrades the spectrin-like protein fodrin and certain other peptides related to the cytoskeleton,

3) this causes a localized membrane reorganization such that normally occluded receptors are exposed,

4) the additional receptors produce potentiated synapses, and

5) additional episodes of intense synaptic activity cause wide-spread activation of calpain leading to changes in the shape of dendritic spines.

The proposed mechanism satisfies certain essential requirements of an intermediate of memory in that it is triggered by physiologically plausible events and produces long-lasting changes that should have physiological consequences. Moreover, behavioral experiments have shown that inhibition of the calcium proteinase produces a selective impairment of certain types of memory (Staubli, U., Baudry, M. and Lynch, G., this meeting). These results will be discussed in light of recent data showing that the proteinase receptor interaction is absent in non-mammalian vertebrates and in mammals is restricted to forebrain structures. This raises the possibility that different forms of memory have different biochemical substrates. (Supported by NSF grant BNS81-12156 to M.B. and NIMH grant 19793 to G.L.)

143.1 EVIDENCE THAT THE NIGROTEGMENTAL GABAERGIC PROJECTION MEDIATES STEREOTYPY INDUCED BY APOMORPHINE AND INTRANIGRAL MUSCIMOL.
J.A. Childs and K. Gale. Dept. of Pharmacology, Georgetown Univ. Schools of Medicine & Dentistry, Washington, D.C. 20007

The substantia nigra (SN) plays a pivotal role in the relay of output from the striatum. One neural pathway from SN projects GABAergic fibers to caudal mesencephalic tegmentum, terminating in the vicinity of the pedunculopontine nucleus (PPN). To evaluate the functional importance of this projection in the mediation of stereotyped behaviors of striatal and nigral origin, we microinjected low doses of the GABA agonist, muscimol, bilaterally into the vicinity of the PPN. Rats receiving 10ng muscimol bilaterally in PPN prior to systemic apomorphine administration (1mg/kg s.c.) showed no observable differences in spontaneous behavior, relative to saline-injected controls. However, this muscimol treatment completely blocked all components of apomorphine-induced stereotyped behavior. Gnawing could not be elicited, even when probes were placed near or inside the rat's mouth. This blockade was evident for the entire duration of apomorphine action. The blockade observed with PPN injections was also seen in rats in which injection of 10ng muscimol was placed 2mm below PPN, suggesting that synapses ventral to PPN may also participate in the apomorphine stereotypy. In contrast, apomorphine-induced stereotyped behavior observed in rats receiving 10ng muscimol placed 1mm above PPN (in dorsal reticular formation) was not significantly different from that obtained in controls.

Stereotypy elicited by bilateral intranigral muscimol was blocked by injection of muscimol into PPN. Rats receiving both 15ng muscimol in PPN and 8ng muscimol in SN exhibited no stereotyped behavior of any sort; instead, they were relatively inactive, sitting still for 50-60 min following intranigral muscimol.

We conclude from our results that there exists in the vicinity of the PPN a population of GABA-receptive neuronal elements which, when inhibited, are capable of blocking all commonly assessed components of apomorphine-induced and intranigral muscimol-induced stereotypy. Since the PPN and the region immediately ventral to it receive GABAergic projections from SN (1), it is likely that stimulation of striatal dopaminergic receptors or of nigral GABAergic receptors acts to reduce GABAergic tone in the terminal field in PPN. Our data shows that the expression of stereotyped hyperactive and dyskinetic motor activities can be prevented by the application of a GABA agonist into PPN; it is therefore reasonable to infer that net disinhibition of target neurons in this region of reticular formation is necessary for the expression of these stereotyped behaviors.

Supported by HHS grants MH32359 and DA02206.

References:

(1) Childs, J.A. and Gale, K., Brain Res. 258:109-114, 1983.

143.2 THE EFFECT OF STABLE "ANTAGONIST" ANALOGS OF SUBSTANCE P ON SUBSTANTIA NIGRA EFFERENT PATHWAYS.
Maria Rosaria Melis* and Karen Gale. Dept.of Pharmacology, Georgetown Univ. Schools of Medicine and Dentistry, Washington, D.C. 20007.

Striatonigral substance P (SP) pathways have been proposed to function as an excitatory component of a neuronal loop which modulates the activity of nigrostriatal dopaminergic (DA) neurons. SP has also been shown to stimulate non-DA cells in pars reticulata of substantia nigra (SN). We therefore examined the effects of two SP analogs (D-Pro^2,-Phe^7,D-Trp^9-SP and D-Pro^2,D-Trp^{7,9}-SP)with purported antagonist characteristics, on the turnover rate of neurotransmitters in the nigrostriatal and nigrotectal projections.

In control rats, microinjection of either of the above SP analogs (20 ug in 1 ul) into SN did not change the Vmax or cofactor affinity of striatal tyrosine hydroxylase (TH), nor was there a change in nigral TH activity.

When haloperidol (Hal) was injected (1 mg/kg i.p.) 10 min after the unilateral intranigral injection of the SP analogs, the Hal-induced increase in the affinity of striatal TH for its pteridine cofactor was blocked in the striatum ipsilateral to the drug microinjection. Associated with this effect was an antagonism of the Hal-induced increase of HVA levels in the striatum.

SP analogs alone caused a small increase in striatal HVA, but unlike the antagonism of the Hal effects, this effect was not limited to the ipsilateral striatum nor was it site-specific. Indeed, a similar increase in striatal HVA was found after injection of SP analogs into the median forebrain bundle (MFB), but this treatment was unable to antagonize the Hal effects on striatal TH and HVA.

To examine the effect of the SP analogs on the activity of non-DA efferents from SN, we measured the turnover rate of GABA in in the terminal field of the nigrotectal GABA projections. The rate of GABA accumulation following local irreversible inhibition of GABA-transaminase by gamma-vinyl-GABA was used as an index of GABA turnover. Intranigral injection of either of the two SP analogs (20 ug in 1 ul) caused a significant (35%) decrease in the rate of GABA accumulation in the deep layers of the superior colliculus ipsilateral to the injected side. No effect was observed when the SP analogues were injected into the MFB.

Our results are consistent with the possibility that the SP analogues we used have SP antagonist activity and that, as a consequence of blocking the excitatory influence of nigral SP, they interfere with the ability of Hal to activate dopamine turnover in the nigrostriatal pathway. Furthermore, our observation that these putative SP antagonists cause a decrease in the turnover rate of GABA in the nigrotectal projections is consistent with the proposal that nigral SP normally provides an excitatory control of GABAergic efferents projecting to the superior colliculus.

Supported by HHS grants MH32359 and DA02206.

143.3 EFFECTS OF SPECIFIC DOPAMINE AND SEROTONIN UPTAKE INHIBITORS ON STRIATAL-NIGRAL SUBSTANCE P ACTIVITY.
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The neuronal circuitry of the striatum and substantia nigra is believed to play an important role in the regulation of certain types of motor activity. A principal pathway in this system is composed of nigral dopamine (DA) neurons which project to the striatum. Some control of this dopaminergic projection is thought to be exerted by feedback striatal-nigral loops. It has been suggested that the excitatory component of these loops employs the neuropeptide, substance P (SP), as its transmitter (Hong et al., Neuropharm. 17[1978]83). If the striatal-nigral SP neurons have a feedback function to the dopaminergic system, changes in dopaminergic activity would likely influence the associated SP pathway. One method for evaluating the activity of the SP striatal-nigral neurons is to measure the concentration of substance P-like immunoreactivity (SPLI) in the substantia nigra, site of the axon terminals of this SP pathway. We have found that subacute administration of the DA agonist, methamphetamine (4X, 10 mg/kg, q6h) elevates nigral SPLI by 30%. However, because methamphetamine (METH) also significantly influences serotonin pathways associated with the striatal-nigral system (Bakhit et al., Eur. J. Pharmacol., 76[1981]229), it is unclear if the METH-induced changes in nigral SPLI are related to the increases in dopaminergic or serotonergic activity caused by this drug. To resolve this question, the responses of the striatal-nigral SP pathway to specific uptake blockers of dopamine and serotonin were studied.

Dose-dependent increases in nigral SPLI concentrations were observed following the subacute injections (4X, q6h) of amfonelic acid, a potent dopamine uptake blocker (12% at 0.1 mg/kg; 26% at 0.5 mg/kg; 60% at 1.0 mg/kg). Increases of 34% in nigral SPLI could also be measured following a single injection of amfonelic acid (2.5 mg/kg). In contrast, subacute treatments with the specific serotonin (5-HT) uptake blockers, citalopram (5X, 2 mg/kg, q6h) and chlorimipramine (5X, 4 mg/kg, q6h) did not alter nigral SPLI concentrations. These results suggest that the METH-induced changes in the striatal-nigral SP pathway are likely dopamine-mediated. However, the possibility that the serotonergic system might also directly or indirectly influence this SP system can not be ruled out since inhibition of 5-HT synthesis 48 hours following a single dose of p-chlorophenylalanine (50 mg/kg) significantly elevated nigral SPLI concentration by 17%.
(Supported by USPHS Research Grants MH 37762 and DA 00869.

143.4 TRAINED CIRCLING RATS SHOW REVERSED LATERALIZATION OF CAUDATE DOPAMINE SYNTHESIS AFTER TURNING STOPS.
Barbara A. Bennett and Curt R. Freed. Depts. of Med. and Pharmacol., Univ. of Colorado Sch. of Med., Denver, Co. 80262

Previous studies in our laboratory using the trained circling rat have revealed increased dopamine synthesis, release and turnover in caudate contralateral to the circling direction. In water deprived rats running in circles for a sucrose/water reward, there was enhanced contralateral dopamine synthesis during the first 20 min of circling when turning was most intense (10-12 rpm) while synthesis returned to baseline levels after 70 min of circling when turns had slowed to 2-3 rpm. There was no change seen in dopamine production in the side of caudate ipsilateral to the circling direction. The dopamine metabolite, dihydroxyphenylacetic acid (DOPAC), followed a similar pattern of synthesis as that seen for dopamine and was increased in contralateral caudate during the first 20 min of circling and declined toward baseline after 70 min. We have now studied caudate dopamine synthesis using a 3H-tyrosine precursor technique in the time period immediately following circling to see if the termination of circling affects caudate dopamine metabolism. Male Sprague-Dawley rats were trained to circle for a water reward. After 7 days of training, animals were cannulated in the lateral cerebral ventricle. On the next day, animals were circled for 70 min and then removed from the circling drum and infused with 250 µCi of 3H-tyrosine (dissolved in 20 µl artificial rat CSF) over 20 min. Results show that after circling stopped, the conversion of 3H-tyrosine into 3H-dopamine and 3H-DOPAC was relatively increased in caudate ipsilateral to the circling direction. Values are Mean ± SEM.

RELATIVE SYNTHETIC ACTIVITY (dpm/nmole/g tissue)

	Contralateral	Ipsilateral
Dopamine	.039 ± .01	.065 ± .01 *
DOPAC	.121 ± .02	.239 ± .06 *

*p <.05 different from the contralateral side, n=8.

These experiments show that the cessation of circling is associated with increased ipsilateral synthesis of caudate dopamine. It is uncertain whether this enhanced ipsilateral dopamine synthesis represents a "stop" signal for turning behavior or whether it is simply the result of ending inhibition of synthesis on the ipsilateral side. Supported by American Parkinson's Disease Association, USPHS Grants GM07063(BAB); RCDA HL00782(CRF); NS099199; and NS18639.

143.5 LATERALIZED DOPAMINE AND SEROTONIN TURNOVER IN RAT LIMBIC STRUCTURES DURING TRAINED CIRCLING BEHAVIOR. Bryan K. Yamamoto and Curt R. Freed. Depts. Med. and Pharm., U. Colo. Sch. Med., Denver, CO 80262

We have previously reported that the trained circling rat has increased dopamine turnover in the caudate contralateral to the circling direction and increased turnover in substantia nigra ipsilateral to the circling direction. We have now extended these observations to the limbic system and have studied the lateral septum (LS) and the ventral tegmental area (VTA). Male Sprague Dawley rats were water deprived for 23 hr and randomly chosen to be trained to turn either left or right for sucrose/water reward (Nature 298: 467, 1982). After a one week training period, regional neurotransmitter activity was studied before, during and after circling. VTA and LS were bilaterally dissected and assayed for dopamine (DA) and DOPAC by HPLC with electrochemical detection. Results were calculated as the ratio of concentrations on the contralateral relative to the ipsilateral side. Ratios greater than 1.0 indicate increased concentrations on the contralateral side.

VENTRAL TEGMENTUM
Contralateral/Ipsilateral Concentration Ratios

	Baseline	Peak Circling	End Circling
Dopamine	1.07±.16	1.91±.36*	1.43±.33
DOPAC	1.00±.09	1.31±.11*	1.62±.27*

LATERAL SEPTUM
Contralateral/Ipsilateral Concentration Ratios

	Baseline	Peak Circling	End Circling
Dopamine	1.03±.04	0.69±.09*	0.83±.08*
DOPAC	0.96±.06	0.65±.09*	0.70±.06*

Values are Mean ± SEM, n=6 at each time point.
*Indicates significantly different from baseline ($p<0.05$).

These data demonstrate increased ipsilateral DA turnover in dopaminergic nerve terminals in LS and increased contralateral DA turnover in the dopamine cell bodies of VTA during circling. These findings are the opposite to those we have seen in the nigrostriatal system. Nerve terminals in caudate showed contralateral increases in DA turnover while cell bodies in substantia nigra had ipsilateral increases. This striking difference in dopamine lateralization in VTA-LS compared to the nigrostriatal system implies a functionally distinct activation of the limbic system compared to the extrapyramidal motor system during voluntary circling behavior. Supported by American Parkinson's Disease Assn.; NIGMS GM07063; RCDA HL00782 (CRF); NIH NS09199; and R01 NS186639.

143.6 SENSORIMOTOR ASYMMETRIES AND TACTILE EXTINCTION IN UNILATERAL FRONTAL CORTEX DAMAGED AND STRIATAL DOPAMINE-DEPLETED RATS. T. Barth*, M. D. Lindner*, and T. Schallert. Dept. Psychology, Univ. Texas at Austin, Austin, TX 78712.

In people, the most characteristic and enduring somatosensory consequence of extensive unilateral neocortex damage is "simultaneous extinction", which is an interhemispheric perceptual interaction that is operationally distinguishable from neglect. A cutaneous stimulus presented on the contralateral side of the body is readily detected when presented singly, but is completely masked during bilateral stimulation.

Analogous tests designed to calibrate sensorimotor asymmetries in rats were used to determine the immediate and chronic effects of frontal cortex damage, which were then compared to the effects of neostriatal damage. We attached small adhesive stimuli to the radial surface of each forelimb bilaterally and simultaneously and recorded the latencies to remove each stimulus.

Although the durations of recovery for each group were markedly different, we found first that no brain-damaged animal contacted or removed the contralateral stimulus until after it removed the ipsilateral stimulus, which was removed immediately. With this test we established both the presence of an ipsilateral sensorimotor bias and the absence of contralateral neglect in every animal.

In a second study, using unremovable tactile stimuli we provided strong evidence that in the presence of the ipsilateral stimulus, the contralateral stimulus was not detected, which is the measure of true extinction. A key feature of the "extinction" was its complete reversibility. Simply by adjusting the sensory fields occupied by the contralateral (C) and ipsilateral (I) stimuli (specifically, by increasing the C/I ratio), the animals contacted both stimuli with equal frequency. In fact, when the C/I ratio was large enough, the sensorimotor bias was totally shifted to contralateral. Thus, extinction appeared to depend in large part on the perceived intensity of the ipsilateral stimulus relative to that of the contralateral stimulus. In additional experiments we examined the effects of damage to other cortical and subcortical areas.

(Supported by N.I.H. grant NS17274 to T. Schallert)

Related reference: Schallert et al., 1982, Pharm. Biochem. Behav., 16:455-462.

143.7 STRIATAL NEURONAL RESPONSES TO DEXAMPHETAMINE ARE SIMILAR IN IMMOBILIZED AND CORTICALLY ABLATED FREELY MOVING RATS. M. W. Warenycia and G. M. McKenzie*, Dept. of Pharmacology, Dalhousie Univ., Halifax, N.S., Canada, B3H 4H7.

In freely moving rats, striatal neurons respond to dexamphetamine (DEX) with excitation as measured by multi-unit recording (Hansen, E.L. and G. M. McKenzie, Neuropharmacology 18: 547, 1979). To ascertain whether feedback from drug-induced behavior might be an important determinant in the response of striatal neurons, a two-fold approach was taken: 1) experiments were carried out in immobilized rats to record responses in the absence of behavior and, 2) experiments were carried out on freely moving cortically ablated rats, since striatal afferents from the cortex presumably mediate feedback arising from DEX-induced behavior.

All experiments were carried out on male Long-Evans rats that had been implanted with bipolar multi-unit electrodes in the striatum 5-7 days prior to the acute experiment. Cortical ablations were performed 21-30 days prior to electrode implantation. In immobilization experiments, tracheotomy was performed during halothane anesthesia, a paralytic dose of succinylcholine given, lidocaine applied to wound surfaces and respiration maintained with 70% N_2O/30% O_2. DEX, 2.5 mg/kg i.p., was given after baseline neuronal activity was established. In freely moving, cortically ablated rats, the same dose of DEX was given once baseline neuronal activity was established.

In immobilized preparations, a multiplicity of striatal neuronal responses (N=28) was observed. Inhibitory (21%), biphasic (36%) and no change (25%) responses accounted for most of the responses to DEX with the incidence of excitation reduced to 18% compared to freely moving controls. Similar results (N=50) were seen in cortically ablated rats where excitation was observed only 24% of the time. Inhibition was most predominant (42%), followed by no change (22%) and biphasic responses (12%). Although the striatal response differed markedly between ablated and non-ablated rats, behavioural responses to DEX were essentially the same. These findings indicate that striatal neuronal responses to DEX are dependent on behaviour and that at least one of the neural substrates subserving feedback from DEX-induced behavior may be striatal afferents of cortical origin.

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143.8 CAFFEINE DECREASES RAT CAUDATE DOPAMINE RELEASE AS MEASURED BY IN VIVO ELECTROCHEMISTRY. M.E. Morgan and R.E. Vestal* Clinical Pharmacology Unit, VA Medical Center, Boise, ID 83702 and Dept. of Med., Univ. of Washington, School of Med., Seattle, WA.

Caffeine (CAF), a ubiquitous dietary constituent, is perhaps the most widely used psychoactive drug in western culture. Despite well known central nervous system (CNS) stimulating effects, the mechanism of action of CAF is not well understood. Recently, several investigators have provided evidence supporting the concept that CAF-induced CNS activation may be due to the antagonism of adenosine at cell surface receptors which inhibit neurotransmitter release. It is not clear, however, if the consequence of adenosine antagonism by CAF is always an increase in neurotransmitter release. Nor is it clear whether CAF affects all neurotransmitter systems in the same manner. The behavioral effects of CAF in rodents are manifested by an alteration in motor movement which may involve the basal ganglia. For these reasons, we have studied the time dependent and dose dependent relationship between CAF and caudate dopamine (DA) release in the rat using in vivo electrochemistry. Male Sprague-Dawley rats (200-300 gm) were anesthetized with urethane (1.0-1.5 gm/kg, i.p.). A 250µ stearic acid carbon paste electrode was inserted stereotaxically into either the left or the right caudate. Electrochemical oxidations were made with a Bioanalytical Systems DCV-5 voltammeter with the electrochemical output processed by semidifferentiation. The electrode was scanned (5mV/sec) every 10 min. over a range of (-) 0.1 to (+)0.38 volt with the peak at (+)0.15 to (+)0.16 volt defined as the DA peak. In vitro experiments indicated no interaction between DA (40µM) and CAF (40µM-120µM). After achieving a steady basal release, animals were injected with either 50mg/kg or 100mg/kg, i.p. CAF. DA release was assessed for 110 min. thereafter.

Per Cent Control of Mean(±SEM) Baseline Peak Heights

Time(min.)	0	30	60	110
Dose(mg/kg)				
50	99±1	70±4*†	82±8†	120±20
100	100±3	42±3*	44±3*	78±2

n = 6 experiments per dose; * $p<0.05$ (30 or 60 min. vs 0 min); † $p<0.05$ (50 mg/kg dose vs 100mg/kg dose)

Caudate DA release was significantly decreased 30 min. after CAF. After the nadir, there was a gradual return to baseline release which was slower in high dose animals compared to low dose animals. These data indicate that there is a significant time dependent and dose dependent CAF-induced decrease in the release of DA from the rat caudate. A CAF-adenosine interaction is a possible mechanism which requires further investigation.

143.9 EFFECTS OF APOMORPHINE AND AMPHETAMINE ON ACUTE ADMINISTRATION OF 6-OHDA TO THE NIGRAL-STRIATAL PATHWAY. M. Cornfeldt*, C. Gailums*, M. Szewczak* and R. Chen* (SPON: S. Fielding). Dept. of Pharmacology, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

Unilateral lesions in rats to the nigral (N) pathway with 6-hydroxydopamine (6-OHDA) has been a valuable tool for assessing the effects of a variety of agents on motor function (Pycock, C.J. Neurosci., 5:461, 1980). Typically, this method involves chronic N lesions. The acute effects of 6-OHDA on N may also have value in determining drug effects on this system. Our purpose was to measure the effects of rotational preference both before and after 6-OHDA lesion and to determine the activity of amphetamine (A) and apomorphine (APO) on rotational preference with an acute lesion. Rats were tested in a rotometer for 16 hrs with 5 mg/kg ip of A before lesion; 48 hrs later, subjects were anesthetized with halothane and 8 µg 6-OHDA in 4 µl saline and ascorbic acid was delivered to the left N at the level of the crus cerebri. After 6-OHDA, rats were immediately placed in a rotometer and given ip saline (I), 1 mg/kg APO (II), 0.5 mg/kg APO (III), 5 mg/kg A (IV) and 2.5 mg/kg A (V). The test for preference before 6-OHDA showed that group III circled left 74% and group IV circled right 61%. All other groups had no directional preference >60%. After 6-OHDA groups I, IV and V had a significant ($p < 0.05$, paired sign rank) ipsilateral preference. APO groups (II and III) had no significant rotational preference after 6-OHDA though there was a tendency to circle ipsilaterally. Both A and APO increased total circling behavior. The postsynaptic effects of APO may have compensated for the acute imbalance to the extrapyramidal system by 6-OHDA.

143.10 INTRASTRIATAL DOPAMINE- AND AMPHETAMINE INDUCED BEHAVIORS ARE MODULATED DIFFERENTIALLY BY ESTRADIOL BENZOATE. C.Van Hartesveldt, J.N.Joyce and E.Montero*. Dept. of Psychology and the Center for Neurobiological Sciences, Univ. of Florida, Gainesville, FL 32611.

We reported in another abstract (Joyce and Van Hartesveldt, this vol.) that intrastriatal dopamine (DA)- and amphetamine (AMPHET)-induced postural deviation and rotation varied in magnitude independently of one another, when measured at several points on the day of proestrus. This suggests that the two behaviors may be under independent hormonal control. To investigate if the two striatal DA-mediated behaviors show different characteristics for estrogen-induced suppression, the following procedure was utilized. Ovariectomized (OVX) Long-Evans hooded rats were implanted with intrastriatal cannulae, then treated with 3 separate hormone regimes. After each hormone regime treatment, animals were tested for unilateral intrastriatal DA- and AMPHET-induced postural deviation and rotation at 1/2,3,24, 48, and 72 hours after the last hormone treatment of any regime. The rats were treated with: (1) two treatments with EB (2µg), 48 hours apart (EB+EB); (2) two treatments with the OIL vehicle for EB (OIL, 0.2ml), 48 hours apart (OIL+OIL); (3) OIL 48 hours before EB (OIL+EB).

The results indicate, first, that the two striatal DA-mediated behaviors have different requirements for suppression by EB. The hormone regimes OIL+EB or EB+EB produced a suppression of intrastriatal DA- and AMPHET-induced postural deviation at 1/2,3, and 24 hours after the last (EB+EB) or only (OIL+EB) EB treatment. The rotational response to the same intrastriatal DA and AMPHET injections showed a reduction only when treated with EB+EB, and then the suppression was not apparent until the 24 hour test. This suggests that the striatal DA-mediated behaviors, rotation and postural deviation, are differentially sensitive to the suppressive effects of estrogen treatment. We also have evidence to believe that the changes in rotational behavior induced by EB treatment do not simply reflect changes in general activity levels of the rats.

Secondly, the results indicate that, during the estrous cycle, the reversal from suppression to enhancement is not mediated by estrogen withdrawal. Rats given the hormone regime EB+EB do show a return to the pre-hormone baseline by 48-72 hours after the last EB treatment, but do not show an enhancement of either the postural deviation or rotational behaviors. Similarly, treatment once with EB (OIL+EB) does not produce an enhancement of either striatal DA-mediated behavior at any of the test intervals. This indicates that the reversal from suppression observed during the estrous cycle, is mediated by one or more other hormones.

143.11 INTRASTRIATAL DOPAMINE- AND AMPHETAMINE-INDUCED BEHAVIORS VARY ACROSS THE ESTROUS CYCLE. J.N.Joyce and C.Van Hartesveldt. Dept. of Psychology and the Center for Neurobiological Sciences, Univ. of Florida, Gainesville, FL 32611.

Previous research has shown that exogenously administered estradiol initially suppresses and later enhances several behaviors thought to be modulated by striatal dopamine (DA). Since plasma estradiol (E_2) varies substantially during the estrous cycle, these behaviors should be suppressed when plasma E_2 is high and enhanced when it is low. However, these results have not been obtained by previous authors, at least in part because behavioral tests have not always been conducted when plasma E_2 peaks on the morning of proestrus. In addition, the behaviors measured may have involved several brain regions. We designed this experiment to test the effects of estrous cycle variations in plasma E_2 on postural deviation and rotation elicited by DA drugs injected directly into the striatum.

Unilateral intrastriatal injections of dopamine (DA; 25µg/.25µl) or amphetamine (AMPHET; 25µg/.25µl) induced contralateral postural deviation and contralateral rotations, that systematically vary in magnitude across the estrous cycle of Long-Evans rats. In experiment 1 it was shown that when tested between 2-6 hours after lights ON (12:12 LD), both behavioral responses were suppressed on proestrus and were enhanced on estrus as compared to all other days of the estrous cycle. In experiment 2 it was shown that the behaviors induced by intrastriatal injections of DA or AMPHET varied independently of one another across the day of proestrus. Intrastriatal DA- and AMPHET-induced postural deviation were suppressed at 4 and 7 hours after lights ON, but were enhanced (equal to the estrus response) at 11 hours after lights ON. In contrast, intrastriatal DA- and AMPHET-induced rotations were suppressed at 4,7 and 11 hours after lights ON, but enhanced by the morning of estrus. These results suggest that striatal DA-elicited postural deviation and rotation are under independent hormonal control.

EXP. 1		PROESTRUS	ESTRUS	DIESTRUS I	DIESTRUS 2
DA	DEV	849±65	1496±121	1177±72	1189±77
	ROT	19±4	69±14	65±11	94±19
AMPHET	DEV	1018±34	1822±117	1508±51	1507±77
	ROT	127±33	332±51	222±69	198±52
EXP. 2		PRO 4 HRS	PRO 7 HRS	PRO 11 HRS	EST 4 HRS
DA	DEV	391±68	370±96	1075±162	1092±159
	ROT	54±16	40±14	43±26	153±37
AMPHET	DEV	753±135	669±153	1671±113	1572±150
	ROT	308±52	278±72	258±73	681±88

143.12 PROGESTERONE REVERSES ESTROGEN SUPPRESSION OF INTRASTRIATAL DOPAMINE-INDUCED POSTURAL DEVIATION. R.L.Smith, J.N.Joyce and C.Van Hartesveldt. Dept. of Psychology and the Center for Neurobiological Sciences, Univ. of Florida, Gainesville, FL 32611.

In other abstracts (this vol.) we indicate that estrogen may mediate the suppression of striatal dopamine (DA)-elicited behaviors observed on the morning of proestrus. Since progesterone (P) can act either synergistically with or antagonistically to estrogen with regard to sexual behavior, we have investigated the effects of the interaction between P and estradiol in intrastriatal DA-postural deviation.

Female Long-Evans hooded rats were bilaterally implanted with 21 GA stainless steel guide cannulae, positioned in the dorsal anterior striatum. Unilateral injections of DA (25µg/0.25µl buffer) were made with 27 GA injection cannulae. After the injections, the animals were placed in a circular chamber and observed for 30 minutes. After completion of the first test, animals were bilaterally ovariectomized (OVX), and assigned to one of two groups. One group was given estradiol benzoate (EB, 4 g/0.2ml oil) twice a day for 7 days. The other group was given EB, as above, and P (.5 mg/.5 ml oil) 6 hours before testing, at 2 and 7 days after OVX.

Animals given EB alone for 7 days exhibited suppression of contralateral deviation at 2 and 7 days after OVX. Animals given EB+P showed significantly enhanced postural deviation at 2 days after OVX. After 7 days of EB treatment, P did not reverse the EB suppression.

These data suggest that P may be involved in estrogen-induced changes of striatal DA-mediated behaviors. P appears able to reverse the suppressive effects of short-term estrogen treatment (2 days) but not after longer treatment (7 days). This effect is similar to the sequential inhibition of estrogen-induced sexual behavior by P found in the female rat (Blaustein, et al. JCPP, 91:752, 1977).

144.1 CORTICAL DISTRIBUTION AND LOCALIZATION OF CALCIUM-BINDING PROTEIN IN RATS AND EPILEPTIC (EL) MICE. K.G. Baimbridge, I. Mody and J.J. Miller. Department of Physiology, University of British Columbia, Vancouver, B.C., V6T 1W5

There is increasing evidence to suggest that many of the effects of calcium in the central nervous system are mediated by intracellular proteins which bind this cation with a high affinity (uM range). One of these, calcium-binding protein (CaBP), has been shown to be neuron specific and to be present in high and unequal concentration in different regions and cell types of the CNS. In view of its possible role as an intraneuronal Ca^{2+}-buffer, CaBP's presence or absence in certain neurons should reflect differences in their ability to regulate Ca^{2+} and consequently neuronal excitability.

Using specific radioimmunoassay (RIA) and immunohistochemical techniques for CaBP the present study was undertaken to examine: 1) the distribution and localization of this protein in cortical areas of rats and mice, and 2) the comparative distribution between different strains of mice, including epileptic (EL) mice, in an attempt to extend our previous observations of a neuronal specific loss of CaBP in kindled rats (Neurosci. Abstr., 8:457, 1982).

1) In the rat cerebral cortex there is a clear dorso-ventral distribution of the protein with ventral areas containing three times more CaBP than dorsal regions (e.g. entorhinal cortex: 1811.9 ng/mg TSP; striate cortex: 602.5 ng/mg TSP). In dorsal cortical areas the protein is mainly localized in terminals projecting to this region, whereas more ventrally it is confined to a dense plexus of fibers and terminals in layers II-IV together with pyramidal cells and their processes. A dorsal to ventral distribution was also observed in phylogenetically older cortical areas, such as the hippocampal formation, where the ventral to dorsal ratio was 1.8:1. A similar pattern of CaBP distribution was found in cortical areas of mice.

2) When compared to control mice, the EL strain had significantly lower amounts of CaBP in the hippocampal formation and cerebral cortical samples particularly the ventral-temporal/entorhinal cortex and dorsal occipital regions. Upon vestibular stimulation leading to seizures a further fall in CaBP levels was observed in the hippocampal formation and ventral-temporal/entorhinal cortex.

In summary, CaBP has an unequal dorso-ventral distribution in the cerebral cortex of the two species examined, and the reduced levels in certain cortical areas of the epileptic (EL) mouse may reflect a genetically altered state in the excitability of the neurons in these regions.

144.2 ALTERED ^{45}Ca UPTAKE IN HIPPOCAMPAL SLICES OF COMMISSURAL AND AMYGDALA KINDLED RATS. I. Mody and J.J. Miller. Department of Physiology, University of British Columbia, Vancouver, B.C., V6T 1W5

Previous observations from our laboratory have indicated that the loss of a neuron-specific calcium binding protein (CaBP) in the hippocampal formation of the rat is correlated with the kindling model of epilepsy (Neurosci. Abstr., 8:457, 1982). Although the role of this protein in the CNS is not clear, it has been suggested to function as an intraneuronal Ca^{2+} buffer.

In order to examine this possibility the present study was undertaken to compare possible alterations in the kinetics of ^{45}Ca uptake in hippocampal slices obtained from control and kindled rats. Although the kinetic analysis of uptake curves does not provide an absolute measurement of intracellular calcium, the relative changes may provide useful information about pools and fluxes of calcium in the system. For our analysis we adopted the open series system model of Uchikawa and Borle (Cell Calcium, 2:173-186, 1981), and the relative pools and fluxes of calcium were derived from a double exponential equation best fitting the ^{45}Ca uptake curve.

As described for other systems, hippocampal ^{45}Ca uptake exhibited a fast and a slow component. When compared to controls, hippocampal slices obtained from either commissural or amygdala kindled rats exhibited significant alterations in ^{45}Ca uptake. Derivation of the fluxes and pools of calcium from the double exponential equations (non-linear least squares analysis) indicates that the enhanced ^{45}Ca uptake in the hippocampal formation of kindled rats is presumably due to an increased intracellular pool of calcium. Similar shifts in the ^{45}Ca uptake curves were observed by Borle (Cell Calcium, 2:187-196, 1981) in kidney cells when mitochondrial Ca-buffering was inhibited by DNP. Although changes in mitochondrial function cannot be excluded in the kindled preparation, our results suggest that following kindling-induced epilepsy and the loss of cytosolic CaBP there is a decrease in the efficiency of a Ca-buffering system resulting in the increase in $[Ca^{2+}]_i$.

These data support the hypothesis that CaBP may have an important role in buffering intracellular calcium and further that the change in $[Ca^{2+}]_i$ associated with a loss of the protein and altered ^{45}Ca uptake may be a contributing factor to the generation of epileptiform activity.

144.3 CHARACTERISTICS OF INHIBITORY PROCESSES IN THE DENTATE GYRUS FOLLOWING KINDLING-INDUCED EPILEPSY. M.W. Oliver* and J.J. Miller. Dept. of Physiology, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

Investigations of the cellular mechanisms underlying epileptiform activity have suggested that seizures may be attributed to inappropriate synchronous neuronal depolarization induced by a reduction or failure of inhibitory processes. The majority of these studies have assessed acute alterations in neuronal activity and have not addressed the question of long-term changes which may characterize 'epileptic' neurons. The present study was undertaken to determine whether inhibitory processes within the dentate gyrus are altered following the chronic induction of kindled seizures.

In vitro hippocampal slices were prepared from either commissural-kindled (stage 5) or control rats. Dentate granule cell excitability was evaluated by delivering paired-pulse stimulation to the perforant pathway and determining the change in the extracellular population spike amplitude at different condition-test (C-T) pulse intervals.

The results indicate that in control slices the test response exhibits a characteristic pattern of excitability consisting of an inhibition-potentiation-inhibition sequence at C-T intervals of 20-40 msec, 40-120 msec and 120 msec-8 sec, respectively. In the presence of bicuculline or low Cl^- medium, the initial inhibition is reversed and replaced by potentiation persisting up to 120 msec. The late inhibitory phase is Cl^- independent and unaltered by GABA antagonists. In contrast, the population spike evoked by the test pulse in kindled slices, maintained in normal medium, is inhibited at all C-T intervals ranging from 20 msec - 8 sec. Perfusion of low Cl^- medium results in immediate potentiation of the test response, however this period is shortlived compared to controls (up to 60 msec) indicating that the late Cl^- independent inhibition is prolonged by decreasing its latency to onset.

These data suggest that the efficacy of inhibitory systems in tissue predisposed to epileptiform activity is augmented rather than reduced, contradicting the general disinhibition hypothesis of epilepsy. The alterations in inhibitory processes appear to be specific for dentate neurons since we have previously demonstrated that inhibition within the CA-1 region remains unchanged following kindling (Neurosci. Abstract, 1982). Finally the results show that enhancement of a late non-GABA mediated inhibition is responsible for the increased inhibition observed in kindled slices in response to paired-pulse stimulation.

144.4 FACILITATION OF ELECTRICAL KINDLING IN VENTRAL HIPPOCAMPUS AND LATERAL SEPTUM IN VASOPRESSIN DEFICIENT RATS (BRATTLEBORO STRAIN). Bonnie J. Gillis and Donald P. Cain, Department of Psychology, U of Western Ontario, London, Ontario, Canada N6A 5C2.

Recent investigations have demonstrated the existence of vasopressin (VP) fine fibre projections to various brain sites including dorsal and ventral hippocampus, lateral septum and several nuclei of the amygdala. The role of VP in central nervous system functioning is not yet clear, however there is some evidence to suggest that VP may play a role in seizure development using a kindling paradigm. Gillis and Cain (in press) found that homozygous Brattleboro rats that have an inherited inability to synthesize VP are severely retarded in their kindling rate when electrically stimulated in the amygdala. Marchand and Hagino (1982) reported a significant decrease in the firing rate of lateral septum neurons after iontophoresis of VP, suggesting the possible role of VP in suppression of neuronal firing in this region. The present study was an examination of electrical kindling of homozygous Brattleboro rats in the lateral septum and the ventral hippocampus.

Adult male homozygous Brattleboro (HO-BRAT) rats and normal Long Evans (N-LE) rats were implanted with bilateral electrodes in the lateral septum or the ventral hippocampus. Kindling stimulation consisted of a 1-sec train of biphasic square wave pulses at 60Hz delivered once a day at 200 μA. The N-LE animals stimulated in the lateral septum reached a stage 5 generalized seizure after a mean of 28.25 afterdischarges (ADs), while the lateral septum HO-BRAT animals took a mean of just 17.33 ADs ($p < .01$) to reach stage 5. The N-LE animals stimulated in the ventral hippocampus reached stage 5 after a mean of 34.8 ADs, while the HO-BRAT ventral hippocampus group had a generalized seizure after a mean of only 13.75 ADs ($p < .001$). There was no difference between HO-BRAT and N-LE lateral septum animals with respect to AD threshold or generalized seizure threshold (GST). However, AD threshold and GST for HO-BRAT animals stimulated in the ventral hippocampus were significantly higher than N-LE controls ($p < .01$; $p < .05$).

These data along with our previous findings suggest that VP may play a role in the kindling of seizures in normal LE rats and that this role may vary depending upon the site of stimulation. The present results favour the role of VP as a neuromodulator which may differentially affect neuronal firing in different brain regions.

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144.5 EFFECT OF PARTIAL AND FULLY GENERALIZED KINDLED SEIZURES ON THYROTROPIN RELEASING HORMONE LEVELS IN SPECIFIC CORTICAL AND SUBCORTICAL REGIONS OF RAT BRAIN. D. Walczak, J.L. Meyerhoff, V.E. Bates, T. Lynch and M.J. Kubek. Neurochemistry & Neuroendocrinology Branch, Dept. Med. Neuroscience, Div. Neuropsych., Walter Reed Army Institute of Research, Washington, DC 20307 and Dept. Anat., Indiana Univ. School of Med., Indianapolis, Indiana IN 46223.

Thyrotropin Releasing Hormone (TRH) has been demonstrated to exist in extrahypothalamic sites and may have neuromodulatory roles in addition to its neuroendocrine function. In a previous study TRH was found to be elevated in amygdala-pyriform complex, hippocampus and cerebral cortex of rats 48 hours after fully generalized kindled seizures (Meyerhoff, Bates & Kubek, Neurosci. Abstr. 8, 457, 1982). We have extended that study to examine effects in specific cortical regions and to compare effects following partially kindled (stage 2-3) seizures vs fully kindled (stage 5) seizures. Nineteen male Sprague-Dawley rats (250-300gm) were used for the study. Rats were stereotaxically implanted bilaterally in the amygdalae with bipolar depth electrodes (250 micron twisted nichrome, coordinates from lambda: 2.2 mm posterior, 4.9mm lateral, 8.5mm ventral, using the atlas of Konig and Klippel). One week after surgery 12 implanted subjects were subjected to a standard kindling regimen of one stimulation every 24 hours (1.0sec train of 60Hz biphasic square wave pulses, 0.1 msec duration, 500 μA peak to peak). Before stimulations were begun, these rats were divided into 2 groups of equal average body weight, to be kindled to stage 5 or only to stage 2-3. The remaining implanted animals were handled but not stimulated and served as sham kindled controls. Forty eight hours after five stage 2-3, or five stage 5 seizures, kindled subjects and their corresponding controls were decapitated and brains were removed. The brains were dissected on ice and the regional samples were immediately frozen. Further subdivisons of cortical and subcortical regions were performed in this study, including separation of amygdaloid nuclei and pyriform cortex. TRH levels were determined in each sample by specific radioimmunoassay.

In comparison to sham kindled subjects, TRH in fully kindled (stage 5) subjects was elevated 6-fold in pyriform cortex, 2.5-fold in cingulate cortex, 2-fold in frontal cortex, 3-fold in the remainder of cortex and 2-fold in both the amygdala and hippocampus. TRH was also elevated in the pyriform cortex of partially kindled subjects (stage 2-3). Elevations of TRH levels in these subjects were intermediate in magnitude between S5 and sham kindled values. It appears from these data that the degree of TRH elevation observed in kindled rats may be related to the stage of kindled seizure elicited. Further studies are under way to determine if the effects of kindling on TRH levels are short term or persistent.

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144.6 KINDLED AMYGDALOID SEIZURES: MODIFICATION BY CYCLAZOCINE AND ETHYLKETOCYCLAZOCINE. T. E. Albertson, R. M. Joy and L. G. Stark. Health Sciences Neurotoxicology Unit. School of Medicine and Veterinary Medicine, Univ. of Calif., Davis, CA 95616.

Sprague-Dawley rats were implanted with chronic cortical and amygdaloid electrodes. Ten days after surgery, rats were given daily i.p. injections of DMA (½ cc/kg, N=8), cyclazocine (CYC) (10 mg/kg, ½ cc/kg in DMA, N=9) or ethylketocyclazocine (EKC) (10 mg/kg, ½ cc/kg in DMA, N=7) twenty minutes before electrical stimulation of the amygdala (biphasic 60 Hz, 1 msec duration and 400 μAmp). After ten daily injections/-stimulations, the rats were rested for 2 days and then stimulated daily without drug pretreatment for another 14 days until generalized kindled amygdaloid seizures (KAS) were produced in all animals (stable rank 5). The average number of stimulations to reach the first stage 5 seizure (mean ± S.E.M.) was DMA = 14 ± 1.1, CYC = 17.2 ± 1.4 and EKC = 16.6 ± 0.9. A significant ($P<0.01$) between group effect, trial effect and trial and group interaction effect was found for afterdischarge duration with CYC. A 10% weight loss occurred in both the CYC and EKC groups and a 5% loss in the control DMA treated group after ten days of daily treatment. Tolerance developed to CYC-induced tail pinch analgesia but not to the elicited stereotypic behavior by the tenth injection. Tolerance developed to a lesser extent to the analgesic and ataxic effects of EKC.

Additional previously kindled rats were utilized to evaluate the effects of various doses of CYC (1-10 mg/kg) and EKC (0.1-10 mg/kg) on suprathreshold (400 μAmp) and threshold (20 μAmp increments) induced seizures. With suprathreshold stimulation, high doses of CYC and EKC increased post-ictal behavioral arrest time and spiking frequency while reducing seizure rank. When threshold stimulation was utilized, CYC significantly increased thresholds and reduced seizure ranks and afterdischarge durations. Naloxone (10 mg/kg) did not reverse and tended to potentiate these effects. EKC reduced the seizure rank and not afterdischarge duration with threshold stimulation.

The mixed opiate antagonist CYC has pronounced anticonvulsant actions against KAS. CYC reduces afterdischarge duration and rank during acquisition of the KAS, as well as, increasing afterdischarge thresholds in the fully KAS. The anticonvulsant properties seen with the kappa agonist EKC are much more limited.

144.7 EVIDENCE IMPLICATING SUBSTANTIA NIGRA IN SEIZURES KINDLED FROM MULTIPLE FOREBRAIN SITES. M. T. Galloway*, L. C. Rigsbee*, S. Legg*, and J. O. McNamara (SPON: D. B. Sanders). Depts. of Medicine (Neurology) and Pharmacology, Duke University, and Epilepsy Centers at Duke University and VA Medical Centers, Durham, N.C. 27705.

Kindling, an animal model of epilepsy, refers to the phenomenon whereby repeated administration of initially subconvulsive stimuli results in progressively more intense limbic and motor seizures. Once established, the effect is permanent. The mechanisms underlying this phenomenon remain poorly understood. Delineation of the underlying anatomic network is a necessary first step in understanding the basic cellular and molecular mechanisms. Our previous studies supported the hypothesis that activation of the substantia nigra (SN) was necessary for the generation of both motor and limbic components of amygdala kindled seizures. We sought to determine whether SN involvement was unique to seizures activated from the amygdala or was common to seizures activated from multiple forebrain sites.

To test this idea, male rats underwent stereotactic implantation of electrodes in one of the following structures: amygdala (n = 8), lateral entorhinal cortex (LEC) (n = 3), olfactory tract (n = 2), olfactory bulb (n = 2), or anterior olfactory nucleus (n = 2). Guide cannulas were placed directed toward the subtantia nigra. Electrode and injection cannula placements were histologically verified in all animals. Electrical stimulations were administered until seizures of consistent intensity were reliably elicited. Motor seizure duration was measured by behavioral observation; limbic seizure duration was quantitated from the afterdischarge recorded from the stimulating electrode.

The effects of microinjection of saline or the GABA agonist muscimol (M) on motor and limbic seizure duration were determined. Animals were gently restrained during injection. Saline injection into SN did not attenuate seizure duration. By contrast, microinjection of M (50 ng in 0.5 μl of saline) bilaterally into SN markedly reduced (80-100% suppression) both motor and limbic seizures elicited from each kindling site. This M effect was highly significant ($p < .001$, Student's t-test). The M effect reversed completely after several hours.

We conclude that the SN is a key site in the network of motor and limbic seizures elicited from multiple forebrain sites. This information should prove useful in delineating the anatomic structures underlying kindled seizures.

144.8 MICROINJECTION OF DYNORPHIN 1-13 INTO SUBSTANTIA NIGRA SUPPRESSES KINDLED SEIZURES. J. O. McNamara, L. C. Rigsbee*, S. Legg*, and M. T. Galloway*. Depts. of Medicine (Neurology) and Pharmacology, Duke University; and Epilepsy Centers, Duke University and VA Medical Centers, Durham, N. C. 27705.

Kindling is an animal model of epilepsy produced by periodic, focal electrical stimulation of the brain. We previously demonstrated that application of either a GABA agonist or a GABA transaminase inhibitor to the substantia nigra (SN) markedly suppressed kindled seizures. This raised the question of whether this effect was unique to GABA or whether other putative neurotransmitters endogenous to SN could also suppress the seizures. Recent immunocytochemical studies demonstrated the presence of dynorphin (DYN) immunoreactivity in fibers within SN. Since the opioid peptides have prominent inhibitory actions, we sought to determine whether microinjection of DYN into SN could suppress the seizures.

To test this idea, male rats underwent stereotactic implantation of an electrode in the amygdala and guide cannulas overlying SN. Electrode and injection cannula placements were histologically verified in all animals. Electrical stimulations were administered until seizures of consistent intensity were reliably elicited. Motor seizure duration was measured by behavioral observation; limbic seizure duration was quantitated from the afterdischarge recorded from the stimulating electrode. Stimulations were administered ten minutes following completion of injection and at hourly intervals thereafter until reversal.

Microinjection of DYN 1-13 (1250 picomoles in 0.5 μl of artificial cerebrospinal fluid (ACSF)) bilaterally into SN markedly suppressed both motor and limbic seizures in six of six rats. (Seizure durations in seconds are expressed as mean ± SEM.) Motor seizure duration pre-injection: 41 ± 4; post-injection 5 ± 5; reversal 39 ± 1. Limbic seizure duration pre-injection: 90 ± 13; post-injection: 7 ± 7; reversal 81 ± 8. The dynorphin effect was highly significant ($p < .001$, Student's t-test). Neither ACSF injection into SN (n = 3) nor DYN injection 100 - 1,000 microns dorsal to SN (n = 4) suppressed the seizures. Pre-treatment with naloxone (10 mg/kg IP) completely prevented the dynorphin effect in two animals, partly attenuated it in a third, and had no effect in a fourth.

We conclude that DYN possesses anticonvulsant properties in this model. The results with naloxone suggest this action may be mediated through an opiate receptor. Endogenous reduction of dynorphinergic neurotransmission in SN could be partly responsible for the abnormal excitability of kindling.

144.9 ATTENUATION OF RAT DENTATE GRANULE CELL EVOKED POTENTIALS FOLLOWING LATERAL ENTORHINAL CORTICAL KINDLING. J. L. Giacchino, G. Somjen and J. O. McNamara. Depts. of Medicine, (Neurology), Pharm. and Physiol., Duke Univ.; Epilepsy Centers, Duke University and VA Medical Centers, Durham, NC 27705.

Kindling is an animal model of epilepsy induced by multiple high frequency electrical stimulations of the brain. It can be elicited by stimulation of many brain areas, including the entorhinal cortex (EC). An initially subconvulsive stimulation eventually results in intense limbic and generalized motor seizures. This enhanced excitability is permanent. The cellular mechanisms responsible for kindling are unknown. One hypothesis is that long-term potentiation (LTP) of excitatory synaptic communication contributes to the kindling effect. Brief high frequency excitation of EC projections to the dentate gyrus results in LTP of granule cell evoked potentials. Therefore, potentiation of dentate granule cell responsivity to test stimuli of specific afferents should appear if LTP underlies the kindling process in these afferents.

LTP was examined in the monosynaptic projections from the lateral entorhinal cortex (LEC) to dentate granule cells (DG) in unrestrained, unanesthetized rats. Population excitatory post-synaptic potentials (pEPSPs) were recorded from the granule cell layer and were evoked by stimulation of permanently implanted LEC bipolar electrodes (monophasic square pulses, .2 pps, .55 msec width). Recording was done via permanently implanted electrodes or via electrodes lowered in chronically mounted microdrives. Input/output (I/O) relationships were determined using single pulse stimulations over a range of intensities. I/O curves were constructed on at least three consecutive days to establish stability of evoked pEPSPs over the range of stimulus intensities. Care was taken to record from alert animals at the same time of day to avoid the complicating influences of behavior and circadian rhythm.

Following the baseline I/O period, animals were kindled (400-1100 μamp, biphasic square wave pulses, 1 msec width, 60 Hz for a total train of 1 sec) via the LEC electrodes. I/O curves were then constructed on consecutive days post-kindling (kindling was considered complete after 3 Class 5 seizures). Controls were unkindled rats recorded from over the same time period as the experimental rats.

Averaged responses from the 4 I/O curves for each of 5 rats post-kindling as compared to averaged pre-kindling values revealed depressed pEPSP amplitudes following kindling. Control rats exhibited no consistent change in pEPSP amplitudes during the study. Thus, the results indicate that LTP of the LEC-DG synapse is not the mechanism of kindling from the LEC.

144.10 MONOAMINERGIC AND LOCAL ANESTHETIC COMPONENTS OF COCAINE'S EFFECT ON KINDLED SEIZURE EXPRESSION. R. D. Russell and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Previous research (Stripling & Hendricks, 1981) has indicated that cocaine (a drug with both monoaminergic and local anesthetic actions) and lidocaine (a local anesthetic) have similar effects on the expression of kindled seizures, suggesting that cocaine's effects are mediated at least in part by its local anesthetic action. The purpose of the present experiments was to explore further the relative involvement of cocaine's two major pharmacological actions on kindled seizure expression.

Male Long-Evans rats were kindled to criterion via daily electrical stimulation of the prepyriform cortex. The expression of kindled seizures was examined in 39 rats following intraperitoneal injection of saline, 20 mg/kg cocaine HCl, 20 mg/kg lidocaine HCl, or 2.5 mg/kg amphetamine sulfate (which causes release of monoamines). Each drug had a different pattern of effects on the various components of a kindled seizure. Cocaine and lidocaine produced a significant reduction in both the latency to forelimb clonus and the persistence of afterdischarge beyond the end of clonus, whereas amphetamine significantly increased the former and was without significant effect on the latter. In contrast, the duration of forelimb clonus was reduced significantly by cocaine and to a lesser (non-significant) extent by amphetamine, while lidocaine produced no decrease at all.

A second experiment further explored the possibility of monoaminergic involvement by examining the effect of cocaine on kindled seizure expression following the administration of four dose levels of the monoamine antagonists haloperidol, prazosin, yohimbine, propranolol, and metergoline (selected for their ability to block dopaminergic, alpha-1-noradrenergic, alpha-2-noradrenergic, beta-noradrenergic, and serotonergic receptors, respectively). None of the antagonists blocked any of cocaine's effects on seizure expression, but prazosin significantly potentiated cocaine's reduction of clonus duration.

The results of these two experiments suggest that cocaine reduces clonus latency and afterdischarge after clonus via a local anesthetic action. Cocaine's reduction of clonus duration does not appear to be mediated by a local anesthetic mechanism but may involve alpha-noradrenergic receptors. Why cocaine (which blocks norepinephrine uptake) and prazosin (which blocks post-synaptic alpha-noradrenergic receptors) should act synergistically in reducing clonus duration is a question for which we have no answer at this time.

144.11 EFFECT OF COCAINE AND LIDOCAINE ON THE DEVELOPMENT OF KINDLED SEIZURES. J. S. Stripling, C. A. Gramlich*, and M. G. Cunningham*. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Cocaine has been reported to have a variety of effects on the expression of previously kindled seizures, including a reduction in the duration of all seizure components (i.e., latency to clonus, clonus duration, and the persistence of afterdischarge beyond the end of clonus). However, its effects on the development of kindling have not yet been studied, although Racine, Livingston, and Joaquin (EEG Clin. Neurophysiol. 38: 355-365, 1975) have reported that the local anesthetic procaine facilitated kindling of the amygdala. The experiment reported here examined the effects of a subconvulsive dose of cocaine on the development of kindling and compared its effects with those of lidocaine, a local anesthetic without pronounced monoaminergic properties.

Male Long-Evans rats were divided into three groups which received daily intraperitoneal injections of either saline, 20 mg/kg cocaine HCl, or 20 mg/kg lidocaine HCl 15 min prior to electrical stimulation of the prepyriform cortex. When an animal reached kindling criterion the drug treatment was discontinued and the transfer of kindling to a non-drug state was assessed by the administration of test stimulations after delays of 1, 5 and 47 days. Both cocaine and lidocaine significantly accelerated the development of kindling. Furthermore, the duration of clonus at kindling criterion was significantly longer in these groups than in the saline group, and the onset of clonus occurred significantly sooner after stimulation in the cocaine group. However, this performance did not transfer fully to the non-drug state. Afterdischarge duration was higher in the two drug groups at all non-drug tests, but clonus duration was no longer significantly elevated, and the latency to clonus rose dramatically.

These results indicate that a subconvulsive dose of cocaine or lidocaine can facilitate the development of kindling when the drug is active at the time of electrical stimulation. Since Racine et al. (1975) also found facilitation of kindling with procaine, this effect appears to be due to a local anesthetic action. The kindling produced in this fashion is not entirely equivalent to kindling produced by electrical stimulation alone. In animals kindled under the influence of local anesthetics, the afterdischarge at the site of stimulation is expressed without reduction in the absence of the drugs, but seizure generalization, as reflected by the latency and duration of clonus, appears to have been artificially enhanced by the drugs and is reduced in their absence.

144.12 ^{3}H-FLUNITRAZEPAM BINDING TO AMYGDALA-KINDLED RAT BRAINS: WASHED VS. UNWASHED TISSUE. H.B. Niznik*, S.J. Kish* and W.M. Burnham (SPON: P.E. Garfinkel). Dept. of Pharmacol., Univ. of Toronto and the Human Brain Lab, Clarke Institute of Psychiatry, Toronto.

We have recently reported (Niznik et al., 1983) that the B_{max} for ^{3}H-flunitrazepam (^{3}H-FLU) binding to crude homogenates of amygdala-kindled rat brain is decreased in the hypothalamus and ipsilateral cortex two weeks following the last seizure. Tuff et al. (1983), however, using a "well-washed" preparation, have failed to observe these changes, and have reported instead an increased B_{max} for ^{3}H-FLU binding in the amygdala and hippocampus. The present study was designed to compare ^{3}H-FLU binding in both washed and unwashed membranes obtained from kindled and control animals 15 days following the 6th stage 5 convulsion. ^{3}H-FLU binding was measured in the hypothalamus, right and left regions of the cortex, amygdala, hippocampus and striatum in either crude homogenate or in extensively washed (7x)/hypotonically lysed (2x)/frozen (-80°C)-thawed (2x) brain membranes. Diazepam or clonazepam were used as displacers. B_{max} and K_d values were estimated from Scatchard plots by linear regression analysis.

In washed membranes, bilateral increases of approximately 20% were observed in the B_{max} for ^{3}H-FLU binding in both the amygdalae and hippocampi of kindled animals. No alterations of the K_d were seen. These results agree with the previous findings of Tuff et al. In unwashed membranes, ^{3}H-FLU binding was unaltered in the amygdalae and hippocampi, but was reduced by approximately 20% in the hypothalamus and right cortex. These results agree with our own previous findings. Similar results were obtained in the presence of clonazepam. ^{3}H-Ro5-4864 binding (2.5 nM) as well as GABA stimulated ^{3}H-FLU binding was unchanged in kindled rat brain.

Thus, long-term alterations in ^{3}H-FLU binding are observed in either washed or unwashed tissue. The pattern of the binding changes, however, depends on the membrane preparation used. In addition, the binding alterations appear to be restricted to the "central-type" benzodiazepine receptor. Possible interpretations of these findings will be presented.

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144.13 UNCHANGED NORADRENALINE LEVELS AND TURNOVER TWO MONTHS AFTER AMYGDALA KINDLING IN THE RAT. M.M. Okazaki*, W.M. Burnham and J.J. Warsh. Dept. of Pharmacology, University of Toronto and Section of Biochemical Psychiatry, Clarke Institute of Psychiatry, Toronto, Canada.

It has been postulated that the noradrenergic system is crucially involved in the kindling phenomenon. The present study was designed to investigate noradrenaline levels and turnover, using the α-methyl-p-tyrosine method.

48 Adult male Royal Victoria hooded rats were implanted with bipolar electrodes in the right amygdala. 24 of them were stimulated once daily (1 sec, 60 Hz, biphasic 1 msec pulses, 400 μa peak-to-peak) until six Stage 4-5 "kindled" seizures had been elicited. The other 24 served as "yoked" controls, receiving identical handling but no electrical stimulation. Each yoked pair was processed in parallel throughout the study. Two months after the completion of kindling, all subjects were treated with α-methyl-p-tyrosine methyl ester (250 mg/kg i.p.). Half of them were sacrificed by decapitation immediately after injection (Time 0), the other half being sacrificed four hours later (Time 4). Immediately after decapitation, each brain was dissected into eight regions (hypothalamus, brainstem, and right and left amygdala, hippocampus and cortex) and stored at -70°C until analysis. Noradrenaline and dopamine levels were simultaneously determined using high performance liquid chromatography with electrochemical detection. Samples were prepared for chromatography using the alumina method of pre-purification and analyzed using a modification of the analytical procedure described by Warsh et al (J. Chromatogr. 228:131-141, 1982). Noradrenaline and dopamine resting levels (Time 0) were unchanged in all regions examined. Noradrenaline and dopamine turnover were also unchanged. These results suggest that there are no long-lasting presynaptic changes in the noradrenergic system after amygdala kindling.

144.14 CHANGES IN A2- AND B-ADRENOCEPTOR BINDING SITES IN AMYGDALA KINDLED RAT BRAIN. John G.R. Jefferys* and S. Clare Stanford* (SPON P.Kirkwood). Sobell Dept, Inst. of Neurology, London WC1N 3BG, and Univ. Lab. of Physiology, Oxford OX1 3PT, U.K..

Kindling occurs when animals become progressively more susceptible to seizures on repeated stimulation of certain brain regions. Several studies have implicated the central noradrenergic system in the development of kindling, although the data have not always been consistent. We report here changes in a2- and B-adrenoceptor binding in the olfactory cortex of kindled rats.

Bipolar Pt/Ir wire electrodes were implanted stereotaxically, under Fluothane anaesthesia, in the left amygdala of thirty 300g Sprague-Dawley rats. Two weeks after operation the rats were stimulated once an hour (100 square, biphasic 400uA, 1ms pulses at 20ms) until they had experienced 3-5 stage 5 seizures; this usually required 24-27 stimulations. Sham controls received identical treatment, but with no current. Unoperated controls from the same batches of rats were housed singly and received as little handling as possible. Experimental rats and their corresponding controls were killed by cervical dislocation 22-26h after the last stimulation, and the brains dissected on ice. A2- and B-adrenoceptor binding followed the protocol of Stanford & Nutt (Neurosci. 7, 1753; 1982), except that nonspecific binding of 3H-clonidine was measured in the presence of 15uM phentolamine and nonspecific binding of 3H-dihydroalprenolol was measured in the presence of 3uM (+/-)propranolol.

Scatchard analysis of the binding data for the olfactory cortex showed the following. (1) The density of a2-adrenoceptors was approximately 50% greater in olfactory cortex than in the remaining cerebral cortex. (2) The numbers of both a2- and B-binding sites were higher in the sham controls than in the unoperated controls. The possibility that this represents a long lasting effect of anaesthesia is currently under investigation. (3) The numbers of both binding sites were significantly and bilaterally reduced by kindling (c.f. sham controls).

These results support previous reports of a down regulation of B-adrenoceptor binding sites following amygdalar kindling; further, they reveal a similar down regulation of a2-binding sites. Suppression of the functional output of central noradrenergic neurones, by either drugs or lesions, has been shown to accelerate the development of kindling, while monoamine oxidase inhibitors delay it. These various observations are consistent with view that the release of noradrenaline has the effect of limiting or preventing seizures. However, the repeated release of noradrenaline and increased binding to adrenoceptors during kindling triggers their down regulation and thus impairs seizure suppression. J.J. supported by the Thorn Trust, S.C.S. by M.R.C.

144.15 **PARALLEL ALTERATIONS IN GABA AND BENZODIAZEPINE RECEPTORS IN FASCIA DENTATA IN THE KINDLING MODEL OF EPILEPSY: A RADIOHISTOCHEMICAL STUDY.** **C. Shin*, H. Pedersen*, and J. O. McNamara (SPON: W. W. Anderson). Departments of Medicine (Neurology) and Pharmacology, Duke University; Epilepsy Centers, Duke University and VA Medical Centers, Durham, N.C. 27705**

Extensive evidence suggests that benzodiazepine receptors (BZR) are coupled to GABA receptors (GABA-R) in a macromolecular complex that includes chloride ion channels. Previous work from this laboratory demonstrated an increased number of BZR in fascia dentata of hippocampal formation following repeated kindled seizures. We subsequently localized the increase to dentate granule cells. The purpose of this work was twofold: 1) to determine whether parallel alterations occurred in GABA-R's and 2) to define the time course of the alteration for both receptor populations. Radiohistochemical methods were used to quantitate GABA-R and BZR binding with radiolabeled muscimol and flunitrazepam.

Male Sprague-Dawley rats received daily stimulations from an electrode in right amygdala and were sacrificed by decapitation 24 hours, 7 days and 28 days after the third Class 5 seizure, along with electrode implanted unstimulated controls (3 pairs at each time point). Frozen sections were thaw-mounted on subbed slides. [^{3}H] sensitive film was exposed to [^{3}H]-ornithine standards and adjacent sections incubated in 10 nM [^{3}H]-muscimol or 2 nM [^{3}H]-flunitrazepam. Developed film was mounted on glass slides and densitometric measurements were quantitated using a standard curve generated with [^{3}H] standards.

At 24 hours, there was a 20% increase in [^{3}H]-muscimol binding in fascia dentata of kindled rats (2394 fmol/mg protein) compared to control rats (1998 fmol/mg protein) ($p < 0.001$; 2 tailed paired t); a 38% increase in [^{3}H]-flunitrazepam binding was found in kindled (1719 fm/mg protein) vs. control (1246 fm/mg protein) rats ($p < 0.001$; 2 tailed paired t). At 7 days and 28 days, neither [^{3}H]-muscimol nor [^{3}H]-flunitrazepam binding exhibited any significant difference between kindled and control rats. Examination of neocortices of kindled and control rats at 24 hours disclosed no significant difference with either ligand. Experiments are under way to differentiate change in affinity from change in number of receptors.

The transient nature of these alterations is consistent with the idea that these represent a consequence of the seizures rather than part of the molecular basis of kindling. The similarities in the time course and direction of the receptor alterations support the idea that the two receptors are coupled within the postulated macromolecular complex.

144.16 ELECTROGRAPHIC AND BEHAVIORAL CONSEQUENCES OF REPEATED KAINIC ACID ADMINISTRATION. Michael Gruenthal and J. Victor Nadler. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710

Intracerebroventricular (ICV) administration of the potent convulsant kainic acid (KA), results in a dose-dependent pattern of selective neuronal degeneration. When KA is administered to rats in doses sufficient to induce prolonged limbic status epilepticus, the resulting neuronal loss closely resembles the pattern of brain damage associated with long-standing temporal lobe epilepsy in man (Ammon's horn sclerosis). In an attempt to develop a model of this disease in the rat, as well as to shed light on the relationship between seizure activity and hippocampal cytopathology, we have studied the behavioral, electrographic and histologic consequences of repeated subthreshold ICV administration of KA.

Awake, freely-moving rats received repeated (every 48 h) infusions of KA through a chronically implanted cannula into the left lateral ventricle while EEG records were obtained from chronically implanted surface and depth electrodes. KA (47 pmol) dissolved in 2.5 ul of artificial CSF was infused at a rate of 0.2 ul/min. Control rats received artificial CSF alone.

The electrographic reaction to the first infusion began after 8-10 min and consisted of a pronounced increase in EEG frequency and/or amplitude recorded from the ipsilateral and/or contralateral dorsal hippocampus with occasional short (30 to 120 s) limbic seizures (i.e., involving the hippocampus and/or amygdala, but not frontal cortex). These EEG changes were typically preceded by increased locomotion, sniffing and defecation. Limbic seizures were associated with reduced locomotor activity and their termination was frequently accompanied by wet dog shakes. These electrographic and behavioral changes lasted 30-60 min.

With repeated infusions of KA the time to onset of an observable reaction increased and the duration of the reaction decreased such that by the fifth infusion no behavioral or EEG alterations were observed. After the development of tolerance to the acute effects of KA, spontaneous limbic seizures were recorded 24-48 h after exposure to KA. The rats died 24-48 h following the fifth or sixth KA infusion. Histologic examination revealed no obvious abnormalities in any limbic region. Control rats showed no observable reaction to as many as 20 infusions of artificial CSF.

These results suggest that repeated ICV administration of KA can induce spontaneous limbic seizures and may provide a model of temporal lobe epilepsy. (Supported by NIH grant NS 17771 and postdoctoral fellowship NS 07018).

144.17 NEONATAL EXPOSURES OF RAT PUPS TO LINDANE FACILITATE THE ACQUISITION OF KINDLED AMYGDALOID SEIZURES IN THE SAME SUBJECTS WHEN ADULT. R. M. Joy, T. E. Albertson and L. G. Stark. Health Sciences Neurotoxicology Unit, Schools of Medicine and Veterinary Medicine, University of California, Davis, CA 95616.

Daily administration of lindane, p.o., to adult male rats facilitates the rate of acquisition of kindled amygdaloid seizures. The degree of facilitation is dose dependent, and the kindled state produced is as stable and persistent as that produced in the absence of lindane, even though it develops in a shorter time. The major mechanism involved appears to be the prolongation of afterdischarge (AD) duration during acquisition trials. Lindane does not modify the total amount of AD accrued in becoming kindled and does not lower AD thresholds. The purpose of this study was to determine whether exposure to lindane during brain development would enhance kindling once subjects were grown.

Nineteen pregnant females gave birth to 104 male offspring who were randomized to mothers. Nine litters were assigned to natal treatment groups which were gavaged daily with 40 mg/kg lindane in corn oil or corn oil for 6 days. The remaining 10 litters were assigned to maternal treatment groups where the mothers were gavaged with 20 mg/kg lindane in oil or oil alone for 10-12 days. All surviving offspring (N=65) were implanted 60 days after weaning and kindled with standard procedures 10 days later.

When kindled as adults no significant effects of natal versus maternal treatments were observed, so data for lindane and control groups were combined. Neonatally lindane-exposed rats kindled faster (9.9 ± 0.5 days, N=18) than did nonexposed rats (11.2 ± 0.6 days, N=25). Lindane-exposed rats tended to have longer and more severe seizures than did nonexposed rats on each trial during acquisition. However, the total AD accrued by the groups in becoming kindled was not different (lindane treated = 254 ± 32 secs, N=18; controls = 212 ± 24 secs, N=25). These findings are of the same type as those observed in adult rats exposed to lindane during the kindling period.

These data demonstrate that exposures to high concentrations of lindane during development can lead to permanent changes in the nervous system that facilitate kindling in the same subjects when adult. The types of changes are similar to those produced in adult rats kindled during concommitant lindane exposure.

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144.18 DEVELOPMENT OF HYPEREXCITABILITY IN MIRROR FOCI OF EPILEPTOGENIC FREEZE LESIONS. D. A. Twombly* and A. V. Delgado-Escueta. Department of Neurology and Brain Research Institute, University of California, Los Angeles, CA 90024

Secondary epileptogenesis is a process whereby discharges of a primary seizure focus enhance the excitability of synaptically related neuronal pools. Via electrocorticographic and evoked potential techniques, the evolution of pathophysiology was examined in developing "mirror" foci of epileptogenic freeze lesions.

Male Sprague-Dawley rats (250gm) were implanted with bipolar stimulating electrodes in left and right somatosensory relay nuclei of the thalamus. The right somatosensory area of the cortex was submitted to focal freezing for 15-30 seconds; diameter of the lesions ranged from 1.0-1.5 mm. Epidural recording electrodes were situated bilaterally over frontal motor and somatosensory cortex, and over the midline of the cerebellum. Electrode leads were attached to a microconnector, and the assembly was secured with dental acrylic. After a seven-day recovery period, spontaneous and thalamically evoked electrocorticographic activity was recorded on a periodic basis. Thalamic stimulation included single-pulse and paired-pulse trials. Averaged evoked potentials were obtained from both right (primary focus) and left (mirror focus) somatosensory cortex.

Focal freezing of neocortex was found to induce chronically active epileptogenic lesions, capable of generating paroxysmal discharges and occasional seizure episodes. Primary foci were abnormally responsive to thalamocortical activation; evoked responses were often followed by brief afterdischarges and, in some cases, seizures. Electrocorticographic recordings in regions contralateral to active primary foci revealed gradual development of hyperexcitability. Ten to fifteen days after induction of freeze lesions, mirror zones began to generate spontaneous epileptiform discharges, as well as augmented thalamocortical evoked potentials. Particularly enhanced were the long-latency components of evoked potentials, which often initiated a series of afterdischarges. Hyperexcitability was also evident in paired-pulse trials, both at long and short interpulse intervals. At intervals which initially produced inhibition in mirror foci, responses to test stimuli frequently exhibited facilitation as epileptiform activity emerged. These data suggest a reduced inhibitory drive in mirror foci of freeze lesions, as well as enhanced cellular synchronization through complex cortical pathways.

144.19 ICTAL TEMPERATURE TRANSIENTS IN THE KINDLED AMYGDALOID FOCUS. T. J. Lynch* and W. J. Jackson* (SPON: R.M. Wylie) Dept. Physiology, Medical College of Georgia, Augusta, GA 30912

Using micro-mini thermistors and voltage clamped polarography, in vivo brain temperature and pO_2 were monitored in left amygdala (LA) foci of Sprague-Dawley rats throughout kindled epileptogenesis. In nine rats, ictal temperature transients were recorded during 173 seizures ranging in severity from the subclinical (stage 0) to the generalized (stage 5). In the free ranging rat, LA temperature showed an average ictal increase on the order of 0.3°F during seizures leading up to and including the first stage 5 convulsion, and on the order of 0.4°F during succeeding stage 5 convulsions. In all rats the ictal heating transients increased as kindling progressed. However, the temperature increases in three out of four stage 5 convulsions elicited under neuromuscular block were 46 to 95% less than the increases observed for the same rats during free ranging convulsions, indicating that warming of the blood by convulsive motor activity is a major contributor to the LA heating transient. Ictal heating transients in the unstimulated, right amygdala (RA) were observed to occur upon the first kindling stimulation of the LA in one rat and upon the first stage 2 seizure in another, indicating either that early kindling metabolic activity is similar in the two sites or that the increase in cerebral blood flow (CBF) in early kindling is not just focal. Throughout the rest of kindling, ictal heating transients in the unstimulated RA were almost identical to those in the stimulated LA. Both LA and RA often underwent rapid postictal cooling to temperatures between 0.3 and 1.5°F below the pre-seizure temperature. Such cooling transients were most often seen in stage 4 and 5 convulsions, but sometimes as early as stage 3. From the consistent increases in ictal pO_2 seen in the kindled amygdala (Lynch and Jackson, Soc. Neurosci. Abst. 7: 187.10) it is inferred that global CBF increases in response to even the first kindling stimulus. From the postictal cooling transients it is inferred that the postictal relationship between focal site heat production and CBF may undergo a particularly large change starting at the stage 3 level of seizure generalization. Ictal heating and pO_2 maxima were well synchronized with the end of the primary AD, but no further increase in either quantity was ever observed during the occurrence of a secondary AD in the same seizure. Only a poor correlation was found between total ictal heating and the duration of the EEG afterdischarge, while the best correlation was found between total ictal heating and the severity of motor convulsive activity. Brain temperature measurement in experimental seizures may be the best on-line index of overall seizure severity since it seems to integrate brain metabolic activation and motor convulsive activity under one measurement. (Supported by NIH grant 1-NS6-2340)

144.20 AFTERDISCHARGE ACCUMULATION DURING AMYGDALA KINDLING IN JUVENILE AND ADULT RATS. Gary G. Buterbaugh and Patricia A. Barditch* (SPON: Naim Khazan). Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Obtaining information relevent to a specific age of development during the kindling of young rats requires more closely spaced stimulations than in adult rats (e.g. hourly vrs. daily) and more afterdischarges (AD) to kindle. Because AD accumulation parallels the acquisition of kindling, we quantitated the AD time during kindling in rats of different ages receiving hourly stimulation as a means of determining the potential usefulness of this kindling protocol for studying developmental aspects of kindling.

Male, Sprague-Dawley rats were implanted with bipolar electrodes in the right amygdala when 23-, 43- and 83-days-old (N = 9 per age). Five days later, kindling was started with hourly stimulations (1 sec of 1 msec, biphasic, square-wave pulses, 60 Hz, 1.5 x threshold), 10 - 12 hours/day, until 2 consecutive stage V convulsions were obtained. The EEG AD from the stimulated amygdala was collected with a Grass Model 7 polygraph.

The three age groups did not differ in the total number of ADs and AD sec to kindle. However, 28-day-old rats accumulated a significant, 5-fold greater number of ADs during stage II. This age also accumulated 32.2% of total kindling AD time in stage II compared to less than 6% for 48- and 88-day-old rats. These ages accumulated more AD time in stage I (46.5 & 36.5%, respect.) than 28-day-old rats (22.3%). No significant differences were found during other stages of kindling. In a separate group of 48-day-old rats receiving daily stimulations, there was no difference in accumulated AD time to kindle compared to hourly stimulated rats (664 ± 53.3 vrs. 727 ± 88.8 sec, respect.). However, they had fewer ADs (13.6 ± 1.2 vrs. 23.4 ± 1.5, respect.), but displayed AD durations 2-fold longer in most stages of kindling. Pretreatment of 28- and 48-day-old rats with reserpine resulted in a 40% decrease in total ADs and AD time to kindle during hourly stimulation, distributed evenly during most stages. However, 28-day-old, but not 48-day-old rats, also showed a marked increase in ADs and AD time during stage III convulsions, a stage seldom observed in untreated rats.

The results indicate the hourly kindling protocol is a useful model for studying developmental aspects of seizure acquisition. Little differences were found in total AD time required to hourly kindle in the three age groups, and in one age compared to daily stimulation. Hourly stimulation revealed differences between 28- and 48-day-old rats, including a different effect by reserpine, during the early stages of kindling. These results suggest that the processes underlying the early acquisition of kindled amygdaloid convulsions are still maturing in rats up to 48 days of age.

144.21 INTRACELLULAR OBSERVATIONS IN IN VITRO HIPPOCAMPUS OF KINDLED RATS. E.W. Kairiss, G.K. Smith and R.J. Racine. Department of Psychology, McMaster University, Hamilton, Ontario, Canada L8S 4K1.

The cellular basis of epileptiform phenomena has proven difficult to identify. Studies of acute seizures (e.g. penicillin-induced) have provided some insights into mechanisms of burst generation and neuronal synchronization associated with the epileptiform spike, but the relevance of these findings to chronic forms of epilepsy remains uncertain. Kindling is an experimental model of epilepsy which has focal characteristics, is progressive, generalizes and eventually develops spontaneous epileptiform activity. In the present study, we have examined, in vitro, the intracellularly-recorded responses of hippocampal neurones from amygdala-kindled rats in an effort to detect abnormal cellular behaviours.

Male Long-Evans rats, under barbiturate anesthesia, were implanted with bipolar stimulating electrodes in the lateral amygdala. Daily kindling stimulations were applied until a class V seizure was elicited. The animals were then sacrificed and hippocampal slices were prepared using standard techniques. The $[K^+]$ of the incubation medium was 3mM and experiments were performed at 34.5°C. Routine electrophysiological techniques were employed for stimulation of stratum radiatum, and recording of extra- and intracellular potentials from the CA1 region.

In agreement with our previous report (Kairiss et al.,Soc. Neurosci. Abstr., 7, 584,1981), slices demonstrated no spontaneous epileptiform activity as reflected in extracellular field potentials. Neuronal recordings revealed normal membrane and spike potentials and input resistance.. Inhibitory postsynaptic potentials and spike afterhyperpolarizations could be recorded in all preparations. However, neurones in kindled slices tended to respond to depolarizing current pulses with an initial high frequency rate of firing, in contrast to the normal non-bursting mode of discharge. This is suggestive of subtle, postsynaptic alterations in excitability and contrasts sharply with the properties of penicillin-induced foci. Whether this behaviour is directly attributable to a kindling-induced epileptogenic process in the hippocampus or results from activation by an extra-hippocampal focus is unclear and will require further in vivo study.

144.22 ANTAGONISM BETWEEN CONCURRENTLY DEVELOPING KINDLED SEIZURE FOCI CAN SUPPRESS KINDLING. J.L. Burchfiel, K. Serpa,* F.H. Duffy. Children's Hospital, Boston, MA 02115

In past work (Duchowny & Burchfiel, EEG Clin Neurophysiol., 51:403-416, 1981; Burchfiel, el al., Exp. Neurol. 75:476-489, 1982) we have shown that concurrent kindling of two limbic foci in rats is associated with a striking antagonism of motor seizure generalization; a phenomenon we call "kindling antagonism." Concurrent kindling is accomplished by electrically stimulating two limbic sites (using chronically implanted electrodes and typical kindling stimulus parameters) in alternation on a trial-by-trial basis, i.e. one stimulus to one site, the next stimulus to the other site, etc. The result of this paradigm is that one focus develops typical, progressive kindling culminating in generalized seizures; whereas, kindling from the alternately stimulated site fails to occur.

In the present study we addressed the question of what type of kindled seizure development is occurring at the non-dominant focus. In general, one could imagine two possibilities. First, the underlying substrate for kindling could be developing, but the actual expression of the kindled state in terms of a generalized seizure is being suppressed. A second possibility is that the kindling process itself is being suppressed, i.e. that little or no increase of seizure susceptibility is being induced by the stimulation. To choose between these possibilities we did the following experiment. Rats were stimulated alternately in the septal area and ipsilateral entorhinal cortex until a state of kindling anatagonism was established in which one site consistently elicited generalized seizures and the other site elicited no motor seizure behavior. Then stimulation of the dominant site was halted and the non-dominant site was stimulated alone on consecutive trials. We found that after alternate stimulation the mean number of additional trials required for the non-dominant site to attain generalized kindled seizures was not significantly different from that required for primary kindling. This result occurred even if a two period without stimulation was interposed at the end of alternate stimulation. These data indicate that during the state of antagonism no significant kindled seizure development occurred at the non-dominant site.

144.23 RETARDATION OF AMYGDALOID KINDLING BY TREATMENT WITH SCOPOLAMINE. V. Westerberg* and M. E. Corcoran. Department of Psychology, University of Victoria, Victoria, B. C., Canada V8W 2Y2.

Studies of the effects of atropine sulfate, a muscarinic antagonist, on the development of amygdaloid kindled seizures have yielded contradictory results. In some studies atropine slowed the rate of amygdaloid kindling (Albright et al., Exp. Neurol., 1979, 66, 409; Arnold et al., Exp. Neurol., 1973, 40, 457), whereas in others it had no effect (Blackwood et al., Psychopharmacology, 1982, 76, 66; Corcoran et al., Exp. Neurol., 1976, 51, 271). In order to clarify this issue we decided to investigate the effects of scopolamine, a muscarinic antagonist more potent than atropine, on the rate of amygdaloid kindling.

Six weeks after implantation of electrodes, male Sprague-Dawley rats received application of unilateral amygdaloid stimulation once every 2 days. Some rats received an IP injection of scopolamine (10 mg/kg) 15 min before each stimulation, and others received injections of the distilled water vehicle. If rats failed to develop bilaterally generalized (stage 5) seizures after 22 afterdischarges (ADs) they were given a 14-day rest without stimulation and then were rekindled without the drug. Scopolamine-treated rats required significantly more total ADs ($M = 22.6$, $SD = 9.5$) than controls ($M = 11.8$, $SD = 3.9$) to develop the first stage-5 seizure. Of the 9 rats treated with scopolamine, 3 failed to develop even unilateral clonic seizures after 22 ADs and required as many additional drug-free ADs to rekindle ($M = 13$, $SD = 2.9$) as controls had required for initial kindling. This suggests that scopolamine can produce a genuine prophylactic effect, in agreement with the findings of Arnold et al. (1973) with atropine.

After completion of kindling, control rats were given injections of scopolamine. There was no effect of scopolamine on the duration or intensity of kindled seizures, suggesting that its prophylactic effects against seizure development were not due to a general antiepileptic action.

The present results support the hypothesis that cholinergic or cholinoceptive neurons play an important role in the development of kindled seizures. Because there are rats that kindled even after treatment with scopolamine, however, the participation of these neurons may not be critical for kindling.

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144.24 REDUCTION OF GAD-POSITIVE CELLS AND TERMINALS AFTER ENTORHINAL-DENTATE KINDLING IN RATS. T. L. Babb, W. J. Brown*, S. Feldblum*, J. Pretorius* and W. Kupfer*. Dept. of Neurology, Division of Neuropathology and Brain Research Institute, UCLA Sch. of Med., Los Angeles, CA 90024.

The hippocampus is known to have an effective recurrent collateral inhibitory circuit which has been shown to involve activation of local inhibitory interneurons by axon collaterals of principal neurons. These interneurons inhibit the principal neurons by secreting GABA at their somata. It has been suggested that epileptogenic hippocampus develops when this GABA-mediated recurrent inhibition is reduced.

The present studies were designed to test if after kindling the hippocampus, there could be a reduction in GABA inhibition as determined by a reduction in the GABA synthesizing enzyme glutamic acid decarboxylase (GAD). GAD anti-body was obtained through the generosity of Dr. I. Kopin (Oertel et al., Neurosci., 6:2689-2700, 1981), and the ABC-HRP method was used to localize GAD in interneurons and at terminals to verify the anatomical loci in normal rats. Group KEC (n=8) were kindled to an average of Stage 3 seizures by 19 stimulations of entorhinal cortex (EC). Group EC (n=8) received 19 EC stimulations each spaced 10 minutes apart and did not kindle. Densitometric analysis of GAD positive terminals in fascia dentata (first synapse) showed a significantly-reduced GAD in the kindled group compared to the non-kindled ($p < .05$).

In a second study kindled rats (n=8) had significantly reduced GAD compared to controls ($p < .01$), whether or not they were sacrificed immediately after the 19th stimulation ($p < .05$) or 24 hours later ($p < .05$). These results indicate that the epileptogenic process of kindling does reduce the rate of synthesis of recurrent GABA-mediated inhibition.

145.1 ORIGIN OF THALAMIC INPUT TO THE SUPPLEMENTARY AND ARCUATE PREMOTOR AREAS. G.R. Schell* and P.L. Strick. V. A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-Upstate, Syracuse, NY 13210.

The premotor areas which project most densely to the motor cortex are: 1) the arcuate premotor area (APA), located in and around the caudal bank of the arcuate sulcus and 2) the supplementary motor area (SMA), located on the medial wall of the hemisphere (Muakkassa and Strick, Brain Res. '79). In the present experiments we determined the origin of thalamic inputs to the SMA and APA as a step toward understanding how subcortical structures might interact with each premotor area.

In 4 monkeys (Macaca mulatta), small (0.025-0.05 µl), multiple injections of 2% wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP, Sigma) were made unilaterally into either the SMA or the APA. For the purpose of comparison, similar injections were made into the motor cortex of another animal. After a 4 day survival period, animals were anesthetized and perfused with phosphate buffered aldehydes. Tissue sections were processed using the TMB technique (Mesulam, '78).

Injections into each cortical area resulted in dense, slab-like accumulations of anterograde and retrograde labeling in the ventrolateral thalamus. Little or no overlap was observed in the thalamic labeling following injections into separate functional areas. APA injections resulted in thalamic labeling which was most dense in area X. In contrast, SMA injections resulted in thalamic labeling which was most dense in VLo. VPLo was most densely labeled following injections into the motor cortex. Thus, the two premotor areas and the motor cortex each received thalamic input from separate, cytoarchitectonically distinct subdivisions of the ventrolateral thalamus.

Previous anatomical studies by others have indicated that each of the three subdivisions of the ventrolateral thalamus receives input from a separate subcortical structure. Area X receives input from caudal portions of the deep cerebellar nuclei (e.g., Stanton, '80; Kalil, '81). VLo receives input from the globus pallidus (e.g., Kim et al., '76; DeVito and Anderson, '82). VPLo receives input from rostral portions of the deep cerebellar nuclei (e.g., Stanton, '80; Kalil, '81). Based on these data and our observations, we propose a new scheme of subcortical-cortical interactions. In this scheme, the output from caudal portions of the deep cerebellar nuclei is focused on the APA; the output from the globus pallidus is focused on the SMA; and the output from rostral portions of the deep cerebellar nuclei is focused on the motor cortex. Supported by funds from the VA Medical Research Service, UPSHS-NS 02957, and the Dept. of Neurosurgery.

145.2 AFFERENT AND EFFERENT CONNECTIONS OF THE POSTARCUATE REGION OF THE MONKEY CEREBRAL CORTEX. M. Godschalk*, R.N. Lemon* and H.G.J.M. Kuypers* (SPON: European Neuroscience Association). Dept. of Anatomy II, Faculty of Medicine, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

We have previously shown that neurons in the region of the arcuate sulcus of the macaque cerebral cortex show pronounced modulation of activity during preparation for specific movements; for some neurons, changes in activity during the preparatory period are clearly related to the trajectory of arm movement required to reach for a visible target. In an attempt to define the anatomical substrate for these characteristic properties, we have now investigated the afferent and efferent connections of the postarcuate region using both anatomical and electrophysiological techniques. For retrograde transport studies we used either HRP or a combination of fluorescent tracers. Results obtained with these experiments were then confirmed by eliciting antidromic or synaptic responses in postarcuate neurons by stimulation of different cortical areas in anesthetized monkeys.

Efferents: There is a pronounced projection from this region to area 4 (motor cortex), particularly from neurons in the posterior bank of the inferior limb of the arcuate sulcus. This projection is topographically organized such that the axonal trajectory of neurons projecting from this area is generally in a postero-medial direction. For example, neurons lying around the spur of the arcuate sulcus project to the hand representation of area 4, whereas those lying in the posterior bank of the inferior limb of the arcuate sulcus project further laterally within area 4. These neurons are concentrated in the upper cortical layers and can be antidromically activated from area 4 with latencies ranging from 0.6-2.1 ms.

Afferents: There is a powerful afferent projection to the post-arcuate region from the antero-lateral part of the inferior parietal lobule (IPL), the banks around the tip of the intra-parietal sulcus and the superior bank of the lateral fissure. This projection comes principally from layer III pyramidal cells, which were filled in great numbers following HRP injections in the posterior bank of the inferior limb of the arcuate sulcus.

Some neurons in the postarcuate region projecting into area 4 responded synaptically to stimulation of the IPL. In contrast, no evidence was found for a direct input from the IPL into area 4. Since neurons in the IPL have been implicated in the processing of visuo-spatial information related to movement, the indirect route from the IPL to area 4 via the arcuate region may provide a means of access for this information to the motor cortex.

145.3 PRIMATE FACE MOTOR CORTEX: REPRESENTATION OF IPSILATERAL AND COMPLEX OROFACIAL MOVEMENTS. M.A. Sirisko* and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto M5G 1G6, Canada.

Previous studies have reported that ipsilateral and complex masticatory-like movements as well as contralateral movements can be evoked from the primate cerebral cortex. These studies have placed the ipsilateral and masticatory-like representations in areas lateral to the contralateral representation. Since our earlier investigations of the "excitable" cortex suggested that ipsilateral and masticatory-like movements might be evoked at some loci within the motor representation of the contralateral orofacial region, we decided to investigate these representations in more detail.

The motor representation of the face, jaw and tongue was determined by examining twitch and complex masticatory-like movements and associated EMG activities evoked by intracortical microstimulation (ICMS) in four unanaesthetized monkeys (Macaca fascicularis). A series of microelectrode penetrations were made 1 mm or less apart in the sensorimotor cortex and in each penetration intracortical sites were stimulated every 250 µ or less over vertical depths of up to 5 mm in precentral cortex and 10 mm in pericentral cortex. Stimuli of less than 35 µA were used to define the "excitable" cortex and its boundaries and the characteristics of evoked responses. Over 70 microelectrode penetrations were made in each animal, and lesions were placed at the bottom of over 20% of the penetrations to aid in the subsequent histological reconstruction.

We confirmed our earlier findings that the face, jaw and tongue representation occurred precentrally, with the facial representation forming a horseshoe around a central core of jaw and tongue movements. Included within this predominantly contralateral representation in area 4 was a region of precentral cortex within which ipsilateral movements could be evoked. This region was characterized as a mediolateral strip, approximately 2 mm wide and up to 9 mm long. Facial movements predominated in this ipsilateral representation and the ipsilateral ICMS thresholds were comparable to those noted for the contralateral movements. Also included within the face motor cortex were loci from which masticatory-like movements could be evoked by continuous ICMS currents as low as 15 µA. These loci tended to be concentrated at two sites that were several mm apart. The masticatory-like movements had a frequency of 1-3 Hz and involved contralateral or ipsilateral deviation of the jaw. These findings suggest that ipsilateral and masticatory-like movements may be represented not only in areas lateral to the face motor cortex, as earlier studies have indicated, but also within the contralateral representation in area 4.

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145.4 SENSORIMOTOR PROPERTIES OF NEURONS IN AREA 5 DURING CONDITIONED ARM MOVEMENTS IN THE MONKEY. C.E. Chapman, L. Busby*, G. Spidalieri* and Y. Lamarre. Centre de recherche en sciences neurologiques, Département de Physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3T8.

Results from lesion studies of area 5, together with its known connections to pre- and postcentral cortex, suggest that it has an important role in sensorimotor integration. We have recorded unitary discharges from area 5 in 2 monkeys trained to perform rapid elbow movements in response to 3 different, and randomly presented, sensory cues: a light, a tone and a small (1-2°), brief (50 ms) perturbation of the elbow.

The discharge of 90/157 neurons was modulated in relation to movement, the earliest change following the onset of EMG activity in 92% of the units (x̄=25 ms before movement onset). The pattern of discharge varied with the direction of movement in 36/38 cells tested, most often in a non-reciprocal manner (30/36). In 50% of the units which showed an excitation (28/56), the movement-related discharge was correlated with physical parameters of the movement. The results suggested that there may be some functional differences within area 5 since units located in the upper part of the anterior bank of the intraparietal sulcus were more often, and more strongly, correlated with movement parameters than those located within the depths of the sulcus.

Forty per cent of the modulated cells (n=38) had a short latency (40 ms) response to the torque pulse. These cells included almost all of the units in which discharge was correlated with movement parameters, suggesting that the correlations could be explained by peripheral afferent feedback. The sensory response was, however, clearly dependent on the subsequent performance of the movement: it was reduced or absent when movement was extinguished by withholding the reward and it was frequently absent in trials with long reaction times. The possibility that changes in the level of alertness could explain the results was ruled out by showing that the same degree of modulation is obtained when the stimulus was repeated immediately after a successful trial: the response was present only when the animal moved to the second cue. The dependence of the sensory response on the motor response is a property which might serve as a neural substrate for stereognosis, a somesthetic function which most likely depends upon concurrent processing of information from both motor centres and peripheral receptors.

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145.5 HINDLIMB MOTOR CORTICAL NEURONAL PATTERNS IN MONKEYS CONDITIONED TO A VARIETY OF TASKS. S.A. Sahrmann, M.H. Clare, E.B. Montgomery JR., W.M. Landau. Dept Neurology, Washington University School of Medicine, St. Louis, MO 63110

Normal rhesus monkeys were conditioned to perform a complex behavioral paradigm that included movements known to be particularly impaired by upper motor neuron lesion. Light signals were used to direct the monkey's performance of 4 self-paced tasks that included small and large sustained forces in plantar and dorsiflexion directions and large phasic forces in both directions. Each of the pseudo-randomly delivered tasks started from rest and required the simultaneous use of both feet. Since the ultimate purpose of the study is to compare the behavior of motor cortical neurons (MCUs) in the normal to that in the monkey with the UMN syndrome, all response patterns were analyzed to describe population characteristics.

Ninety percent of the 226 analyzed MCUs showed increased activity temporally related to force and EMG. Ten percent showed decreased activity exclusively. The 203 MCUs showing increased activity differed in their directional preference: half were active only in relation to development of force in one direction (unidirectional) and the remainder with active forces in both directions (bidirectional). Only 28/101 of the bidirectional units were symmetric (equal activity with force in both directions). The majority were asymmetric with a greater degree of activity in one direction. Both unidirectional and bidirectional (symmetric and asymmetric) MCUs often developed increased activity not only with active agonist forces but also with relaxation of opposite "antagonistic" force (relaxing force change). Seventy-eight percent of the MCUs showing increased activity during the prolonged force holds. Thirty-eight percent of the units were more active with larger force and 14% with smaller force; 48% had comparable activity with both force levels. None of these characteristics was related to directionality. The most striking finding evident with our multitask behavioral paradigm was the spectrum of MCU behaviors, ranging from units which increased or decreased discharge with force in one direction, to those responding with active and relaxing force change in both directions. The complexity of the patterns of MCU activity was not simply interpretable in terms of rigid linkage to the anterior horn cells of the final common path.

145.6 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. VIII. CELL DISCHARGE IN MOTOR CORTEX AND AREA 5 VARIES WITH MOVEMENT DIRECTION, NOT WITH FINAL POSITION OF THE ARM. J.F. Kalaska, A.P. Georgopoulos and R. Caminiti. The Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins University, School of Medicine, Baltimore, MD 21205.

The hypothesis was tested that the frequency of discharge of motor cortical or area 5 cells during the reaction time (RT) might predict the final position of the arm in aimed movements. Rhesus monkeys were trained to move their arm to the same endpoint in a two-dimensional space using an articulated manipulandum. The movements started from 8 different points located equidistantly on the circumference of a circle of 8 cm radius and ended on the center of the circle in a reaction time task. Thus the movement endpoint was dissociated from the movement direction since all movements ended on the same point but were made in 8 different directions. The movements exhibited a dome-shaped velocity curve with a peak value of 200-400 mm/sec in different movements. The activity of 154 motor cortical and 55 area 5 cells was studied in the task during microelectrode penetrations into 5 hemispheres of 3 monkeys. These cells were related to arm movements and changed activity during the RT in the task. The relations between the changes in cell discharge during the RT and the direction of movement (variant) or the movement endpoint (invariant) were evaluated using an analysis of variance. The RT discharge rate of 98% of cells in either structure varied in association with the direction of movement. Therefore, these cells were related to movement direction and not to its endpoint. The remaining 2% of motor cortical and area 5 cells showed uniform changes in activity for all the directions tested and could be related to the invariant final position of the arm. However, the same uniformity in the changes of cell discharge was observed when the animals moved, in a different task, from the same starting point (center of the circle) to 8 different points on the circle. This suggests that the changes in discharge rate that were observed in that small proportion of cells were related to neither the direction of movement nor to its endpoint. Similar results were obtained for the movement time. In summary, cell discharge in motor cortex and area 5 relates to the direction of movement despite the same final arm position; indeed no cells were found that were related exclusively to the movement endpoint. It appears that if the final arm position is indeed the controlled spatial variable in aimed arm movements (cf. Polit and Bizzi, J. Neurophysiol. 42: 183, 1979), that variable is not reflected in the changes in discharge rate preceding movement in the cortical areas studied. Instead, these changes are related to the movement vector itself. (Supported by USPHS Grants NS17413 and NS07226).

145.7 CORTICAL MECHANISMS OF TWO-DIMENSIONAL AIMED ARM MOVEMENTS. IX. STATIC (POSITIONAL) FACTORS CANNOT ACCOUNT FULLY FOR MOVEMENT-RELATED CELL DISCHARGE IN MOTOR CORTEX AND AREA 5. A.P. Georgopoulos, J.F. Kalaska, J.T. Massey and R. Caminiti. The Bard Laboratories of Neurophysiology, Department of Neuroscience, The Johns Hopkins Un., School of Medicine, Baltimore, MD 21205.

The frequency of discharge of arm-related motor cortical and area 5 cells varies in an orderly fashion with the direction of movement (Georgopoulos et al., J. Neurosci. 2: 1527, 1982; Kalaska et al., Exp. Brain Res., 1983, in press). On the other hand, the steady-state discharge rate of about 70% of these cells in either structure varies with the position of the arm in space (Caminiti et al., Soc. Neurosci. Abstr. 7: 563, 1981). (The remaining 30% of cells show changes in activity with movement in the absence of positional effects.) The positional variation is such that when the hand is held at various points in a two-dimensional space, the cell response surface describes a plane. Given that during movement the hand occupies successively different positions in space, it could be argued that the directional variation in discharge observed in these cells during movement reflects simply the change in position. We examined this idea by performing the following analysis on experimental results obtained from 78 motor cortical and 63 area 5 cells which exhibited planar static (positional) response surfaces. 1) The plane equation was derived from the static data. It was of the form, $d = d_o + \alpha x + \beta y$, where d is the discharge rate of a cell at coordinates (x,y), and d_o, α and β are constants that differ for different cells. 2) Given two-dimensional movement trajectories made by monkeys in a reaction time task (see Georgopoulos et al., J. Neurosci. 2: 1527, 1982), we calculated, using the plane equation above, the changes in discharge rate that would have occurred during these movements based on changes of hand position alone, that is, if the movements were of infinitely low speed. The spatial trajectories, of course, would be the same under both the actual and the hypothetical conditions. 3) These predicted "positional" curves were then compared with the changes in cell discharge actually observed during movement. In most cases the observed changes led the predicted ones in time, differed significantly from them in magnitude and time course but were similar in the direction of change in cell activity. Moreover, brisk changes in discharge were frequently observed even when the hand moved perpendicularly to the axis of maximum static slope; that is, when the predicted "positional" change was zero. Finally, these "dynamic" changes in cell discharge (calculated as: actually observed - "positionally predicted" responses) varied in an orderly fashion with the direction of movement. (Supported by USPHS Grants NS17413 and NS07226).

145.8 CHANGES IN ELECTRICAL EXCITABILITY OF PRIMATE PYRAMIDAL TRACT AXONS DURING ACTIVE WRIST MOVEMENTS. A. Schmied and E. E. Fetz, Dept. of Physiology & Biophysics, and Regional Primate Research Center, University of Washington, Seattle, WA 98195.

In monkeys performing alternating wrist movements the threshold for electrical activation of pyramidal tract (PT) axons was found to change systematically during the wrist movements. Tested randomly, the antidromic thresholds of PT neurons were usually lower during the phase of movement in which the cell discharged. Two mechanisms previously documented in anesthetized animals may explain this effect. The axonal excitability of corticofugal neurons has been shown to be transiently increased by peripheral stimulation in anesthetized cats (1,2), suggesting the possibility of presynaptic modulation of transmission in corticofugal pathways. Second, the electrical threshold of an axon fluctuates following conduction of an action potential (3). In awake, behaving primates we found evidence for both spike-related and task-related changes in axonal threshold of precentral PT neurons.

Electrical excitability of PT axons was tested in macaques performing ramp-and-hold wrist movements against an elastic load. Antidromic action potentials were evoked by 0.2-ms biphasic stimuli via a concentric bipolar electrode placed near the medullary pyramids. The proportion of antidromic action potentials evoked by near-threshold stimuli of constant intensity ($n > 100$) was taken as an index of electrical excitability. (Suprathreshold stimuli invariably evoked antidromic spikes, so the failures were due to fluctuations of axonal threshold.) Delivering PT stimuli at fixed delays after orthodromic spikes revealed a repeatable time course for the postspike change in excitability. After the collision interval, excitability quickly rose to a peak within several milliseconds and subsequently dropped to a plateau level after 15-25 ms. The tested PT neurons all exhibited a similar time course of postspike excitability, suggesting an intrinsic membrane mechanism (3). Such an increase in excitability following an orthodromic action potential may enhance conduction of subsequent action potentials into terminal branches; indeed, the facilitation of the second of two corticomotoneuronal EPSPs has a similar time course (4).

Independently of the postspike threshold changes, most PT neurons also exhibited additional excitability changes related to motor activity. Such task-related changes were not consistently related to the phase of movement in which the PT neuron fired. PT stimuli delivered at a fixed postspike latency often evoked more antidromic responses during the active movement that involved the greatest force. Such task-related threshold changes may reflect depolarization of terminals within electrotonic conduction distance of the stimulation site (1,2).

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145.9 THE PRIMARY MOTOR CORTEX OF THE OWL MONKEY: A MICROSTIMULATION MAPPING STUDY OF THE LEG AND ARM AREAS. E.J. Neafsey, E.L. Bold*, C.F. Sievert*, R.R. Terreberry* and G.J. Quirk*. Dept. of Anatomy, Loyola Univ. Medical Center, Maywood, IL 60153.

A number of recent studies have provided detailed maps of the visual, auditory and somatosensory cortices of the owl monkey (Aotus trivirgatus), revealing in each area multiple representations of the receptor surface. The owl monkey is particularly suited for these studies since its lissencephalic brain facilitates the mapping process. The present study sought to exploit this same advantage in an intracortical microstimulation study of the primary motor cortex.

During both surgical preparation and stimulation each of the three owl monkeys studied was anesthetized with ketamine HCl. End-expired CO_2, blood pressure and body temperature were maintained near normal values. After placing the animal in a stereotaxic frame, a wide craniotomy was made, exposing frontal and parietal cortex on one side. An acrylic dam was constructed around the opening, the dura was removed, and the dam filled with mineral oil. A photograph of the surface was used to plot the location of the 100-200 penetrations made in each experiment. The tracks were .5-1 mm apart, were perpendicular to the surface, and were made to a depth of 1.5 mm. Stimulus parameters were a 50 msec train of .25 msec constant current negative pulses at 350 Hz. The maximum current was 30 μamps. Marking lesions were made by passing 10 μamps DC for 10 sec at selected points. At the conclusion of the experiment the animal was perfused with formalin and the brain sectioned.

Based on histological reconstruction of electrode tracks, the primary motor cortex defined by stimulation coincides with cytoarchitectonic area 4. The leg motor area of all three monkeys appeared to contain at least two distinct areas where toe movements were evoked. In two animals these areaswere separated from each other by cortex where more proximal knee or hip movements were elicited. In the other animal a single large patch of cortex was devoted to toe movements; however, within this area as one moved from caudal to rostral the sequence of movements repeated itself: toes flex, toes extend, toes flex, toes extend. It was common to find zones for flexion and extension movements about a joint adjacent to each other. These same observations also held true for the digit representations in the arm area, as has been reported for the squirrel monkey by Strick and Preston (JNP 48, 1982). In both the leg and arm areas there were also multiple representations of more proximal movements, similar to the pattern seen by Murphy and his coworkers in the macaque (JNP 41, 1978). The existence of multiple representations of the same movement in primary motor cortex is well established; its significance is yet to be discovered. (Supported by NIH grant NS16146 to EJN.)

145.10 MOTOR CORTICAL MECHANISMS MEDIATING RECIPROCAL ACTIVATION AND CO-CONTRACTION OF FOREARM FLEXOR AND EXTENSOR MUSCLES IN THE PRIMATE. R.J. Kasser* and P.D. Cheney. Departments of Anatomy and Physiology, University of Kansas Medical Center, Kansas City, Kansas. 66103.

Based on their pattern of covariation during volitional motor responses, the activity of flexor and extensor muscles acting at a joint can be divided into two basic types: 1) reciprocal and 2) co-activation. Reciprocal activation is associated with tasks in which the activity of antagonist muscles decreases as that of agonists increases. In contrast, co-activation involves simultaneous contraction of flexor and extensor muscles to stabilize the joint in a fixed position. The purpose of this study was to determine if separate motor cortex cells mediate these two patterns of muscle activity or if some cells participate in both. Rhesus monkeys were trained on two motor tasks: 1) an alternating movement task which involved reciprocal activation of forearm flexor and extensor muscles, and 2) a power grip task which involved co-activation of forearm flexors and extensors. The activity of single motor cortex cells and EMGs of 6-12 wrist and digit forearm muscles were recorded simultaneously during task performance. Cells were isolated during both tasks. Spike-triggered averaging of rectified EMG activity was used to identify corticomotoneuronal (CM) cells and the sign (facilitation or suppression) of their output effects on motoneurons of agonist and antagonist muscles (Cheney, P.D. et al. Brain Res. 247:164, 1982). Forty-eight task related motor cortex cells were recorded in two monkeys. Of these, 11 were identified as CM cells based on their postspike facilitation of agonist muscle EMG activity. Two CM cells had reciprocal output effects and 9 facilitated agonists but had no effect on antagonists (pure facilitatory cells). In all cases tested (7), stimulus-triggered averages computed from single 5-15 uA stimuli applied to the site where the CM cell was recorded confirmed the cell's facilitation of agonists. Stimulus-triggered averages also confirmed suppression of antagonist muscles at the site of both reciprocal cells. One reciprocal cell increased its activity during wrist flexion of alternating movements; the other during extension, but both cells decreased their activity during the power grip task. These relations are consistent with the fact that suppression of antagonist muscles from these cells would interfere with co-activation during power grip. Of the 9 pure facilitatory CM cells, 3 increased their activity only during wrist flexion, 3 only during wrist extension and 3 during both alternating movements and power grip. Of 37 task related non-CM cells, only one showed an increase in activity specific for power grip; 23 increased their activity only for alternating movements, and 13 showed increased activity for both alternating movements and power grip. In conclusion, these results suggest that co-activation is mediated largely by nonreciprocal facilitatory CM cells which are also involved in alternating movements.

145.11 SPATIAL DISTRIBUTION OF NEURONS WITH MONOSYNAPTIC INPUTS TO ADJACENT NEURONS IN THE PRIMATE MOTOR CORTEX. M. Matsumura* and K. Kubota. Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, 484 JAPAN

How close a cortical neuron receives excitatory and inibitory monosynaptic inputs was investigated by a technique of spike triggered averaging of intracellular membrane potentials. Monkeys, sitting in a primate chair with their heads tightly fixed to the chair frame , were weakly anesthetized with 0.5% Halothane. A pair of glass micro-electrodes,one for intracellular recording (filled with KCl or K-acetate),the other for extracellular unit recording (filled with Na-glutamate),were inserted into the motor cortex (area 4)through a drilled hole in the skull. The horizontal distances of the two electrodes were adjusted in each recording session between 0.2 and 1.5mm. To identify pyramidal tract neurons (PTNs), a concentric bipolar electrode was implanted in the pontine pyramid.

Thirty-one intracellular recordings (14 PTNs, 6 non-PTNs and 11 unidentified neurons) were obtained from 20 penetration tracks. Fifty-six extracellular units, satisfying a criterion of at least 100 successive spike activities (usually in a period of 10 to 30s), were simultaneously recorded at cortical depths of between 1.2 and 2.0mm (corresponding to the lower part of layer III to layer V).

Of these unit activities, twenty-two (in 5 penetration tracks) were recorded within areas of 0.2 to 0.5mm horizontally from the intracellular recording sites at a cortical depth of 1.2 to 2.0mm. In seventeen of these 22 (77%), spikes triggered averaged EPSPs with amplitudes of 96 + 35μV/spike (mean + SDs) ranging from 60 to 150μV with the shortest latency of 0.7ms. Twenty-three units(in 9 tracks) were recorded within areas of 0.6 to 1.0mm horizontally at depths of 1.2 to 2.5mm. Nine of these 23 (39%)showed averaged EPSPs with amplitudes of 83 + 44μV ranging from 60 to 140μV, and one showed IPSP of 100μV. Eleven units (in 6 tracks) with horizontal distances from the electrode tips of more than 1.1mm, however,did not show any averaged potential deflections. No significant differences of PSP amplitudes were observed in respects of horizontal and vertical differences of distances of the two recording sites. The ratio of innervating neurons was higher in neurons within 0.2 to 0.5mm of horizomtal distances. These results support anatomical observations of vertical columnar organization of motor cortex.

145.12 CORTICOSTRIATE AND CORTICOTHALAMIC PROJECTIONS FROM AREA 6 IN THE RACCOON. Sharleen T. Sakai and Duke Tanaka Jr. Dept of Anatomy, Michigan State University, East Lansing, MI. 48824.

Several electrophysiological studies in carnivores have shown that area 6, located within the anterior sigmoid gyrus (ASG), contains both an eye movement area located medially and the supplementary motor area located laterally and caudally. In both the dog and raccoon, these functional subdivisions correspond in location to cytoarchitectonically distinct sectors of area 6. However, the cytoarchitectonic and functional differences seen within divisions of area 6 are not reflected by topographically organized subcortical projections. For example, in the dog and raccoon, thalamic afferents to area 6aα located medially and to area 6aβ located more laterally do not arise from topographically distinct bands of neurons but rather from neurons organized into wide partially overlapping bands. The purpose of this study was to determine whether neostriatal and thalamic projections from area 6 also show similar widespread termination patterns in the raccoon.

Discrete single injections of tritiated leucine and proline (1.0 μl; 50 μCi) were made into each of two area 6 subdivisions on the ASG in the raccoon. A comparison of matched sections showed considerable overlap between corticostriate projections arising from area 6aα and 6aβ. In both cases, patches of dense label were located bilaterally over wide areas of the caudate nucleus immediately adjacent to the internal capsule. However, after injections of area 6aβ, the label extended slightly more dorsal in the caudate nucleus than that observed following area 6aα injections. Heavy label was also noted bilaterally over the dorsal and medial parts of the putamen.

Following injections of area 6aα or 6aβ, bands of silver grains were located over the medial half of the ventral anterior and ventral lateral nuclei as well as over the central and ventrolateral parts of the mediodorsal nucleus. Dense label was also present over the ventral medial nucleus and intralaminar nuclei; these nuclei also contained light contralateral label. Although the label observed after area 6aα injections was situated slightly medial to that seen after area 6aβ injections, extensive overlap between the two projections was noted.

These data suggest that, although the corticostriate and corticothalamic projections of areas 6aα and 6aβ show slight topography, they are best characterized by their widespread and overlapping termination patterns.

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145.13 THE INTERRELATION OF CALLOSAL AND CORTICOSPINAL NEURONS IN THE SENSORIMOTOR REGION OF THE RAT. P. Herron and J. Miller*. Div. of Neurosci. and Behavior, Dept. of Psych., Univ. of Massachusetts, Amherst, MA 01060

We utilized double-labelling techniques in order to determine the spatial interrelationship of the cells of origin for the corticospinal and callosal fiber systems in the sensorimotor region of rats. We wanted to determine the following: 1) if single cells send collateral to both target regions, and 2) if the distribution of callosal neurons were congruent with the distribution of corticospinal neurons. We used a combination of the retrograde tracers nuclear yellow and fast blue, nuclear yellow and horseradish peroxidase, or fast blue and horseradish peroxidase. Injections of 0.5-1.0 ul were made in the lumbar or cervical region of the spinal cord in combination with injections in the contralateral sensorimortor region. Survival times ranged from 1 wk (for fast blue) to 24 hr. The brain and spinal cord were removed and sectioned at 30 or 40 um and the HRP sections reacted with tetramethylbenzidine. All sections were examined for fluorescent labelling using an Olympus excitation filter and reflected light attachment and the horseradish peroxidase was examined using light microscopy.

We did not observe any double-labelled neurons. Both the corticospinal and callosal systems were topographical organized. Although the distribution of corticospinal and callosal neurons overlapped considerably in both the horizontal and vertical dimensions, there were regions which contained many corticospinal neurons and relatively few callosal neurons. The bulk of the callosal neurons were restricted to the agranular regions whereas the corticospinal neurons were located in both the agranular and granular regions. Consequently, the distribution of corticospinal neurons were not congruent with the distribution of callosal neurons.

The corticospinal neurons formed a well-defined lamina in the ventral part of layer V whereas the callosal cells were in all layers except layer I with the maximum concentrations in layers III and V. The density of corticospinal neurons was relatively constant throughout the area of distribution for corticospinal neurons. In contrast, the density of callosal neurons varied considerably throughout the area of distribution for callosal neurons. The maximum density of corticospinal neurons as observed in 30 or 40um sections was between 175-225/mm^2 whereas the density of callosal neurons was as high as 1000/mm^2. In the region of overlap between the corticospinal and callosal neurons the number of callosal neurons was generally greater than the concentration of corticospinal neurons in layer V. We concluded that parts of the corticospinal and callosal neurons receive similar inputs, other parts do not.

145.14 RECIPROCAL SYNAPTIC RELATIONSHIPS OF MsI PYRAMIDAL NEURONS WHICH PROJECT TRANSCALLOSALLY IN THE MOUSE. L. L. Porter* E. L. White and G. R. Belford. Dept. of Anat., Boston Univ. Sch. Med., Boston 02118.

The vibrissal region of the mouse primary motor cortex (MsI) is reciprocally connected with the corresponding region in the contralateral hemisphere (Porter and White, J. Comp. Neurol. 214;279, 1983). The purpose of the present investigation was to study the synaptic relationships between callosal projection neurons in the motor cortex and callosal afferents from contralateral MsI.

Injections of horseradish peroxidase (HRP) were placed into the vibrissal region of MsI in adult male CD-1 mice in order to retrogradely label cells in the contralateral cortex. On the following day, the cortex at the injection site was aspirated to identify callosal afferents by lesion-induced degeneration. Four days post-lesion, the animals were perfused with aldehydes and the MsI cortex contralateral to the lesion/injection site was tissue chopped in the coronal plane and reacted for HRP. After light microscopic analysis, labeled cells from layers II-III were prepared for electron microscopic examination. Unbroken series of thin sections were cut through the soma and the entire length of the apical shaft and spines. Electron micrographs of all labeled profiles of the apical dendrite were montaged and graphically reconstructed. The locations of all synapses with the shaft and spines of the apical dendrite were noted on the reconstruction.

The callosal projection neurons were pyramidal cells whose somata were located predominately in cortical layers II-III and V. Derse HRP reaction product filled the dendrites and spines of these cells. The apical dendrites of labeled cells located in both cortical tiers exhibited variable concentrations of dendritic spines and extended close to the pial surface. The dendritic shaft formed both symmetric and asymmetric synapses; most synapses were symmetric onto spines. A small proportion of the synaptic contacts with the dendritic spines were formed by degenerating callosal axon terminals. Preliminary results from the examination of one neuron showed that callosal terminals formed 6.0% of all asymmetric axospinous synapses on the apical dendrite of this cell.

Thus, these data imply that cells in the primary motor cortex project to the homotopic area of the contralateral cortex and in turn receive synapses from the same area.

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145.15 NEOCORTICAL COMMISSURAL SIZE & SEX DIFFERENCES IN PRIMATE BRAIN. M. C. de Lacoste and D.J. Woodward, Dept. Cell Bio., Univ. Texas Health Science Center, Dallas, TX

Our study was undertaken (1) to delineate interspecific correlative effects of brain size (BR) on the cross-sectional surface areas of both the corpus callosum (CCAREA) and the anterior commissure (ACAREA) as well as on other callosal measurements; and (2) to determine if there is sex-linked intraspecific variation in these correlative relationships.

Mid-sagittal sections (N=84) of brains of both sexes from a variety of prosimian (PRO) and anthropoid species (N=30) were photographed at the Max-Planck Inst. for Brain Research (Frankfurt). BR values ranged from 1.78g (Microcebus m.) to 570g (gorilla). The photographs were used for computer-assisted planimetric measurements of CCAREA and ACAREA and for measurements of the dorsoventral widths of the splenium (SPLW) and the body of the corpus callosum. Previous data from human material were added to the primate sample. In a preliminary analysis, we identified species with median values for BR within PRO, Ceboids (CEB), Cercopithecoids (CERCO) and pongids (PON). For each of the selected species, mean values for BR, CCAREA, ACAREA, and SPLW were calculated and then entered in log/log regression analyses of BR vs. CCAREA (R=.9989), ACAREA (R=.98), and SPLW (R=.96). Residuals (Observed values/expected values from regression equation) were used to examine interspecific patterns as well as intraspecific sexual dimorphism in a small number of species: Homo sapiens (N=14), PONG— Gorilla g. (N=10) and Pan t. (N=3), CERCO— Papio h. (N=4) and Macaca m. (N=2), CEB, Saimiri s. (N=2) and Callithrix j. (N=2), and PRO— Nycticebus c. (N=4), Galago d. (N=4) and Cheirogaleus m. (N=2).

Results suggest that CCAREA and ACAREA appear to follow different phylogenetic growth rates: CCAREA is on the average 1.5X larger than expected in humans while ACAREA is only .4RX its expected size on the basis of brain weight. In terms of sex differences, the above PRO species show a marked tendency, similar to that found in humans, for callosal sexual dimorphism, with observed values for CCAREA and SPLW higher than expected, and, conversely, lower than expected in respectively females and males. Similar patterns were found for CCAREA but less consistently for SPLW in CERCO and PON. CEBs are the least consistent for both CCAREA and SPLW. No distinct pattern of sexual dimorphism in ACAREA was observed in all species.

Although a complete evolutionary picture of callosal dimorphism within the primate order remains to be delineated, we can conclude that at least some non-human primates manifest sex-linked variation in CCAREA and SPLW. Supported by the Biological Humanics Foundation.

145.16 CALLOSAL CONNECTIONS OF THE SUPERIOR PARIETAL LOBULE IN THE MONKEY. R. Caminiti and A. Sbriccoli*. Institute of Human Physiology, University of Rome and Institute of Neurology, Catholic University, Rome, Italy.

The callosal connections of the superior parietal lobule (SPL) of the monkey were studied using the axonal transport of HRP in four M. nemestrina and one M. fascicularis. In two of these animals, lectin conjugated horseradish peroxidase (HRP-WGA) was used. In all cases, multiple injections of the enzyme were made in the various architectonic divisions of SPL(Pandya, D.N. and Seltzer, B., J. comp. Neurol., 204: 196, 1982). The brains were processed with tetramethyl benzidine.

Callosal neurons giving rise to homotopic connections are pyramidal cells of the deeper part of layer III as well as pyramidal and fusiform cells of layer VI. They occupy the entire tangential extent of SPL. Commissural cells are present in the cortex of the dorsal and rostral part of SPL up to its posterior and apical sector, including the cortex of the medial surface of the hemisphere. This callosal zone is co-extensive with architectonic areas PE and PEc. In the ventral part of SPL callosal cells occupy, at all mediolateral levels, the cortex of the upper bank of the intraparietal sulcus (IPS), in area PEa.

Commissural fibers of SPL cross the midline in the posterior half of the body of the Corpus Callosum (CC). Labeling of callosal axons extends from the level of the fornical and posterior commissures up to the rostral limit of the splenium of the CC. They enter the cortex of SPL, and terminate in radial patches or columns, of various shape and size, spanning layers I-IV. These characteristic dust-like patches are particularly evident in the cortex of the upper bank of IPS. Callosal neurons are found within as well as between these columns.

Differently from the primary sensory and motor areas, where large parts of peripheral representations are free of callosal connections, the cortex of SPL projects profusely to the opposite hemisphere. This callosal projection may be important for the interhemispheric integration of sensory and/or motor information across the midline and the patches of callosal terminals may represent an anatomical substrate for the representation of the ipsilateral periphery. The callosal system of SPL may also represent an anatomical substrate for the intermanual transfer as well as for the interhemispheric integration of sensory and/or motor inputs working in a synchronous and combined way.

145.17 STRUCTURAL CHANGES IN BRAINS OF MICE WITH AGENESIS OF THE CORPUS CALLOSUM. D. Wahlsten and G. B. Jones*. Dept. of Psychology, Univ. of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

From 10 to 20% of adult mice of the inbred strain BALB/cCF have either total absence or substantial deficiency of transcortical fibres in the corpus callosum (CC). Littermates with normal CC provide good controls for comparison with sibs having abnormal CC because both kinds of mice have the same genotype. It is thus possible to specify what other abnormalities of the forebrain are associated with the CC defect and to detect unusual pathways of CC axons which are prevented from crossing midplane. Analysis of 99 littermate pairs matched for age and brain weight suggested that axons which are normally part of the CC do not reroute via the anterior commissure (CA) when CC formation is disrupted. As indicated in the table, cross-sectional areas at midplane of the anterior and posterior parts of CA (designated CAA and CAP, respectively) as well as the hippocampal commissure (CFV) did not differ significantly between sibling pairs with normal and deficient CC cross-sectional area.

	CC Size of Sibs			
Variable	Normal	Abnormal	t	P(2-tail)
Area of CC	1.180mm^2	.478mm^2	22.91	--
Area of CAA	.079	.079	0.70	.48
Area of CAP	.057	.056	1.54	.13
Area of CFV	.267	.270	-0.99	.32

Projections from cortical neurons were traced in over 100 BALB/c brains by placing small electrolytic lesions in one of ten different locations in the dorsal cerebral cortex, perfusing the mouse five days after the lesion and then staining for degenerating axons and terminals using the Giolli and Pope modification (J. Comp. Neurol., 147:129, 1973) of the Fink-Heimer silver stain. Although ipsilateral projections to cerebral cortex, caudate nucleus and thalamus were clearly revealed, no degenerating axons were found in CAA or CAP of any mouse, regardless of presence or absence of CC. Axons were traced for long distances through the longitudinal Probst bundles when CC was totally absent or very small at midplane, but in cases of moderate deficiency of CC the axons usually found their way to the contralateral cortex and the size of the Probst bundles was substantially smaller.

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145.18 PHYSIOLOGICAL AND MORPHOLOGICAL PROPERTIES OF PYRAMIDAL TRACT NEURONS IN THE RAT. P. Landry*, C. J. Wilson and S. T. Kitai (SPON: M. Jones) Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

Intracellularly recorded pyramidal tract (PT) neurons in rat sensorimotor cortex were identified by antidromic stimulation of the pyramidal tract and injected with horseradish peroxidase (HRP). A recurrent IPSP that lasted from 25-55 ms and that could be reversed with hyperpolarizing current was observed in most neurons regardless of the occurrence of antidromic action potentials. The latency of this IPSP ranged from 2.3-5.5 ms (mean = 3.2, SD = 0.8, N = 13). Antidromic latencies from the same stimulation ranged from 1.1-3.3 ms (mean = 1.6, SD = 0.5, N = 25). A similar response was also observed in most layer V cells, including those not responding antidromically. The IPSP was followed by a prolonged period of inhibition of action potentials which could be followed by a rebound excitation at latencies of about 180-200 ms. In only a few cells was there an EPSP preceding the initial IPSP. A period of excitation following the onset of the IPSP and lasting from 40-50 ms was occasionally isolated from the IPSP. In most cells a similar excitatory potential could be revealed by passing hyperpolarizing current. The earliest latency to the first spike evoked by this excitation ranged from 4-12 ms (mean = 7.9, SD = 2.2, N = 12). To insure that this excitation did not arise from stimulation of medial lemniscus, a knife was passed through the entire thalamus caudal to most of the ventroposterior nuclei and sectioning the medial lemniscus. The latencies of the IPSP and the excitatory period were not changed by this procedure. Staining by intracellular injection of HRP revealed the presence of two types of PT cells having the same conduction velocities. Most of the injected neurons (11 of 12) had extensive intracortical axonal arborizations restricted to layers IV, V and VI and extending 1-2 mm from the soma horizontally. The axon of the second type was like that described by Donoghue and Kitai (JCN, 201:1-13, 1981). Its axon collaterals were limited to an area 0.5 mm around the cell body horizontally, but extended radially as far as layer I. Cells of both types were observed to send an axon collateral to neostriatum. An aspinous stellate cell identified after injection with HRP exhibited a large and monosynaptic EPSP with a latency of 2.8 ms evoked by pyramidal tract stimulation. The duration of this EPSP was comparable to that of the short latency IPSP in PT cells, but not the longer inhibition.

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145.19 INVESTIGATION INTO THE ELECTRICAL EXCITABILITY OF THE CEREBRAL CORTEX: THE FIRST HALF CENTURY. KL Tyler* (SPON: HR Tyler). Neurology Dept., Massachusetts General Hosp., Boston, Mass. 02114

In 1870 Eduard Hitzig noted that galvanic stimulation through certain portions of the intact skull in an awake patient produced involuntary eye movements. These results led him, with the assistance of Gustav Fritsch to conduct a series of experiments in dogs, on the electrical excitability of the exposed brain. The resulting discovery that electrical stimulation produced muscular contractions of the opposite half of the body, "Localized to specific, strictly limited, muscle groups," and that these responses were obtained from centres in discrete brain regions; was seminal.

Within three years of Hitzig's report, David Ferrier published the first in a series of reports on cerebral localization. His work was summarized in his Croonian and Gulstonian Lectures, and in the two editions of his book, The Functions of the Brain. He dedicated this to Hughlings Jackson, noting that he had,"Anticipated many of the more important results of recent experimental investigation into the functions of the cerebral hemispheres." Using improved technical methods, and extending his work to the monkey, Ferrier significantly expanded knowledge of brain function. In Sherrington's words he provided a, "Solid basis of experimental fact," to the, "Hitherto disputed existence of 'localisation' of cerebral function." Ferrier explicitly extended his localisation schema to man by transposing it onto a map of the human brain.

In 1874 Roberts Bartholow demonstrated for the first time in man that the cortex was electrically excitable. Bartholow was aware of the experiments of Fritsch and Hitzig and Ferrier, and referred to them in his paper. His patient was his housemaid, who suffered from an eroding cancer of the skull. Placing one insulated needle into her left 'posterior lobe' and another a 1/4 inch away, in contact with the dura, electrical stimulation produced muscular contraction and a, "Feeling of tingling," in the right arm and leg. Postmortem examination of the fixed brain showed that the stimulating electrode had penetrated over an inch into the brain, in the region of the upper postcentral gyrus.

Subsequent pioneering observations on the excitable cortex in primates were conducted by Gotch, Beevor, Horsley, Grunbaum (Leyton) and Sherrington. The great German neurosurgeon Feodor Krause extended Bartholin's previous observations in man, and accurately mapped out the "focal fields" of many limb movements on the cortex of awake patients undergoing neurosurgical procedures. These investigations set the stage for the subsequent work of Foerster and Penfield which ushered in the second half-century of investigation into the excitable cortex of man.

146.1 PHASE-LOCKING TO AUDITORY STIMULI BY EIGHTH NERVE FIBERS OF A NEOTROPICAL TREEFROG. C. M. Hillery* and P. M. Narins (SPON: C. Clemente). Department of Biology, University of California, Los Angeles, Los Angeles, CA 90024.

The phase-locked response pattern of eighth nerve fibers was investigated in Eleutherodactylus coqui. Period histograms, triggered by the positive zero-crossing of the stimulus waveform, accumulated spike counts relative to a single cycle of a pure tone stimulus. The degree of synchronization, measured as vector strength (VS), and the phase angle (i.e. the median of the period histogram's distribution) were measured for each unit at best frequency (BF) and as many other frequencies and intensities as time permitted. We found that below 1.0 kHz, neurons showed a high degree of phase-locking to stimulus waveforms, often at sound levels well below (10-15 dB) their discharge rate thresholds.

Within the frequency response range of a neuron, VS showed no systematic correlation with stimulus intensity. The VS of the response was, however, clearly frequency dependent showing the greatest synchronization at the lowest test frequencies. This correlation was independent of the neuron's BF. The frequency dependence of phase-locking was described as a negative linear function that was similar for low and mid-frequency fibers deriving from the amphibian papilla (a.p.). Although fibers innervating the basilar papilla in anurans have BFs well above the frequency range for which phase-locked responses occur, initial observations suggest that these fibers exhibit phase-locked responses to sinusoidal stimuli at frequencies below 1.0 kHz. The preferred phase angle shows an increasing shift (lag) with stimulus frequency. The slope of this function is similar for all a.p. neurons studied and suggests a constant time delay for stimulation of all a.p. units.

Increasing the level of simultaneously-presented broadband noise (0.05 to 6.5 kHz) did not effect the phase angle, however, the distribution of spike occurrences broadened, thereby reducing the VS. It is notable that even at the highest noise spectrum levels tested (64 dB/Hz) phase-locking was still prominent and could, at low frequencies, provide a potential source of information about stimulus waveform structure. This could provide the animal with cues about stimulus frequency (coded temporally), sound source localization (coded by relative phase shifts) or overall temporal features of a stimulus. The upper frequency limit for phase-locking (1.0 kHz) corresponds to measures determined for fish and reptiles but is significantly lower than the 4 - 6 kHz cut-off frequency determined for birds and mammals.

146.2 RAPID AND SHORT-TERM ADAPTATION IN GERBIL AUDITORY NERVE RESPONSES. L. A. Westerman* and R. L. Smith (SPON: A. Ecker). Inst. Sensory Res., Syracuse Univ., Syracuse NY 13210.

The firing rate of a nerve fiber in the auditory periphery declines rapidly and markedly following the initial brisk response at the onset of a constant-intensity tonal stimulus. In gerbil, this decay appears to involve two components which occur within the first several hundred milliseconds. Hence at a given intensity the decay can be characterized by the equation

$$R = A e^{-Bt} + C e^{-Dt} + E$$

where R is the instantaneous response at time t after the onset peak, A and B characterize the rapid decay which occurs within the first few milliseconds, C and D characterize the short term decay, and E is the steady-state rate. In order to determine the dependence of these components on sound intensity, the equation was fitted to PST histograms by a least-squares procedure. An intensity range of 40 dB was used, with the minimum intensity for each fiber set at 3 dB above audio-visual threshold.

Short-term adaptation had a time constant of c. 70 msec which varied little with stimulus intensity. When the intensity was varied, the magnitude of the short-term component of the response varied in proportion to the steady-state rate, and saturated over the same limited operating range (c. 20 dB re threshold). The relative magnitudes of the short-term and steady-state components varied among fibers: in our sample of 19 fibers, the ratio ranged from 0.5 to 3.5, measured at the highest intensity for each fiber.

The rapid component grew more rapidly with intensity than did the steady-state rate, and continued to increase at intensities which produced saturation in the steady-state rate. In some fibers, the rapid component saturated at intensities 20-30 dB higher than did the steady-state rate. This saturation occurred at an onset rate limited by the absolute refractory period of each fiber. The saturated onset response approached the limiting probability of unity, that a spike occurred within a specified narrow window at the onset of the tone-burst stimulus. In some fibers, the onset response grew more slowly with intensity and did not reach saturation at the highest intensity tested.

At high intensities, the time constants of rapid adaptation ranged from 0.8 to 4.5 msec in our sample. The rapid time constant for most fibers declined with increasing intensity and for some individual fibers varied by more than a factor of 3 over the intensity range tested. The measured effects of intensity on the time constants of adaptation match the analytical solution to Smith and Brachman's (Biol. Cybern. 44:107-120, 1982) phenomenological model of peripheral adaptation.

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146.3 CONTINUOUS BACKGROUND NOISE AND AVERAGE DISCHARGE RATE OF AUDITORY-NERVE FIBERS IN THE CAT. J. A. Costalupes and E. D. Young. Dept. of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

This study reports that addition of broadband background noise extends the overall operating range of auditory-nerve fibers. Average discharge rates in response to 200 ms best frequency (BF) tones in the presence of continuous noise backgrounds (noise turned on at least 15 s before tone and left on continuously while testing) and simultaneously gated noise backgrounds (noise turned on and off simultaneously with tone) were recorded from auditory-nerve fibers in anesthetized cats. Inspection of BF rate versus level functions obtained in noise over a wide range of noise levels reveals two effects of continuous noise backgrounds. First, previous exposure to noise results in a decrement in average discharge rate that is independent of the level of the tone stimulus. Second, the range of tone intensities over which the fiber is sensitive to changes in tone level -- the dynamic range of the fiber -- shifts to higher tone intensities. The shift occurs at an average rate of .68 dB of shift for each 1 dB increment of noise level and is independent of the fiber's best frequency and spontaneous discharge rate. Simultaneously gated and continuous noise backgrounds at a given intensity produce the same amount of shift.

Effects of continuous noise backgrounds on discharge rate to BF tones can be summarized by expressing a fiber's rate versus level function in continuous noise, $R_I(i)$, in terms of its rate versus level function in quiet, $R_q(i)$, by the equation

$$R_I(i) = S\left[R_q(i-i_H) - R_{sp}\right] + R_{No} - D_I$$

where i is the level of the BF tone, R_{sp} is spontaneous discharge rate, I is the level of the background noise, R_{No} is the driven response to the noise burst alone, D_I is the rate decrement produced by previous noise exposure, and S is the fractional response rate of the fiber in noise relative to its response rate in quiet.

This extension of operating ranges of auditory-nerve fibers will be considered in light of behavioral measures of the detection of tones in noise by cats.

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146.4 MORPHOLOGY OF AUDITORY NERVE FIBER INNERVATION OF THE CAT COCHLEAR NUCLEUS IN RELATION TO SPONTANEOUS RATE ACTIVITY. E.M. Rouiller*, R. Cronin Schreiber*, D.M. Fekete and D.K. Ryugo (SPON: J.P. Hornung). Dept. of Anatomy, Harvard Med. Sch. and Eaton-Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA.

The acoustic receptors in the cochlea contact the peripheral processes of spiral ganglion neurons; the central processes of the neurons constitute the auditory nerve (AN) and project into the cochlear nucleus (CN). We have been studying the structure-function relationships of type I AN fibers in mature cats (Ryugo et al., Anat. Rec., 205:171A, 1983). The characteristic frequency (CF) and spontaneous rate (SR) of single fibers were determined by intracellular recordings, and then each fiber was labelled by injecting HRP through the recording pipette. This report describes morphological characteristics of en passant swellings and terminal endings within the CN, and their relationship to SR. The central innervation of 22 AN fibers, ranging in CF from 0.3-28 kHz, has been analyzed from camera lucida reconstructions of serial sections. All fibers bifurcate and form an ascending and descending branch. The number of primary collaterals ranges from 12-34 ($\bar{x}$=23), independent of CF or SR. These collaterals ramify further and display en passant swellings and terminal endings. Both have been examined by EM and exhibit synaptic specializations (membrane densities and numerous clear, round vesicles approximately 50 nm in diameter). In our population of fibers, the ratio of terminal endings to en passant swellings is relatively constant, being 60:40. The terminal endings grade in size and complexity from simple boutons to large endbulbs of Held; in contrast, the en passant swellings are less variable in size and shape. We have counted every swelling and terminal and measured their cross-sectional areas (magnification=1250x). Our working hypothesis is that these measures will provide an estimate of synaptic density for each fiber. Neither the number nor the size of endings and swellings is related to CF, but both are related to the SR classification scheme of Liberman (JASA, 63:442, 1978). Low and medium SR fibers have a larger number ($p<0.01$) of simple boutons ($\bar{x}_b$=69) and en passant swellings ($\bar{x}_p$=82) when compared to high SR fibers ($\bar{x}_b$=42, $\bar{x}_p$=41). Furthermore, the simple boutons of low and medium SR fibers are smaller in size ($p<0.001$). In contrast to the simple boutons, the number and size of complex terminal endings (i.e., endbulb of Held) do not differ across SR groups. As a first approximation, AN fibers of different SR groups appear qualitatively similar to one another with respect to the types and spatial distribution of their terminal endings, but differ quantitatively in the number and size of endings of certain classes. (Supported by NIH grant NS 13126 and the Swiss Foundation for Medicine and Biology).

146.5 A GOLGI IMPREGNATION STUDY OF THE ACOUSTIC TUBERCLE NUCLEI IN THE RED-EARED TURTLE, CHRYSEMYS SCRIPTA ELEGANS. D. Marbey and R.H. Browner. Department of Anatomy. New York Medical College, Valhalla, NY 10595.

The cytoarchitecture of the acoustic tubercle (AT) was examined in 10 turtles. Following transcardiac perfusion in 5% buffered glutaraldehyde, 0.1 M phosphate buffer, brains were removed and processed for Golgi impregnation using the Golgi-Kopsch technique. The specimens were embedded in araldite and serial sectioned at 150 um intervals. Coronal sections of cresyl violet stained brains(3), 15 um thick, were also examined.

The AT was a superficial trinuclear complex located in the dorsomedial medulla. It contained two primary auditory nuclei, Nucleus Magnocellularis (NM) and Nucleus Angularis (NA), and a secondary auditory nucleus, Nucleus Laminaris (NL).

The NM extended from the caudal end of the NA to just inferior to the caudal posterior root fibers of the eighth nerve. This nucleus consisted of larger spherical cells (9-24 um) with round eccentric nuclei among clusters of smaller round cells (6-12um) which had central round nuclei. The larger spherical cells had 3 to 5 primary dendrites. The primary dendrites of a population of larger spherical cells, exhibited only secondary branches. Some of the higher order branches were long and formed an elaborate dendritic pattern. Thorn-like spines and swellings were present on many branches of the dendritic tree. A characteristic dendritic orientation did not exist in the NM. The smaller round cells had a single dendrite which could project in any plane. Higher order branching was very rare. Dendritic spines or swellings were not on the surface of the primary dendrites.

The NA was located in the rostral end of the AT caudal to the cerebellomedullary junction. It contained medium sized spherical neurons (15-24 um) with round eccentric nuclei. The 3 to 5 primary dendrites were usually oriented mediolaterally. A few neurons had secondary branches. Many thorn-like dendritic spines and dendritic swellings were present throughout the dendritic tree.

The NL was located ventromedial to the NM and was populated by medium sized fusiform neurons (12-21 um). The nuclei were generally central and somewhat elongated. They exhibited smooth single primary dendrites from each soma tip. There were only secondary branches from the distal ends of the main dendrite. Dendritic spines were rare and, when present, were distally located and thorn-like.

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146.6 EXCITATION AND SUPPRESSION BOUNDARIES OF UNITS IN THE AUDITORY NERVE AND ANTEROVENTRAL COCHLEAR NUCLEUS OF THE CAT. R.A. Schmiedt and J.C. Adams. Department of Otolaryngology, Medical University of South Carolina, Charleston, SC 29425.

Threshold boundaries to single- and two-tone excitation and two-tone suppression were mapped for single fibers in the auditory nerve and for cells in the cochlear nucleus of the cat. The boundaries were obtained with the isoresponse tracking procedure developed by Kiang and Moxon (J. Acoust. Soc. Am., 55:620, 1974). Three stimulus paradigms were used. One incorporated a single excitatory tone and was used to obtain a standard tuning curve at a fixed criterion response. In the second paradigm, the tuning curve was retaken in the presence of a second tone placed above the characteristic frequency (CF) of the unit at a moderate level (70 to 80 dB SPL). The second tone by itself was not excitatory. In the third paradigm, a second tone was placed at the unit's CF about 15 dB above threshold and a criterion amount of suppression of the CF tone was tracked across frequency. The same measures were made from cells in the anteroventral cochlear nucleus. Most of the cells studied responded with "chopper" or "primary-notch" types of PST histograms to CF tone bursts and had no prepotentials in their spike waveforms.

Our results indicate that there is often great similarity between the responses obtained from the first-order fibers and these second-order cell bodies with regard to the three types of isoresponse boundaries. Tuning curve shapes associated with cells are nearly identical to those of fibers obtained with the single-tone paradigm. As in the nerve, the majority of the cell bodies respond to the $2f_1$-f_2 distortion product under the excitatory two-tone condition, yielding a secondary minimum above CF in the tuning curve. Further, the second tone above CF can raise the tips and often shifts the tails of the tuning curves of the cell bodies, usually as happens with the first order fibers. However, the response boundaries of the cell bodies sometimes are not predictable from the nerve responses for two-tone stimuli. For example, CF thresholds may not be raised with the addition of the second tone above CF, even though these same cells exhibit normal suppression boundaries above CF. Finally, the absolute position of the suppression boundaries obtained with tones below CF is fairly stable for fibers in the nerve regardless of fiber CF, a result that confirms what has been found previously for fibers in the gerbil auditory nerve. (R.A. Schmiedt, Hearing Res., 7:335, 1982). (Supported by NSF grants BNS 82-10233 and BNS 81-10222).

146.7 COCHLEAR NUCLEUS UNITS SPECIALIZED FOR ENCODING AMPLITUDE MODULATION ARE TUNED TO DIFFERENT MODULATION FREQUENCIES. R.D. Frisina, R.L. Smith, and S.C. Chamberlain. Institute for Sensory Research, Syracuse Univ., Syracuse, NY 13210.

The function of certain divisions of the cochlear nucleus may include the preferential encoding of modulations in sound amplitude. We have studied CHOPPERS in the gerbil ventral cochlear nucleus (VCN) and found that many units show phase-locked responses to amplitude modulation (AM) over an intensity range 50 dB greater than that of auditory-nerve fibers [Frisina, R.D., Smith, R.L., and Chamberlain, S.C., J. Acoust. Soc. Am., 73:S81 (1983)]. At a single modulation frequency (150 Hz) and a high average intensity level, CHOPPERS show phase-locked responses that span a 30 dB range of response amplitude. We suggest that each CHOPPER may be tuned to a different modulation frequency to which it is maximally responsive.

To test this hypothesis, we measured modulation transfer functions (MTFs) for CHOPPERS in the VCN. Analyses of the responses of 14 units, for modulation frequencies from 20 to 1000 Hz, show that CHOPPERS are tuned to frequencies between 150 and 400 Hz. At an intensity of 50 dB above threshold, the response at the peak of the MTF is approximately an order of magnitude greater than that reported for auditory-nerve fibers. The shape of the MTF changes as a function of average intensity. Near threshold, the MTF resembles a low-pass filter with a small peak near the high cutoff frequency. As the average intensity increases, the MTF takes on the shape of a band-pass filter. No relation was found between natural chopping frequency, characteristic frequency, and the frequency at the peak of the MTF.

Our findings suggest that CHOPPERS in the gerbil VCN are specialized for encoding AM over a wide dynamic range for frequencies between 150 and 400 Hz. Presently, we are further investigating the mechanism which produces this functionally relevant response property. [Supported by NSF and NIH]

146.8 HIGH FREQUENCY PHASE CODING IN THE COCHLEAR NUCLEUS OF THE BARN OWL. W. E. Sullivan and M. Konishi (SPON: K. Nakai). Div. of Biol., Calif. Inst. of Tech., Pasadena, CA 91125.

Ongoing time or phase differences between the two ears are utilized by Barn owls to localize high frequency sounds (5-9 kHz) along the horizontal axis. For the auditory system to measure time differences in high frequency waveforms, waveform timing must be preserved at the monaural level. Since studies of mammalian auditory systems have failed to demonstrate phase or time coding at frequencies greater than 4 to 5 kHz, we investigated phase coding in the owl's cochlear nucleus to see if waveform timing is preserved at frequencies where binaural time sensitivity can be shown.

Single unit responses were obtained from output fibers of the Nucleus Magnocellularis. This nucleus is known to project bilaterally to N. Laminaris, the first brainstem site where binaural time sensitivity can be found. Best frequencies of units ranged from 2.0 to 9.0 kHz. Virtually all units with BFs from 2 to 6 or 7 kHz showed clear phase coding. In contrast to cat and monkey data, there does not seem to be a dramatic decline in phase locking ability over this range. Further, clear examples of phase coding could be seen in the range of 7 to 9 kHz. At these frequencies, the period length is from 110 to 140 microseconds. Analysis of cycle histogram peak width shows that these neurons reliably respond within a range of ±25 to 40 microseconds about the mean response phase.

These observations indicate that the owl's auditory system is capable of encoding waveform timing at frequencies over an octave higher than the maximum frequencies where phase locking is seen in mammalian acoustic nerve fibers. This observation provides a basis for the earlier finding that owls can use binaural time or phase information for localization of high frequency sounds. It also raises the question of how the auditory system is able to code stimulus timing with an accuracy in the range of tens of microseconds.

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146.9 INTRACELLULAR RECORDING AND STAINING OF CELLS IN BRAIN SLICE PREPARATIONS OF THE MOUSE COCHLEAR NUCLEI. S.H. Wu* and D. Oertel. Dept. of Neurophysiology, Madison, WI 53706.

Intracellular injections of horseradish peroxidase (HRP) were made into cells which responded to electrical stimulation of the auditory nerve in parasagittally cut brain slices of the mouse cochlear nuclei. Two conclusions can be made from the results obtained thus far. (1) The morphology of cells in brain slices resembles that of Golgi-stained cells that were fixed in situ. (2) Some stellate cells in the ventral cochlear nucleus have Type I electrical characteristics (Oertel, J. Neuroscience, in press).

The morphology of HRP-filled cells in brain slice preparations is similar to the morphology of cells in Golgi-stained tissue described by Webster and Trune (1982, Am.J. Anat. 163:103-130). We have injected 2 Purkinje-like cells in the dorsal cochlear nucleus; 15 stellate cells and 1 granule cell in the anteroventral cochlear nucleus; 2 stellate cells in the globular cell region; and 18 stellate cells, 1 bushy cell, and 3 granule cells in the posteroventral cochlear nucleus. Stellate cells may comprise more than a single cell type since stellate cells vary greatly in their anatomical features. Their diameters range from 13 to 21μm. Some cells have long thick dendrites with highly branched endings whereas others have thin tapering dendrites which end with terminal beads. Some have dendrites which extend only 100 μm from the cell body while others have dendrites that extend over 300 μm in the parasagittal plane. Some dendrites are aligned parallel to the auditory nerve fiber fascicles whereas others cross the fiber bundles. Some cells have somatic and dendritic spines while others are smooth.

It was difficult to obtain good recordings with HRP-filled microelectrodes so that it was not possible to characterize most cells. Three recordings from stellate cells revealed that at least some stellate cells respond to suprathreshold depolarization with large, regularly firing, action potentials and have linear current-voltage relationships in the subthreshold voltage range showing that they have Type I characteristics (Oertel, J. Neuroscience, in press). We have not yet made a good recording from a cell with Type II characteristics with HRP-filled electrodes.

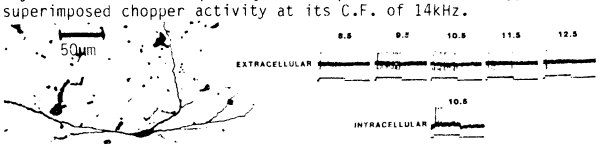
This work was supported by a grant from NIH NS 17590

146.10 INTRACELLULAR STUDIES IN CAT COCHLEAR NUCLEUS: PHYSIOLOGICAL RESPONSES OF MORPHOLOGICALLY IDENTIFIED NEURONS. R.H. Britt, G.T. Rossi, and †D.K. Morest. Div. of Neurosurgery, Stanford Univ. Sch. of Med., Stanford, CA 94305 and †Dept.of Anatomy, Univ. of Conn. Health Center, Farmington, CT 06032.

Intracellular recording techniques were used to study the physiological responses of cochlear nucleus neurons in 32 pentobarbital anesthetized cats. Electrodes were filled with 2 to 4% horseradish peroxidase (HRP) in 0.5M KCl. Each neuron was studied extracellularly by obtaining a tuning curve. After determining the characteristic frequency (CF), a peristimulus time histogram(PSTH) was generated by averaging the respones to 40 250msec tone bursts at the CF. After intracellular penetration, the PSTH at the CF was repeated to determine if any change in response had occurred. HRP was then iontophoretically injected intracellularly. Serial transverse frozen sections were incubated using a diaminobenzidine reaction intensified by preincubation with cobalt chloride.

In the anteroventral cochlear nucleus (AVCN) three HRP-filled neurons were physiologically characterized. A spherical bushy cell located in the AA region had a primarylike response at its CF of 4kHz. An elongated stellate cell in the PD region of the AVCN had a primarylike response at its CF of 10kHz at 90dB, but at lower intensities (80 to 60dB) the unit showed a progressive change to a classic chopper response. This unit also had tone-evoked suppression of spontaneous activity at 14-15kHz, however, no inhibitory post-synaptic potentials were observed during intracellular recordings. A small stellate cell along the medial border of PV in the AVCN showed an onset response at its CF of 4kHz.

In the dorsal cochlear nucleus (DCN) a horizontal giant cell was successfully marked. Extracellular recordings (biphasic spikes) showed a change from an onset pattern (8.5kHz) to onset-sustained response (10.5kHz) at its CF (see figure below). With intracellular penetration the resting membrane level dropped to -50mV, the spikes became monophasic, and there was a sustained dc shift that lasted for the duration of the tone burst. Importantly, the response patterns remained unchanged after intracellular penetration. The second HRP-marked cell in the DCN was a small broadly tuned (7-18kHz) granule cell recorded in the molecular layer which showed a primarylike response with some suggestion of superimposed chopper activity at its C.F. of 14kHz.



[Supported by NIH/NINCDS NS15860 and VA Merit Review Grants].

146.11 ORGANIZATION OF DESCENDING PROJECTIONS FROM THE SUPERIOR OLIVARY COMPLEX TO THE COCHLEAR NUCLEI IN THE CAT. K. S. Spangler*[1], C. K. Henkel[2] and Nell Cant[3]. Department of Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC[1,2] and Duke University School of Medicine, Durham, NC[3].

Descending auditory pathways from the superior olivary complex to the cochlear nuclei were investigated in the cat using retrograde and anterograde tracing methods. Injections of HRP were made in regions of the cochlear nuclei in a large number of cats. Irrespective of the size or location of these injection sites, labeled cells were seen in all periolivary regions except the ventromedial periolivary nucleus. No retrograde labeling was found in either the lateral (LSO) or medial superior olivary nuclei (MSO). Three groups of cells were prominently labeled. The first, containing the most number of labeled cells, was the ipsilateral lateral periolivary region including the anterolateral, lateral and *posterior periolivary nuclei*. The labeled cells in the lateral region on the contralateral side had the same topography but were far fewer in number. The region containing the second greatest number of labeled cells was the contralateral ventral nucleus of the trapezoid body. The third largest source of efferents to the cochlear nucleus was the ipsilateral dorsal periolivary region. No labeled cells were found within the dorsal hilus of LSO. Nine cats received injections of tritiated leucine in the periolivary regions. Fibers projected to the cochlear nucleus via the trapezoid body and dorsal acoustic stria in all cases. Rostral fibers in the trapezoid body entered the anteroventral cochlear nucleus directly. Fibers in AVCN terminated lightly in the anterior part of the anterior division and more heavily in the posterior and posterodorsal parts of the anterior division of AVCN. Only scattered axons were seen in the posterior division of AVCN. Most of the silver grains were found in the neuropil of AVCN although perisomatic labeling could be seen. Caudal trapezoid body fibers entered the cochlear nucleus from a ventromedial direction and fanned out to supply the posterior and dorsal cochlear nuclei. Axons and terminals in the posterior nucleus were sparse, but when seen were often perisomatic or peridendritic. The dorsal cochlear nucleus (DCN) was primarily labeled in the fusiform and deep cell layers, with fewer silver grains in the molecular layer. The results of both methods lead to the conclusion that the different periolivary nuclei project widely within the territory of the cochlear nuclei, but in much the same pattern, not heterotopically as might have been expected.

Supported by NIH Grant NS 18627-01.

146.12 THE EFFECTS OF STIMULATION OF MEDIAL OLIVOCOCHLEAR NEURONS ON IPSILATERAL AND CONTRALATERAL COCHLEAR RESPONSES. M.L. Gifford* and J.J. Guinan. EECS Dept., M.I.T., Cambridge, MA; Eaton-Peabody Lab, Mass. Eye & Ear, 243 Charles St., Boston, MA 02114.

Olivocochlear (OC) cells are divisible into two groups according to the location of their cell bodies. The medial OC (MOC) group has cell bodies medial to the medial superior olive and projects predominantly to outer hair cells of both cochleas. With an electrode in the MOC, we have stimulated MOC neurons in the cat and monitored the effect of this stimulation on auditory-nerve compound action potential (N_1) and cochlear microphonic (CM) in response to clicks and on endocochlear potential (EP) as measured in scala media. MOC stimulation reduced N_1 amplitude, increased N_1 latency and increased CM amplitude in both ipsilateral and contralateral ears. These effects were reduced following an intravenous injection of strychnine sulfate. MOC stimulation also reduced EP in both ears. At least 20 shocks at a rate of 200 to 400 shocks/s were needed to produce large decreases in N_1 amplitude. Effects were maximum at the end of a train of shocks and decreased over several hundred milliseconds after the shocks. For experiments in which we feel that the stimulating electrode was well placed, MOC stimulation typically shifted N_1 amplitude vs. click level functions to higher sound levels by 10 to 15 dB in the contralateral ear and 4 to 6 dB in the ipsilateral ear. In contrast, stimulation of the olivocochlear bundle (OCB) at the midline of the floor of the fourth ventricle typically shifted these level functions by 20 to 25 dB in both ears. MOC stimulation typically increased CM amplitude by 1.5 dB in the contralateral ear and 0.5 dB in the ipsilateral ear; midline OCB stimulation typically increased CM amplitude in both ears by 2 dB. The ratio of MOC effects seen in the contralateral ear to effects seen in the ipsilateral ear is approximately equal to the ratio of MOC neurons projecting to the contralateral cochlea to MOC neurons projecting to the ipsilateral cochlea (Guinan, Warr and Norris, in prep.). In order to see if MOC effects on the ipsilateral ear were due to excitation (e.g. transsynaptically) of contralateral MOC cells with crossed projections, OC fibers were cut in the floor of the fourth ventricle just medial to the left facial genua. Following this cut, stimulation of the left MOC had no effect on either ear; stimulation of the right MOC had no effect on the left (contralateral) ear but typical effects were present in the right (ipsilateral) ear. We conclude that the effects of MOC stimulation on responses of both ipsilateral and contralateral ears are similar to those previously reported for OCB stimulation at the midline of the floor of the fourth ventricle.

146.13 DEVELOPMENTAL CHANGES IN THE DISTRIBUTION OF THE GRANULE CELL OF THE HUMAN COCHLEAR NUCLEUS. T.D. Heiman-Patterson* and N. L. Strominger. Department of Anatomy, Albany Medical College, Albany New York.

The primate cochlear nuclear complex undergoes several characteristic morphogenetic changes during the course of primate phylogenesis from lorisidae through hominidae. The most striking alterations occur in the organization of the dorsal cochlear nucleus in which the laminar pattern becomes progressively obscured in the adult. Granule cells form an external granule layer as well as being intermixed within the molecular and pyramidal layers in slow loris, squirrel monkey and rhesus. While a prominent external granular layer remains in the chimpanzee, granule cells are scant in other portions of the nucleus. In human adult, the external granule cell layer is absent. A small number of granule cells persist but with inconstant distribution. The present investigation was undertaken to elucidate in greater detail the organizational variations of the cochlear complex in developing human brains.

A series of human brains of varying age, from fetus to adult was studied to establish whether progressive changes could be observed that might reflect phylogenetic trends. In the human adult, granule cells are sparse and the superficial granule cell layer non-existent. However in the fetus a prominent superficial granule cell layer is present. It occurs lateral to both the anteroventral and dorsal divisions of the cochlear nucleus and surrounds the entire posterior end of the cochlear complex. It is continuous with a thin lamina which extends ventrally and medially around the entire cochlear complex. In neonatal brainstems a reduced superficial granule cell layer exists; it is markedly thinner than in the fetus. The granule cell layer along the medial aspect of the complex disappears. By 18 months the molecular layer resembles the adult form. Thus, a substantial developmental reorganization or attrition of these neurons must occur. It has been shown in the rat that the granule cells of the dorsal cochlear nucleus develop and comigrate with those of the cerebellum (Pierce, E.T., J. Comp. Neurol., 131: 323, 1967). The presence of granule cells in the fetal dorsal cochlear nucleus and their subsequent disappearance suggests that a programmed cell death may be responsible for this phenomenon and would seem to preclude the possibility that a defect in migration is the principal mechanism. Additionally, evidence indicates that there is a reduction in the olivocochlear bundle in human brainstems (Moore, J.K. and Osen, K.K., Am. J. Anat., 154: 393, 1979) which provides afferent input into the granule cell layer. It may be that a lack of afferent input triggers the process of programmed cell death in these cells.

146.14 AN ANOMALOUS INTER-COCHLEAR NUCLEUS PATHWAY INDUCED IN CHICK EMBRYOS BY UNILATERAL REMOVAL OF THE OTOCYST. Hunter Jackson and Thomas N. Parks. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

Neurons in nucleus magnocellularis (NM) normally receive large calycine endbulbs from the cochlear nerve. NM neurons send two branches of their axon to form sprays of boutonal endings in nucleus laminaris (NL). One branch of each NM axon innervates the dorsal dendrites of the ipsilateral NL and the other branch travels in the crossed dorsal cochlear tract (CTrX) to end on the ventral dendrites of the contralateral NL. Surgical removal of the otocyst on the third day of incubation (E3) in chick embryos prevents formation of the input to NM. To compare the axon arbors in NL of normal and "deafferented" NM neurons, the right otocyst was surgically destroyed on E3 in a number of embryos. Then, using an in vitro brain stem preparation previously described (e.g., Jackson & Parks, J. Neurosci 2:1736-1743, 1982), we iontophoretically injected horseradish peroxidase (HRP) into the CTrX in operated embryos at E11-12 (n=6), E13-14 (n=5), and E17-18 (n=4). Several normal control animals were also injected at these ages. After 2-3 hr survival periods, the brains were fixed, rinsed, cryoprotected, sectioned and reacted for HRP by a cobalt-intensified DAB method. These procedures typically result in retrogradely filled cell bodies bilaterally in NM and anterograde labeling of NM axon arbors bilaterally in NL. To our surprise, we consistently observed an anomalous branching of the CTrX axons coming from the left (normally innervated) NM in operated animals. As these fibers approach the medial margin of the right NL, the main branch continues laterally along the ventral neuropil but anomalous axon collaterals branch at right angles and ascend to end in sprays of terminal boutons around cell bodies throughout NM and in the dorsal NL neuropil. These anomalous branches form a dense projection to the right NM in every operated animal but are never seen in the left NM or in normal animals. It remains possible, of course, that a small transient normal projection exists prior to E11. In electron micrographs of the right NM small boutonal endings are seen forming asymmetric junctions with neuronal cell bodies; these anomalous endings closely resemble the normal NM endings in NL (Parks et al., JCN 214:32-42, 1983). Preliminary electrophysiological recordings of responses in the right NM to electrical stimulation of the left NM suggest that these anomalous endings form functional synapses. This new afferent to NM cells deprived of their cochlear nerve input may explain why only 30% of NM cells ultimately die after otocyst removal (Parks, JCN 183:665-678, 1979).

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146.15 SENSITIVITY TO ELECTRICAL STIMULATION OF AUDITORY NUCLEI IS ALTERED BY CONTINUOUS SOUND. G.M. Gerken* and S.S. Saunders* (SPON: R.D. Stillman), Callier Center for Communication Disorders, Univ. of Texas at Dallas, Dallas, TX 75235.

Subjects were 4 cats with a total of 12 PtIr electrodes permanently implanted in cochlear nucleus (CN) and inferior colliculus (IC). Thresholds for the detection of cathodal electrical pulses were measured behaviorally by means of a self-paced operant task. Using a counterbalanced design, electrical stimulation thresholds (in dB re 1.0 μA) were measured in the presence of continuous tones (0.5, 2.0, 4.0 kHz) of 80 dB SPL and in quiet. In separate sessions, the voltage (μV rms) of the ongoing activity recorded from the 12 electrodes was measured during the same conditions of tone and quiet. Finally, 14 ms phase-locked tone bursts of several frequencies at 80 dB SPL were used to elicit frequency following responses.

The behaviorally-measured electrical-stimulation thresholds obtained in the quiet condition were compared with the thresholds obtained during continuous tone. Nine instances of electrical stimulation threshold decreases (re quiet) of 10 dB or more occurred for particular combinations of electrode and tone frequency. Sixteen instances of threshold decreases between 2.0 and 9.9 dB occurred, and there were 8 instances of essentially no threshold shift (± 1.9 dB). There were only 3 occurrences of a masking-like threshold increase of 2.0 dB or more during the continuous tone. Thus, the effect of the continuous tone on the detection of electrical stimulation was generally facilitory. The continuous tone also produced both increases and decreases in ongoing brain activity as recorded from the 12 electrodes. The correlation coefficients for electrical-stimulation threshold-shift vs. change in ongoing activity was 0.13 for CN and 0.69 for IC. Large, neurally-based frequency-following responses were obtained for frequencies near 2 kHz from some CN electrodes but the presence of these responses did not affect electrical stimulation threshold.

The results indicate that in central auditory structures a continuous tone may produce strong, sustained effects on certain neural populations.

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146.16 SPECIALIZATIONS OF GLIAL STRUCTURE IN THE COCHLEAR NUCLEI J. K. Moore. Department of Anatomical Sciences, SUNY at Stony Brook, Stony Brook, NY 11794.

Investigations of the density and disposition of astrocytes in the cochlear nuclei of the cat and guinea pig, done by GFA (glial fibrillary acidic protein) immunohistochemistry and Golgi-Hortega impregnation of the nuclei, have shown two regions of specialized concentration and morphology of astrocytes in the cochlear complex. First, there is a concentration of astrocytic elements within the granule cell regions of the nuclei, including the superficial granule cell layer of the ventral nucleus, the granule-fusiform cell layer of the dorsal nucleus, and the lamina of granule cells separating the two nuclei. The increased density of astrocytic processes in these regions may be related to glial investment of glomerular synaptic complexes involving granule cell dendrites and afferent mossy terminals (Mugnaini et al., J. Neurocytol., 9:537-570, 1980). Secondly, there is an increased concentration of astrocytes within the molecular layer of the dorsal nucleus, with astrocytes in this area characterized by lamellate or spiny expansions similar to those seen on Bergmann glial processes of the cerebellum (Rakic, J. Comp. Neurol., 141:283-312, 1971) and radial astrocytes of the hippocampal formation (Duffy and Rakic, J. Comp. Neurol., 214:224-237, 1983). These lamellate expansions are demonstrated by Golgi impregnation, but not by the GFA immunohistochemical technique, indicating that they do not contain glial fibrils. In all areas where such lamellate expansions have been noted, they are believed to be related to synaptic junctions between molecular layer axons and dendrites of related neurons.

Similar investigations of the cochlear complex in the macaque monkey show that the glial structure in the simian cochlear nuclei is significantly different from what is seen in the nonprimate species. No astrocytic formations typical of granular or molecular layers are seen, though staining and impregnation of glia throughout the cochlear complex and brainstem is otherwise completely comparable to what is seen in other mammals. These differences reflect the alterations in cytoarchitecture of the cochlear complex which accompany regression of the cochlear granule cell population and related neuronal populations in higher primates (Moore, J. Comp. Neurol., 193:609-629, 1980). A further distinctive feature in the macaque is the presences of a structure analagous to the human pontobulbar body: this superficial structure is seen to consist of a dense mass of astrocytic somas and processes.

147.1 CA SPIKE INITIATION IN THE SOMATA OF CRAYFISH GIANT MOTOR NEURON: STUDY WITH ARSENAZO III. T. Shimahara, G. Czternasty*, J.Stinnakre and J. Bruner*. Lab. de Neurobiologie Cellulaire, C.N.R.S., 91190 Gif sur Yvette and Lab. de Neurobiologie, Université de Picardie, 80039 Amiens*

In normal conditions, a depolarizing pulse cannot trigger a regenerative action potential in the somata of the giant motor neuron (MoG) of crayfish Procambarus. However a Ca^{2+} dependent slow action potential (SAP) appears with the depolarizing pulse if it is preceeded by a conditioning depolarization (Czternasty & Bruner, 1981). This may be due to the reduction of the K-outward current or to the facilitation of the Ca-current, or both,by the conditioning pulse. To answer these questions, further experiments were performed in the same preparation using the metallochromic dye Arsenazo III (AzIII). A brief current pulse (≃5ms) does not initiate neither a regenerative action potential nor is accompanied by a change in AzIII absorbance. If the test pulse is preceeded by a long depolarizing pulse (e.g. 8 second to -40 mV), the SAP appears. The plateau phase of the SAP is accompanied by a continuous increase in AzIII absorbance. Furthermore, tetraethylammonium ions (TEA) either applied extra- or intracellularly, give the same results without the need for a conditioning depolarization. These results suggested the existence of a Ca current during the SAP. Under voltage clamp, a transient increase in AzIII absorbance is detected during a 300 ms depolarization test pulse above -30 mV. This signal increases as the voltage become more positive and peaks around + 10 mV. Above there is a slight reduction but the signal cannot be suppressed even when the membrane potential is brought up to +150 mV. When a conditioning pulse is present the absorbance transient is larger at any one membrane potential between -40 and about + 40. In this case either a suppression potential cannot be demonstrated. In the presence of TEA, the effect of conditioning pulse on the AzIII transient is no longer observed and a clear suppression potential is now obtained around +130/+140 mV. These results show that a facilitation of the Ca current does not seem to explain the appearence of the SAP, rather a reduction of the K current is an essential factor.

147.2 SPIKE DURATION AND CALCIUM COMPONENT OF ACTION POTENTIALS IN RAT SUPRAOPTIC NEUROSECRETORY NEURONS ARE ACTIVITY DEPENDENT. C.W. Bourque* and L.P.Renaud. (Spon: B.Esplin)Neurosciences unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada H3G 1A4.

The magnocellular neurosecretory neurons of the mammalian hypothalamic supraoptic nucleus respond to osmotic stimuli by an increase in firing rate and/or the evolution of phasic or bursting activity patterns in-vivo and in-vitro. While the latter patterns of activity have been determined to be most efficient at inducing vasopressin release on a per-spike basis, the mechanisms underlying such an advantage have yet to be explained.

Intracellular recordings from 53 supraoptic neurons obtained in 22 intravascularly perfused explants of rat hypothalamus have revealed that the duration of their action potentials (APs) is activity-dependent. In any given neuron, the duration of an AP (measured at 1/3 amplitude) occurring during active firing (5-25 spikes/sec) can be as much as 150% longer (2.9±0.3 vs 1.7±0.2 msec, S.D.;n=43) than during quite periods (fewer than 0.1 spikes/sec). Furthermore, phasic firing is characterized by the progressive increase in duration of the initial 15-30 APs of a burst following which spike duration is observed to correlate negatively with the duration of the preceding interspike interval (ISI), indicating that recovery from events associated with an AP can influence a subsequent APs' duration. Antidromic or current evoked APs following termination of a burst retain their increased duration for as long as 10-15 seconds before full recovery of the shortest AP durations.

Perfusion of the explant with media containing either 0 Ca^{++} + 5mM EGTA, 2mM Co^{++} or 0.2mM Cd^{++} abolishes the tetrodotoxin (TTX; 0.1 uM) resistant (Ca^{++}) spike; in the absence of TTX, this treatment causes a 5-25% reduction in spike amplitude, a decrease in spike duration and the elimination of the shoulder normally found on the repolarization phase of the AP. Under these conditions the TTX sensitive (Na^{+}) spike fails to display either activity-dependent changes in duration or spike broadening at the onset of a burst. All of the above effects are reversible.

These results indicate that in these neurons activity-dependent changes in spike duration result in variations in the calcium component of the action potential. The high incidence of short ISIs as a consequence of osmotic or neurotransmitter induced firing and bursting or phasic activity is therefore predicted to enhance Ca^{++} influx during the action potential. The existence of similar events at terminals of neurosecretory neurons could participate in the facilitation of hormone secretion.

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147.3 THE ELECTROPHYSIOLOGICAL EFFECTS OF VARIOUS ALCOHOLS AND NALOXONE ON THE CA^{++} COMPONENT OF ACTION POTENTIALS. S. A. Eskuri* and R. S. Pozos. Dept. of Physiology, Univ. of Minn., Duluth, Sch. of Med., Duluth, MN 55812.

It has been reported that ethanol can modify Ca^{++} movements in action potentials of neurons chemically manipulated to represent presynaptic terminals (Oakes and Pozos, Dev. Brain Res., 5 (1982), 251-255). There is also evidence that naloxone inhibits ethanol's (and to a lesser extent, other alcohols') depletion of brain Ca^{++} (Ross, Ann. N.Y. Acad. Sci., 273 (1976), 280-294) and ethanol dependence (Blum and Futterman, Nature 265 (1977), 49-51). Therefore, various alcohols were tested using the presynaptic model to determine if their effects compared to ethanol and whether naloxone inhibited the effects.

The alcohols were made up in solutions such that their thermodynamic activities in water were equal. Dose response curves were constructed for each (% decrease in the duration of the Ca^{++} plateau vs. concentration) and a concentration corresponding to an activity of 8.04×10^{-2} was used for the naloxone study. Electrophysiological measurements were performed using a bridge circuit to simultaneously stimulate and record from a single microelectrode. Application of the drugs was accomplished by either pressure ejection or diffusion from a micropipette.

It was found that when thermodynamic activities were identical for the alcohols, they affected Ca^{++} movement to the same degree. The dose range of the activities corresponded to ethanol concentrations between 3 and 110 mM. Naloxone (10^{-4} M) was found to significantly inhibit ethanol's effect. The percent decrease in Ca^{++} movement with ethanol alone was 38.1 ± 2.9% and with ethanol and naloxone together it was 20.5 ± 3.5%. However, naloxone did not inhibit the other alcohols to the same degree. When naloxone was applied alone, no effect was detected.

From this study, it appears that short, straight chained alcohols (C_1-C_5) have identical effects on Ca^{++} currents if their concentrations in water are made equivalent. Also, the data suggests that naloxone does inhibit some of the effects of ethanol. However, the antagonism was not as complete as what has been reported for the enkephalins (Werz and MacDonald, Brain Res., 239 (1982), 315-321) or what Ross reported for brain Ca^{++} depletion. Further, these initial findings do support the proposal that the structure of an alcohol may determine whether its action may be antagonized by naloxone.

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147.4 SUPERNORMAL CONDUCTION VELOCITY IN EARTHWORM GIANT AXON: ROLE OF DIVALENT CATIONS AND NA/K PUMP. B. S. Glick* and S. A. George. Neurosci. Prog., Amherst College, Amherst, MA 01002.

In the giant axons of the earthworm, the second of two impulses spaced 3 to 100 msec apart travels at a higher conduction velocity (CV) than the first. We sought to discover the mechanism of this supernormality (SN). The possibility that SN results from reverberating synaptic activity was tested by applying saline with 20 mM Mg^{++} to the nerve. SN (defined as percent increase in second spike CV at a given interval between stimuli, usually 15 msec) was not reduced in high Mg^{++}, indicating that synaptic activity is not involved. The accumulation of K^{+} ions around axons has been proposed as a possible explanation for SN, but we found that applied increases in $[K^{+}]_o$ affected neither first spike CV nor SN. Two other possible explanations involve Ca^{++} ions. A decrease in $[Ca^{++}]_o$ or an increase in $[Ca^{++}]_i$, resulting from Ca currents during a spike, could lead to increased axon excitability via effects on membrane surface potentials; in turn, these could increase the CV of the second spike. These possibilities were investigated by bathing the nerve cord in saline containing $[Ca^{++}]$ ranging from 0 to 15 mM (normal $[Ca^{++}]$ = 1.8 mM). First spike CV decreased with increasing $[Ca^{++}]_o$, as expected from known effects of divalent cations on membrane excitability. SN showed the opposite dependence on $[Ca^{++}]_o$, decreasing by up to 90% in solutions containing very low $[Ca^{++}]$; this suggests a role for Ca currents in SN. However, SN was not reduced by lanthanum ions, suggesting that factors other than Ca^{++} may be involved. The role of active transport in SN was then investigated by adding ouabain to the bathing solution, and by replacing Na^{+} by Li^{+}. Both procedures abolished SN without affecting first spike conduction velocity.

In conjunction with these experiments, the Hodgkin - Huxley equations for impulses in squid axon were solved by computer in order to study the above mechanisms. A shift in the K^{+} equilibrium potential in a depolarizing direction did not produce an increase in computed CV, in agreement with the experimental result in the earthworm axon. Changes in $[Ca^{++}]$ were modelled by assuming a change in the dependence of Hodgkin-Huxley parameters upon membrane voltage. A decrease in $[Ca^{++}]_o$ or an increase in $[Ca^{++}]_i$ would be expected to shift the dependence in a depolarizing direction. For a 5 mV parameter shift, the equations predict a 1% increase in CV in squid axon. If the equations are separately modified to include a hyperpolarizing pump current of 3.5 ua/cm^2, a CV increase of 0.5% was computed. When the Ca and pump effects were combined, the predicted CV increase was 3.5%.

In conclusion, both experimental and theoretical studies support a combined contribution of Ca^{++} currents and electrogenic pumping in the generation of supernormal conduction velocity aftereffects.

147.5 A "SLOW" VOLTAGE-DEPENDENT Ca^{2+}-DEPENDENT CONDUCTANCE IS PRESENT IN SOME CULTURED MOUSE SPINAL AND HYPOTHALAMIC NEURONS. D.G. Owen*, M. Segal* and J.L. Barker (Spon: P. Sonderegger), Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20205

Voltage-dependent membrane properties of neurons cultured from mouse spinal cord and rat hypothalamus were studied using single electrode current-clamp and two electrode voltage-clamp techniques. All recordings were made with KCl-filled microelectrodes in media containing micromolar TTX and 20mM TEA. Brief depolarizing current pulses often triggered Ca^{2+} spikes which in some cells were followed by long-lasting (up to 20 sec.) depolarizations of up to 25mV in amplitude (Fig. 1A). Both rapid and delayed events were activated by depolarizing the cell to about -40mV for as little as 1 msec., and were accompanied by an increase in membrane conductance. Both events were absent in solutions nominally free of Ca^{2+} and both were blocked by 0.2mM Cd^{2+} (Fig. 1A). Under voltage-clamp, brief commands to potentials more depolarized than -40mV evoked long-lasting inward current responses upon stepping back to the holding potential (E_{K+}) (Fig. 1B). The inward current was eliminated by 0.2mM Cd^{2+} (Fig. 1B). Similar voltage-jump protocols have shown that the slow inward current response is: 1)dependent on $[Ca^{2+}]_o$; 2) increased in amplitude and prolonged by Ba^{2+}_o; 3) blocked by Co^{2+}_o, Ni^{2+}_o, and Mn^{2+}_o; and 4) reverses in polarity between -15mV and +15mV. We tentatively conclude that the slow inward current response observed under voltage-clamp, underlies the long-lasting depolarizing event seen under current-clamp. This Ca^{2+}-dependent conductance occured in a minority (~10 percent) of spinal cord neurons, which were relatively large (~ 25μ diameter cell body) with extensive process formation and in 4 of 9 hypothalamic neurons. Slow Ca^{2+}-dependent conductances were noticeably absent in neurons cultured from rat hippocampus, suggesting that this membrane property may be regionally specific. Its presence in cultured neurons derived from the spinal cord and hypothalamus should allow us to examine in detail the membrane mechanisms responsible for its genesis, as well as its role in neuronal excitability.

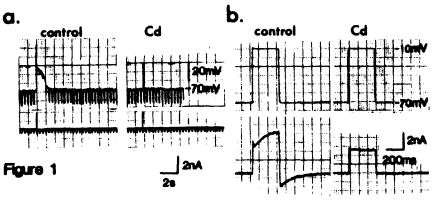

Figure 1

147.6 SLOW VOLTAGE-DEPENDENT POTASSIUM CONDUCTANCE IN SENSORY NEURONS IS MUSCARINE INSENSITIVE.

Susan R. Barry, Mary Ann Werz and Robert L. Macdonald. Dept. of Neurology, University of Michigan, Ann Arbor MI 48109

We have identified a slow voltage-dependent potassium conductance in mouse dorsal root ganglion (DRG) neurons in cell culture. This channel shares similar kinetics and voltage dependency to the muscarine-sensitive m-channel in spinal cord and sympathetic ganglion neurons. However, the slow potassium conductance in DRG neurons is not blocked by muscarine but is sensitive to tetraethylammonium ions (TEA^+).

Intracellular and single electrode voltage-clamp recordings were made from neurons bathed in tris-buffered saline at 30-35°C. TEA and muscarine were applied to the neuronal surface by pressure pulses from blunt glass micropipettes.

Application of TEA^+ to DRGs produced membrane depolarization associated with a decrease in membrane conductance. TEA^+ responses increased in amplitude as the membrane was depolarized from about -60 to -20 mV. At potentials more negative than -60 mV, no response to TEA^+ was observed. In voltage clamp experiments, TEA^+ evoked a net inward current which increased with depolarization of the membrane from -60 to -20 mV.

To determine further what channels were involved in the TEA^+ response, voltage jump experiments were performed. DRG neurons were voltage-clamped at -30 mV and then stepped to -50 mV, producing an early inward current followed by a slow inward current relaxation. Upon repolarizing the cell to -30 mV, an early outward current preceded a slow outward current relaxation. Current relations were blocked by TEA^+. In high (40 mM) K^+ media, a voltage jump from -30 to -50 mV produced an early inward current followed by an outward current relaxation. Thus, as expected for a K^+ current, increasing the external K^+ concentration reduced the K^+ equilibrium potential and reversed the direction of the current relaxation. The current relaxation following the voltage jump from -30 to -50 mV may result from the closing of a voltage-sensitive K^+ channel. Upon repolarizing the cell, this channel may be activated, causing an outward current relaxation. Since TEA^+ blocked the current relaxations, TEA^+ may block this voltagesensitive K^+ channel.

Muscarine at a concentration of 25 μM, depolarized spinal cord neurons but had no effect on the membrane potential of DRG cells. Thus, DRG neurons, like sympathetic and spinal cord cells, possess a voltage-sensitive K^+ channel activated at potentials close to the resting potential. In contrast to sympathetic and spinal cord neurons, the slow voltage-dependent K^+ channel in DRG neurons was not blocked by muscarine.

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147.7 THREE TYPES OF TRANSIENT OUTWARD CURRENTS ARE PRESENT IN CULTURED CENTRAL MAMMALIAN NEURONS. J.L. Barker and M. Segal, (Spon: E. Yadin), Lab. of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

Neurons cultured from dissociated rat hippocampus (HPC) and hypothalamus (HTH) and mouse spinal cord (SC) were studied using the two-electrode voltage clamp technique. The recording medium contained 0.5μM tetrodotoxin (TTX) and 20mM tetraethylammonium (TEA) to block some of the Na^+ and K^+ conductances commonly recorded in cultured CNS neurons. Cells were impaled with KCl-filled microelectrodes, clamped at about resting potential and stepped to depolarizing and hyperpolarizing potentials for various durations. Rapid transient outward currents (TOCs) were recorded in the majority of the cells when stepping from -90mV to potentials depolarized to -45mV. These TOCs could be divided into three distinct types. Type I was identified in 13 of 19 SC, 5 of 15 HPC and 4 of 6 HTH cells. The TOC peaked in 5 msec. and decayed in 15-40 msec. Cd^{2+} or Mn^{2+} enhanced the TOC by up to 50 percent without changing its time constant of decay. This effect was evident when the membrane was stepped to potentials positive to -20mV, and was independent of the presence of a transient inward Ca^{2+} current. Type II TOC had similar kinetics to that of type I. Cd^{2+} or Mn^{2+} blocked this TOC by up to 50 percent when the TOC was evoked from -90mV but not when the TOC was already half-inactivated (e.g., when the membrane was held at -70mV). Cd^{2+} also slowed the time constant of decay of this TOC. Type II TOC was recorded in 5 of 19 SC and 10 of 15 HPC neurons. Both types of TOC were blocked by 4-aminopyridine (4-AP) and Ba^{2+}. Type III TOC was found mainly in SC and in some HTH cells. Its time constant of decay was slow, 100-200 msec. Furthermore, it was insensitive to Cd^{2+} or 4-AP. Type III TOC coexisted with the more rapidly decaying TOCs and when recorded together with a faster TOC, usually accounted for 10-20% of the total current transiently evoked by the depolarization. The roles of the TOCs in controlling membrane potential behavior were studied in the presence of TTX and TEA by applying a depolarizing current-jump protocol to neurons current-clamped at -60mV. A transient (ca. 30 msec.) rectification of voltage responses was recorded at potentials depolarized to -45mV. These rectifications were reduced by 4-AP and/or Cd^{2+}.

The results indicate that central neurons derived from both spinal and supraspinal regions of the CNS exhibit at least three different types of TOC, two of which may be regulated by Ca^{2+}. Since 4-AP- and Cd^{2+}-sensitive rectifications were observed over the membrane potential range subthreshold to action potential genera- tion, it is probable that one of the physiological roles of these rapid TOCs involves regulation of the rate of membrane potential depolarization and subsequent spike discharge in response to a rapid depolarizing stimulus.

147.8 EVIDENCE FOR A CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE IN SEROTONERGIC DORSAL RAPHE NEURONS. C. P. VanderMaelen and G. K. Aghajanian. Depts. of Psychiat. and Pharmacol., Yale Univ. Sch. of Med., New Haven, CT 06508.

Calcium-activated potassium conductances have now been demonstrated in a variety of invertebrate and vertebrate neurons. In these cells increased intracellular free Ca^{2+}, usually caused by Ca^{2+} entry during an action potential, triggers a potassium conductance, g_K, which causes hyperpolarization of the membrane. Since serotonergic neurons of the rat dorsal raphe (DR) nucleus exhibit pacemaker potentials during which each spike is followed by a prominent after-hyperpolarization (AHP) (Aghajanian & VanderMaelen, Brain Research, 1982, 238, 463-469), the present study was aimed at determining if a calcium-activated g_K might be present in these neurons, and might play a role in these pacemaker potentials.

Intracellular recordings were obtained from presumed serotonergic DR neurons in rat brain slices as previously described (VanderMaelen & Aghajanian, Neurosci. Abst., 1982, 8, p.482; Brain Research, in press). Slices were maintained in flowing Ringers solution at 33° - 37 °C containing in mM: NaCl, 130; KCl, 5.0; NaH_2PO_4, 1.25; $NaHCO_3$, 24.0; $CaCl_2$, 2.5 or 4.0; $MgSO_4$, 1.5; D-glucose, 10.0; and aerated with humidified 95% O_2, 5% CO_2.

Single spikes (spontaneous or evoked) were followed by 10-20 mV AHP's which lasted 200 - 800 msec. A burst of spikes driven by an intracellularly injected depolarizing pulse was followed by an AHP greater in amplitude and duration than the AHP following a single spike. The reversal potential for the AHP was in the neighborhood of the expected equilibrium potential for K^+ (-75 to -94 mV). Recording with KCl rather than K-acetate microelectrodes did not shift the AHP reversal potential in the depolarizing direction. A decrease in input resistance accompanied the AHP, and was not due to anomalous rectification. The AHP reversal potential varied with the extracellular K^+ concentration in reasonably close agreement with the Nernst equation. The removal of calcium ions from the bath, with a concommitant increase in Mg^{2+}, resulted in reductions in the amplitude of AHP's of up to about 50%, and even greater reductions in their durations. It also resulted in an increased rate of discharge for these cells. Taken together, these results indicate that the AHP is mediated by an increase in g_K, and that at least a good portion of this is calcium dependent.

It is concluded that a calcium activated g_K, triggered by Ca^{+2} entry during the spike, is probably an important contributor to the pacemaker potentials of serotonergic DR neurons, and plays an important role in helping to maintain the slow and steady discharge so characteristic of these neurons. Supported by Grants MH-17871, MH-14276, and the State of Connecticut.

147.9 INTRACELLULAR INJECTION OF A CA^{++}-DEPENDENT PROTEIN KINASE AMPLIFIES CA^{++}-MEDIATED INACTIVATION OF A TRANSIENT K^+ CURRENT (I_A) IN HERMISSENDA GIANT NEURONS. J. Acosta-Urquidi, D.L. Alkon, J.A. Connor and J.T. Neary. Section on Neural Systems, Lab. of Biophysics, NINCDS-NIH, MBL, Woods Hole, MA 02543, and Bell Labs, Murray Hill, NJ 07974.

The early transient outward K^+ current (I_A) in Hermissenda Type B photoreceptors exhibits a Ca^{++}-mediated inactivation [hA (Ca^{++})] lasting tens of seconds. Previous studies show that this inactivation persists on retention days following associative conditioning (Alkon et al., Biophys. J., and Science, 1982). To probe possible biochemical steps in the long term hA(Ca^{++}) we have previously iontophoretically injected exogenous protein kinases into B cells. A Ca^{++}-dependent protein kinase (viz. phosphorylase kinase, PhK) amplified, in a Ca^{++}-dependent manner, the magnitude and duration of hA(Ca^{++}) in Type B photoreceptors (Acosta-Urquidi et al., Soc. Neurosci. Abstr. 8, 825, 1982). We have now extended these findings to central g_ant neurons LP1-3 in the left pedal ganglion of Hermissenda, which exhibit a 4-AP (5-10 mM) sensitive I_A, an $I_{K(Ca^{++})}$ and other delayed I_K(s). The I_A exhibits hA(Ca^{++}) comparable to that of B photoreceptors. In LP1-3 cells hA(Ca^{++}) varies from cell to cell and usually lasts 20-90 sec, depending on the magnitude of the Ca^{++} load (12-20 sec depolarizing commands to 0 to +75 mV), in either 0-Na^+ ASW, or in cut-axon preparations in SW, which show little or no impulse activity. I_A recovery roughly parallels the return of the elevated Ca_i^{++} to pre-load levels, as measured by differential absorption spectrophotometry (660-690 nM) using the Ca^{++} indicator ARZ. III. Ca^{++}-free ASW, Ba^{++} substitution or Cd^{++} addition reduce or abolish hA(Ca^{++}). Iontophoretic EGTA (ca. 1M, pH 7.3) injection reduces hA(Ca^{++}) or greatly speeds its recovery, and also reduces or abolishes $I_{K(Ca^{++})}$. Iontophoretic PhK (Sigma and purified, courtesy of Dr. E.G. Krebs) injections (0.5-1.0 mg/ml, 3-6 nA, 1-4 min) reduced I_A and potentiated hA(Ca^{++})(123%, N=9, P< 01); full I_A recovery was abolished. PhK injection also enhanced $I_{K(Ca^{++})}$ and reduced other delayed I_K(s) (41.7 ± 4.9 SEM, N=5, P<.005). Control injections of inactivated PhK were without significant effects on I_A, delayed I_K(s) and $I_{K(Ca^{++})}$. The possibility that PhK exerts its effects by increasing $I_{Ca^{++}}$ directly or by modulating its Ca^{++}-mediated inactivation is being considered.

147.10 A COMPARISON OF THE NUMBERS OF NITRENDIPINE BINDING SITES WITH THE NUMBER OF VOLTAGE DEPENDENT CALCIUM CHANNELS OF PC12 CELLS. M.J. Hawkes, S.L. Hamilton, D.L. Kunze and A.M. Brown. (SPON: K.-W. Yau). Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Dihydropyridines such as nitrendipine block voltage dependent calcium channels in nerve and cardiac tissue. Using [^{3}H]-nitrendipine, a single class of binding sites is found in the rat pheochromocytoma (PC12) cell line. In agreement with Toll (J. Biol. Chem. 257, 13189-13192 (1982)), nitrendipine binds with a dissociation constant of 1.1 nM. The site is located on the plasma membrane, as its purification parallels the purification of ouabain sensitive Na^+/K^+ ATPase activity and [^{3}H]-methyl-α-neurotoxin binding. It is calculated that there are 5000-8000 nitrendipine binding sites per PC12 cell. For comparison, we calculate approximately 2200 α-neurotoxin binding sites per PC12 cell.

Functional Ca^{++} channel density was estimated electrophysiologically from single Ca channel and whole cell Ca current recordings using the patch clamp method. Na^+ and K^+ currents were suppressed and 40 mM Ca was used to enhance single channel current amplitudes. At -20 mV, single channel current had a range of 0.35 to 0.45 pA and whole cell peak currents, which inactivated very slowly ranged from 150 to 200 pA. In this solution, at this potential the opening probability in Helix neurons is 0.05 (Brown, et al., J. Physiol., 1983, in press). Assuming this value for PC-12 cells, there are between 3500 to 8500 voltage-gated functional Ca channels per cell. Hence dihydropyridine receptors and functional Ca channels have similar densities in PC12 plasma membranes and we suggest tentatively that there is one receptor site per channel. (Supported by NIH grant NS-11453).

147.11 $^{45}Ca^{++}$ UPTAKE INTO RAT WHOLE BRAIN SYNAPTOSOMES ALTERED BY DIHYDROPYRIDINE CALCIUM ANTAGONISTS. Laura C. Daniell*, Edward M. Barr*, and Steven W. Leslie, Division of Pharmacology, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712.

Voltage-dependent $^{45}Ca^{++}$ uptake in rat whole brain synaptosomes was measured after 3 second KCl-induced depolarization to investigate possible inhibitory effects of calcium antagonists, nitrendipine, nimodipine, and nisoldipine. Synaptosomes, isolated on Ficoll density gradients, were pre-incubated in a buffered physiological salt solution with various concentrations of Ca^{++} and drug. $^{45}Ca^{++}$, in high or low KCl solutions, was added to each sample. $^{45}Ca^{++}$ uptake was stopped by the addition of an EGTA solution. When incubated in the presence of 1.2 mM Ca^{++}, nitrendipine, in concentrations ranging from 0.1 nM to 10 uM, had no effect on $^{45}Ca^{++}$ uptake. However, when the Ca^{++} concentration was lowered to 0.06 and 0.12 mM, 10 uM nitrendipine inhibited $^{45}Ca^{++}$ uptake in response to 109 mM KCl depolarization. When the depolarizing solution contained 65 mM KCl, nitrendipine (10 uM) inhibited $^{45}Ca^{++}$ uptake only when the Ca^{++} concentration was lowered to 0.06 mM. The same concentration of nitrendipine failed to inhibit $^{45}Ca^{++}$ uptake with 30 KCl depolarization at all Ca^{++} concentrations examined (0.06, 0.12, 0.6, and 1.2 mM). Similarly, nimodipine and nisoldipine did not inhibit $^{45}Ca^{++}$ uptake, in concentrations ranging from 0.1 nM to 10 uM, when synaptosomes were depolarized by 30 mM KCl. Our studies show that large concentrations of nitrendipine are required to demonstrate a small degree of inhibition of $^{45}Ca^{++}$ uptake into synaptosomes. These results indicate that the dihydropyridine calcium antagonists are considerably less potent in brain tissue than in peripheral tissue.

147.12 DRUG INHIBITION OF CALCIUM UPTAKE BY RAT BRAIN SYNAPTOSOMES. Wayne Hoss and Robert S. Aronstam (SPON: B.D. Goldstein). Center for Brain Research, Univ. of Rochester, Rochester, NY 14642 and Dept. of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Depolarization-induced calcium uptake by rat brain synaptosomes was measured by placing synaptosomes in a high (65 mM) potassium medium containing ^{45}Ca. Uptake was terminated by filtration on glass fiber filters which were washed with 5 ml of a calcium-free medium. Synaptosomes were prepared by sucrose density centrifugation and then equilibrated in a medium containing 5 mM KCl, 130 mM NaCl, 12 mM Na_2PO_4, 10 mM glucose and 20 mM Tris-Cl, pH 7.4. The ability of synaptosomes to exclude ^{45}Ca in the resting (i.e., nondepolarized) state increased for 2 h after final dilution, while stimulated (i.e., depolarization-induced) uptake was stable for 4 h. Stimulated uptake was essentially complete within 20 s and was linearly related to potassium concentration between 10 and 80 mM. The level of stimulated uptake gradually declined while resting uptake increased, so that after 40 min synaptosomal calcium content was the same in high and low potassium media. Calcium uptake reached a plateau at 5.4 nmoles/mg protein in the presence of 10 mM calcium. The apparent Km for this process was 0.56 mM.

Depolarization-induced calcium uptake was inhibited by tricyclic antidepressant drugs, including imipramine, desipramine, amitriptyline, nortriptyline, doxepin and chloripramine, with IC50's in the micromolar range. Anti-depressant drugs which were secondary and tertiary amines were equally effective at inhibiting calcium uptake. A wide range of neuroleptic drugs, including phenothiazines, haloperidol and butaclamol, also inhibited stimulated calcium uptake in a dose-depressant manner. Certain local anesthetics (e.g., dibucaine) inhibited calcium uptake while others (e.g., lidocaine and tetracaine) were ineffective. Among the compounds which did not affect depolarization induced calcium uptake were 4-aminopyridine, phenobarbital, diphenhydramine, cyclazocine, phencyclidine, histrionicotoxin, tetraethylammonium, pentazocine, pumiliotoxin, gephyrotoxin, valproic acid and various neurotransmitters. These results suggest that inhibition of calcium transport underlies the the actions of certain classes of psychoactive drugs. (Supported by PHS grants DA-01851 and DA-03303).

147.13 EFFECTS OF DECYLTRIMETHYLAMMONIUM ON IONIC CHANNELS OF THE SQUID GIANT AXON. H. HORIE, T. TAKENAKA, H. HORI*& T. MAEDA*. Dept. of Physiol. Sch. of Med. Yokohama City Univ., Minamiku, Yokohama, 232 JAPAN

Decyltrimethylammonium chloride is an analogue of TMA^+ and has a hydrocarbon chain with 10 carbon atoms. This agent is very soluble in water and its effects on inward-currents and outward-currents of nerve membranes were studied by using a voltage clamp in squid giant axons. When the agent was applied internally at concentrations of 0.1 mM, the resting potential of the membrane was not changed but the maximum inward-current was reduced to 50% and late outward-current also decreased. These values reached steady-state at 5 min after application of the agent. The potential level at the maximum value of inward-currents scaresly shifted. These effects of the agents were reversible. When the agnet was applied externally to the axon at a concentration of more than 1 mM, the agent affected both values of peak inward-current and late outward-current.However, when the agent at concentration of 5 mM was externally applied to the axon perfused intracellularly, both values of the peak inward-current and late outward-current gradually decreased and reached the steady-state value at 20-30 min after application of it. The shift of the potential level at the maximum inward-current was about 20 mV in the direction of depolarization. When the intracellular perfusion was stopped after reaching the steady state, late outward-currents decreased comparing to peak inward-currents, but the potential level at the maximum inward-current scaresly shifted. When intracellular perfusion started again, the values were recovered to those in the steady state. However, by washing externally with control solution, the potential shift recovered to the control level and the value of the maximum inward-current recovered, but the values of the late outward-current recovered little. These data show that when the agent was applied externally, it affects not only external side but also internal side of the membrane. This suggests that the agent might penetrate the membrane from outside into inside of the axon and inhibit the late outward-currents. TMA^+ itself has no effect on sodium and potassium currents in concentrations of 10 mM. However, the agent inhibited both sodium and potassium currents in a low concentration of 0.1 mM. This might be considered that the agent gets into the lipid layer of the membrane and attaches a TMA^+ site of the agent to a potassium channel.

147.14 K^+-CHANNEL FROM SARCOPLASMIC RETICULUM : A STUDY OF ITS PROPERTIES IN THE NATIVE MEMBRANE. A. M. Garcia*. (SPON: E. Marder). Biophysics Program, Dept. of Biol., Brandeis University, Waltham, MA 02254.

A contribution to the study of ionic channels has been the fusion of membrane vesicles into planar bilayers membranes. Although this technique has provided useful information about the channels studied, a question still remains: Are the properties of the channel the same in the planar as in the native membrane? The main difficulty in answering this question has been measuring ion fluxes in vesicles, in a time scale of significance. Moore and Raftery (P.N.A.S. 77:4509, 1980) have developed a method to measure fluxes on the millisecond time scale: If a fluorescent dye trapped in a vesicle is rapidly mixed with a Tl^+ solution, the fluorescence is quenched as Tl^+ enters the vesicles. We have used this method to study the K^+-channel from sarcoplasmic reticulum (SR) in its native membrane. Isolated SR vesicles are loaded with the fluorescent dye pyrene tetrasulfonate (choline salt) and a "test cation". The loaded vesicles are mixed with Tl^+ in a stopped-flow apparatus and the fluorescence quenching monitored. It has been found that the rate of Tl^+-test cation exchange follows a double exponential relaxation. The slow exponential corresponds to about 30% of the total exchange and it is independent of the test cation used. The rate of fast exchange depends on the ion present, following the same pattern of selectivity as the channel in the bilayer, namely: $K^+ \equiv Na^+ > Li^+ > Diethylamine^+ > Choline^+$. The rate constants found (K^+, Na^+>300 s^{-1}; Li^+=40 s^{-1}) are in good agreement with the expected values. Furthermore, blockers of the channel in the bilayer also block in the vesicle, with a lower affinity, but with the same sidedness. In conclusion, using the same ions that are known to permeate the K^+-channel in the bilayer, it has been found that the channel is present in a major population of SR vesicles and that its properties are similar to those found in the bilayer.

147.15 EFFECTS OF PHOSPHOLIPID SURFACE CHARGE ON ION CONDUCTION IN THE K^+ CHANNEL OF SARCOPLASMIC RETICULUM. Joan Bell* (SPON: I. Levitan) Dept. of Biochemistry, Brandeis Univ. Waltham, Mass. 02254.

Single-channel currents through sarcoplasmic reticulum K^+ channels were compared when reconstituted into planar bilayers formed from neutral or negatively charged phospholipids. In neutral bilayers the K^+ conductance saturates with K^+ concentration according to a rectangular hyperbola, with half-saturation at 40 mM K^+, and maximum conductance of 220pS. In negatively charged bilayers, the conductance is, at a given K^+ concentration, higher than in neutral bilayers. This effect of negative surface charge is increasingly pronounced at lower ionic strength. The maximum conductance at high K^+ approaches 220pS in negative bilayers and the K^+/Na^+ selectivity is unaffected by lipid charge. The divalent channel blocker,"bisQ11", causes discrete blocking events in both neutral and negatively charged bilayers; the apparent rate constant of blocking is sensitive to surface charge, while the unblocking rate constant is largely unaffected. Bilayers containing a positively charged phosphatidyl-choline analogue lead to K^+ conductances lower than those seen in neutral bilayers. The results are consistent with a simple mechanism in which the local K^+ concentration sensed by the channels entryway is determined by both the bulk K^+ concentration and the bulk lipid surface potnential, as given by the Gouy-Chapman model of the electrified interface. In order to be described by this approach, the channel's entryway must be assumed to be located 1-2nm away from the lipid surface, on both sides of the membrane.

147.16 THE EFFECTS OF 4-AMINOPYRIDINE AND 3,4-DIAMINOPYRIDINE ON RAT MYELINATED PERIPHERAL NERVE AND CEREBELLAR CORTEX. D. L. Eng* and J. D. Kocsis. (SPON: R. W. Angel) Dept. of Neurology, Stanford Univ. Medical School, and Veterans Administration Medical Center, Palo Alto, CA 94304

The aminopyridines have been shown to increase transmitter output at the neuromuscular junction and to have convulsant effects on cortical tissue. Voltage clamp studies on squid axon indicate that the aminopyridines block voltage-dependent potassium conductance (g_K). However, application of 4-AP to mature mammalian myelinated axons has minimal effect on their action potential characteristics. In contrast, young myelinated axons are exquisitely sensitive to 4-AP and they give rise to a delayed membrane depolarization and spike burst activity in the presence of 4-AP (Kocsis et al, J. Neurophysiol, in press). Thesleff (Neuroscience 5, 1980) states that the aminopyridine analog 3,4-diaminopyridine (3,4-DAP) is particularly potent in the neuromuscular junction. In this study we compared the effects of 4-AP and 3,4-DAP on young myelinated axons and on the excitability properties of the cerebellar cortex. Sciatic nerves of 6 week old rats were removed, desheathed, and placed in a sucrose gap chamber from which compound action potentials could be recorded. Intra-axonal recordings were simultaneously obtained with glass microelectrodes filled with 2M KCl. Application of either 4-AP or 3,4-DAP led to the development of burst activity as determined by intra-axonal recording and from occlusion experiments carried out with whole nerve recording. The area of the compound action potential after drug application (A_T) over the area before application (A_C) was plotted versus drug concentration (10^{-6}M to 10^{-3}M). The increase in this ratio (A_T/A_C) was dose-related in a similar manner for both 4-AP and 3,4-DAP. Neither compound altered resting membrane potential. In another set of experiments, field potentials and extracellular potassium concentrations $[K^+]_o$ were recorded from the molecular layer of the cerebellar cortex with ion selective microelectrodes. As has been previously reported, a single stimulus to the cerebellar surface in the presence of 4-AP elicits an increase in $[K^+]_o$ from resting levels of 3.0 mM to above 10 mM. There is a transient cessation of presynaptic and postsynaptic components of the field potential during this period of high $[K^+]_o$. A similar cessation of neuronal activity after a single stimulus was observed in the presence of 3,4-DAP. These results indicate that the aminopyridine analogs, 4-AP and 3,4-DAP, have similar electrophysiological action on myelinated mammalian axons and on excitability properties of the cerebellar cortex. Supported by the National Multiple Sclerosis Society and the Veterans Administration.

147.17 PASSIVE ELECTRICAL PROPERTIES OF PERIPHERAL MYELINATED AXONS: DUAL INTRA-AXONAL MICROELECTRODES REVEAL PROMINENT SLOW (INTERNODAL) COMPONENT. K.A. Scappaticci and E.F. Barrett. Dept. of Physiology and Biophysics, Univ. of Miami Sch. of Med., Miami, FLA 33101.

Single peripheral myelinated axons coursing through the dewlap extensor muscle of lizards (Anolis sagrei) were impaled with two separate micropipettes (50-200 MΩ, filled with 0.2 M K_2SO_4), one for recording voltage, the other for passing current. Similar recordings were made from isolated frog sciatic axons. Input resistances of fibers selected for study ranged from 35-100 MΩ in the linear (hyperpolarized) region of the current-voltage relationship. The voltage response to an injected current step showed distinct fast and slow (>20 msec) components. The fast components are due to current flow through nodal membrane and internodal series leakage resistance, while the slow component represents charging of the internodal axon membrane (see Barret', E.F. and Barrett, J.N., J. Physiol. 323:117, 1982). The magnitude and time constant of the slow component varied with membrane potential. In hyperpolarized axons the slow component accounted for 40-85% of the total voltage transient in four lizard axons (30% in one frog axon). These percentages give a lower-bound estimate of the relative contribution of the internodal axon membrane to the resting properties of the myelinated axon. The relative contribution of the slow component to the passive voltage transient decreased as the axon was depolarized. The time constant of decay of the slow component ranged from 90-250 msec in hyperpolarized lizard and frog axons; this time constant decreased with depolarization.

In both frog and lizard axons action potentials evoked by stimulating the nerve trunk are followed by a depolarizing afterpotential. The time constant of decay of this depolarizing afterpotential usually showed the same voltage dependence as the time constant of the slow component of the passive response, suggesting that both reflect charging of internodal membrane.

When doubly impaled axons were perfused with the potassium channel blocker tetraethylammonium (TEA, 1-5 mM), axonal input resistance increased at both depolarized and hyperpolarized potentials, and the magnitude and duration of both the slow component of the passive voltage transient and the depolarizing afterpotential increased. In addition, a slow anomalous rectification seen in hyperpolarized lizard axons became more prominent in TEA.

We conclude, in agreement with Chiu's voltage clamp studies in frog (Soc. Neurosci. Abs., 8:253, 1982), that voltage-dependent, TEA-sensitive potassium channels in the internodal membrane make a major contribution to the resting properties, as well as to the depolarizing afterpotential, in frog and lizard myelinated axons. Supported by the Muscular Dystrophy Association and NS 12404.

147.18 SPONTANEOUS OSCILLATIONS OF $[K^+]_o$ IN NEONATAL RAT OPTIC NERVES IN Cl^- DEFICIENT SOLUTIONS. B.R. Ransom and B.W. Connors (SPON: M.W. Siegel). Dept. of Neurology, Stanford University Sch. of Med., Stanford, CA 94305.

Neonatal rat optic nerves display unusally large increases in extracellular K^+ concentration ($[K^+]_o$; up to 20 mM) when stimulated repetitively (Connors et al., Science 216:1341, 1982). We report here that these nerves exhibit spontaneous and recurring waves of markedly elevated $[K^+]_o$ when bathed in Cl^--deficient solutions.

Rat optic nerves from animals ranging in age from 1 to 25 days were maintained in a perfusion chamber at 37° in a solution with 5 mM K^+. Nerves were stimulated supramaximally with suction electrodes, and double-barrel K^+-sensitive microelectrodes were used to record $[K^+]_o$ and the compound action potential. When 1-5 day old nerves were exposed to solutions in which propionate was substituted for Cl^-, $[K^+]_o$ increased by 2-3 mM over several minutes. In most nerves, $[K^+]_o$ then rapidly and spontaneously increased to a peak of 10-15 mM before falling more slowly toward the normal baseline level. This pattern of $[K^+]_o$ oscillation, or "cycling", usually continued with a period of 1-2 min per cycle, often for more than an hour. Nerves older than 5 days did not spontaneously oscillate, but showed signs of hyperexcitability.

We sought the mechanism for $[K^+]_o$ cycling. Tetrodotoxin (1 μM) immediately blocked the fluctuations, indicating that action potential activity plays a critical role. Reducing the bathing $[K^+]$ to 3-4 mM slowed the oscillations; 1-2 mM blocked them completely. When 9 different anions of varying hydrated radii were substituted for Cl^-, cycling of $[K^+]_o$ occurred only with anions the size of HCO_3^- or larger, but not with anions the size of BrO_3^- or smaller. The amount of spontaneous axonal firing within the nerve was estimated from the size of the maximal evoked field potential: the field was largest at the $[K^+]_o$ trough of the cycle, diminished in size during the slow initial $[K^+]_o$ increase, was abolished during the rapid $[K^+]_o$ rise and returned during the falling limb of the cycle.

We hypothesize that neonatal optic axons possess an anion-selective membrane channel which is open at rest. This conductance is abolished by deleting permeant anions, rendering the membranes hyperexcitable. Spontaneous firing of some axons leads to an increase in $[K^+]_o$ which facilitates the firing of adjacent axons until $[K^+]_o$ rises to very high levels. Firing eventually subsides because of Na^+ channel inactivation and/or direct K^+ effects and $[K^+]_o$ declines. This enhancement of axonal excitability in Cl^- deficient solutions may be similar to that of myotonic muscle, which results from a genetically determined reduction in Cl^- channels.

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147.19 ACTIVATION OF ROD OUTER SEGMENT SHEDDING: THE UNIMPORTANCE OF MEMBRANE POTENTIAL. David S. Williams*, Chester Wilson* and Steven K. Fisher* (SPON: George M. Austin). Dept. of Biological Sciences, University of California, Santa Barbara, CA 93106.

In the retinae of lower vertebrates, the daily shedding of rod outer segment discs is maximal following dawn, and is largely a light-evoked event (Basinger, S. et al., Science 194: 1074, 1976). We have been interested in the physiological changes resulting from light stimulation that might be responsible for the activation of shedding. Our first experiments were designed to test if the hyperpolarisation of rods or retinal pigment epithelial (RPE) cells by light is pertinent to the shedding response. Previous work is compatible with this hypothesis: isobutylmethylxanthine has been shown to depolarise rod photoreceptors (Lipton, S.A. et al., J. Gen. Physiol. 70: 771, 1977) and inhibit the light-evoked shedding response (Besharse, J.C. et al., J. Gen. Physiol. 79: 775, 1982). We exposed retinae to other conditions that depolarise rod and RPE cells, and to conditions that hyperpolarise them. Several hours before the usual time of the onset of light, eyecups of the frog, Xenopus laevis, were placed in a bicarbonate-buffered salt solution, supplemented with amino acids and vitamins. Twenty minutes prior to lights-on, the eyecups were transferred under dim red light to either fresh normal medium (controls) or the experimental medium. Two hours after lights-on, the eyecups were fixed and processed for light microscopy. The extent of shedding was quantified by counting the phagosomes in the RPE. When Na^+ was replaced by choline, and the eyecups were not exposed to light, the shedding response was significantly greater than that in control eyes kept in darkness, and almost as large as that in control eyes exposed to light. The addition of 0.5 mM ouabain to the normal medium, with or without exposure to light, induces a shedding response that is significantly greater than that in control eyecups even exposed to light. Replacement of Na^+ by choline hyperpolarises the rods (Brown, J.E. and Pinto, L.H., J. Physiol. (Lond.) 236: 575, 1974), but, by inhibiting Na^+, K^+-ATPase activity, ouabain should quickly depolarise retinal cells, including the rod and RPE cells. Because both these conditions induced shedding in darkness, we conclude that membrane potential is irrelevant to shedding. Our findings are consistent with the likelihood that Na^+, K^+-ATPase activity of rod inner segments and RPE apical membrane is inhibited by the onset of light: that of rods by a lowering of the internal Na^+ concentration, and that of both the rods and RPE by a lowering of the K^+ concentration in the subretinal space (cf. Oakley, B., Invest. Ophthal. Vis. Sci. 24 (Suppl.): 179, 1983). Supported by NIH grants EY04670 and EY00888.

147.20 ELECTROANATOMICAL STUDIES OF A SPIKING AMACRINE CELL. R. F. Miller. Departments of Ophthalmology, Physiology and Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

Intracellular recordings obtained from the retinas of a variety of species have demonstrated the presence of on-off amacrine cells which respond to light stimulation with large amplitude EPSPs and spikes. Two different spike mechanisms, of soma and dendritic origin have been suggested. Both types of spikes are activated by depolarization of the soma, and both are blocked by TTX. The EPSPs can be as large as 60 Mv and constitute one of the largest PSPs reported in the central nervous system. Intracellular recordings from ganglian cells sometimes reveal two types of inhibitory events, one of which includes fast IPSPs which have properties and a time course similar to the dendritic spikes seen in on-off amacrine cells. This evidence coupled with two electrode recording experiments suggests that on-off amacrines are inhibitory. HRP staining of on-off amacrine cells in the mudpuppy was combined with measurements of input resistance and time constant. In addition peeled time constants were analyzed to predict electrotonic length of the dendrites. HRP stained on-off amacrines show a relatively simple dendritic structure with 3 to 6 primary dendrites. Diameter and length measurements show that these structures do not branch in a "conventional" dendritic fashion but bear a closer resemblance to some axonal branching characteristics, which I have referred to as "trunk" branching. Computer simulations were carried out to evaluate possible sites of origin of dendritic spike activity. These studies suggest the following: Dendritic spikes are probably initiated close to the soma, and may simultaneously activate the entire dendritic tree in a centripetal direction. Consistent with this suggestion is the observation that extracellularly recorded dendritic spikes are purely negative. The soma spike may help to insure that dendrite spikes are propagated in a centripetal fashion. The large amplitude PSPs together with "fast" Na dependent spikes appear to be designed for evoking powerful inhibitory influences predominately onto ganglion cells and other amacrines.

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147.21 PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF CHOLINERGIC ENTERIC NEURONS GROWN IN CELL CULTURE. Alan L. Willard* and Rae Nishi (SPON: S.G.Matsumoto). Dept. of Neurobiology, Harvard Med. School, 25 Shattuck St., Boston, MA 02115.

The enteric nervous system (ENS) contains several classes of neurons that can be distinguished by electrophysiological, pharmacological, morphological and immunohistochemical criteria. We are using cell cultures of neurons dissociated from small intestines of newborn rats to correlate some of these properties in single cells. Cholinergic neurons are a major subpopulation in the ENS. They are also a major class (30-50%) in our cultures. Here we report some of the electrophysiological, pharmacological and immunohistochemical properties of cultured cholinergic enteric neurons.

Neurons in cultures 2-10 weeks old were impaled with intracellular electrodes and tested for their ability to cause fast nicotinic epsps in nearby neurons. After a neuron was physiologically identified as cholinergic, the ionic basis of its action potential (AP) was tested, its ability to fire repetitively was examined, its responses to putative transmitters were tested and it was then fixed and examined for VIP-like immunoreactivity (VIP-LIR).

The somata of cholinergic neurons shared electrophysiological properties with both types of neurons described in adult guinea pig ENS (e.g. North, Neurosci. 7: 315 (1982)); most had APs with both Na and Ca components but a few appeared to have totally Na dependent APs. Afterhyperpolarizations (AHs) following single APs were less than 100 msec long; prolonged AHs did not follow single APs. TTX rarely blocked APs in cell bodies but it always blocked cholinergic synaptic transmission, suggesting that the neuronal processes do not have propagating Ca dependent APs. Spontaneous synaptic activity was common, but no pacemaker drivers were observed, suggesting that the spontaneous activity was largely a network property (spontaneous epsps were often suprathreshold). No cholinergic neurons were observed to fire continuously when depolarized; rather they fired 1-10 APs at the onset of a depolarizing pulse.

Responses to putative transmitters were tested by pressure ejection of test compounds from blunt micropipets. Cholinergic neurons were excited by ACh, serotonin, Substance P and VIP. They were inhibited by GABA, norepinephrine and Met-enkephalin.

To relocate identified neurons, dishes were marked so that they could be placed back on the microscope stage in the same position and the field of view containing the physiologically and pharmacologically characterized neuron(s) was photographed. The cultures were then fixed and processed for VIP staining. Two of 14 identified cholinergic neurons stained positively for VIP-LIR. Thus there appear to be at least 2 categories of cholinergic neurons in our cultures. Studies are currently underway to test for other classes of cholinergic enteric neurons. Support: MDA, AHA and NIH.

147.22 PATCH CLAMP STUDIES OF RAT SYMPATHETIC NEURONS IN TISSUE CULTURE. Joseph E. Freschi. Physiology Dept., Armed Forces Radiobiol. Res. Inst., Bethesda, MD 20814

Using the gigohm-seal patch clamp technique (Hamill et al, Pflugers Arch 391: 85, 1981) I studied electrophysiological properties of neonatal rat sympathetic neurons grown in tissue culture. I used cell-attached patch, detached patch, and whole-cell recording techniques. Access resistances of 5-20 gigohm were usually obtained with standard salines in bath and pipette. These values reflected conductances of similar orders of magnitude through the patch membrane and through the pipette-membrane seal, for example, 17 pS through the patch and 83 pS through the seal. Because, however, many channels in the patch could be active at the resting potential of the cell (around -60 mV), the established patch resistance could vary considerably, and sudden large jumps in patch conductance could resemble loss of pipette-membrane sealing. Spontaneous action potentials (APs) frequently were seen through the passive RC elements of the patch membrane. Their shape and amplitude were functions of the patch conductance. APs were small (4-5) pA and biphasic when patch conductance was low, and were large (60-80) and monophasic when patch conductance was high. Particularly in high conductance patches, however, APs were often followed by large inward currents of 20-60 pA amplitude. Changing the potential across the patch membrane had no discernable effect on action potential frequency or configuration. This suggests that the ratio of patch to whole cell conductance is usually very small. This may not be the case, however, for high conductance patches with many channels. Whole cell recording in the current clamp mode confirmed that the total cell conductance is usually several hundred times greater than the patch conductance. Cell input resistance, estimated by measuring the slope resistance near resting potential, was around 100 megohms. This value is significantly higher than the 30-50 megohm range found using conventional microelectrodes, but not as great as the order of magnitude differences seen in some cells (Fenwick et al, J. Physiol. 331: 577, 1982). Many types of single channels were apparent in membrane patches of cultured sympathetic neurons. Of particular interest is a channel that had a decreasing probability of opening when hyperpolarized from rest and an increasing frequency of opening following depolarization. This is similar to the conductance-voltage relationship of the muscarine-sensitive channel which is seen when macroscopic currents are studied by voltage clamping these cultured neurons (Freschi, Soc. Neurosci. Abstr. 8: 123, 1982). Ionic selectivity and pharmacological studies will establish whether this is the correct identity of this channel.

147.23 VOLTAGE-DEPENDENT MEMBRANE CURRENTS OF RAT PHEOCHROMOCYTOMA CELLS (PC12). G.G. Schofield and F. F. Weight, Laboratory of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20852.

The rat pheochromocytoma (PC12) clonal cell line has been shown to display a variety of neuronal properties. Current-clamp recordings from PC12 cells grown in the absence of nerve growth factor (NGF) reveal a rectifiying response to depolarizing current pulses suggesting the presence of a delayed potassium conductance (Dichter et al, Nature 268: 501, 1977). The authors also reported that PC12 cells grown in the presence of NGF developed tetrodotoxin (TTX)-sensitive action potentials. A study on large chemically-fused PC12 cells demonstrated both a tetraethylammonium (TEA)-sensitive and a calcium-dependent potassium conductance, as well as a regenerative calcium spike in non-NGF treated cells (O'Lague et al, P.N.A.S., 77: 1701, 1980). Cells treated with NGF (200 ng/ml) displayed TTX-sensitive action potentials, in addition to conductances similar to those in non-NGF treated cells. We have studied membrane currents in PC12 cells in the presence and absence of NGF (50 ng/ml) using the gigaohm seal technique in the whole cell voltage-clamp configuration. Untreated PC12 cells in standard physiological solutions displayed a voltage-dependent, TEA-sensitive outward membrane current which activated at potentials more depolarized than -40 mV. Typical slope conductance was 20 nS between -30 and +30 mV. The addition of TEA (1 mM) reversibly reduced the slope conductance of this outward current by 50%. In addition to the outward current described, PC12 cells grown in the presence of NGF showed a transient inward current during command potentials between -40 and +20 mV from a holding potential of -80 mV. This transient inward current activated and decayed in less than 10 msec. In the presence of TEA (10 mM), the net inward current reached a maxium near -20 mV, decreased with further depolarization, and reversed near +40 mV. The transient inward current was inactivated with holding potentials positive to -40 mV. These observations indicated that PC12 cells exhibit voltage-dependent conductances that resemble conductances reported previously in various types of neurons.

147.24 **EXPRESSION OF EXCITABLE SODIUM CHANNELS IN DEVELOPING MOUSE CEREBELLUM**, R.B. Rogart*, M. Willinger*, and G. Kuzma.* Depts. of Neuroscience and Neuropathology, Children's Hospital and Department of Medicine, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115. (SPON: P. Mullenix)

Saxitoxin (*STX) is a radiolabeled neurotoxin which has been used as a high-affinity ligand for studying the Na^+ channel in nerve and skeletal muscle membrane. The ability of this labeled ligand to bind to the channel at concentrations which correlate well with the physiological block of Na^+ current, allows the characterization of membrane channels as ligand receptors. We have used this approach to study the expression of Na^+ channels in the developing mouse cerebellum as a model for the development of membrane excitability in the mammalian CNS.

We observe *STX binding to a high affinity receptor with a K_d= 1-1.5 nM starting at postnatal day 6. The density of high affinity receptors rises most steeply between days 15 and 22. The site density increases 10-fold, from 25 to 250 fmoles *STX bound per mg/wet weight of cerebellum. This increase in channel density occurs during a period of extensive synaptogenic activity in the cerebellum.

We observe a second population of Na^+ channel with a low affinity receptor for *STX, present in cerebellum from postnatal day 6 to day 14. This corresponds to a period of neurogenesis and morphogenic activity. The low affinity receptor has a K_d= 12-16 nM for *STX, and a K_d= 300 nM for tetrodotoxin, a neurotoxin similar to STX. This receptor is similar to a low affinity receptor found in newborn and denervated mammalian muscle and mammalian heart. In these latter preparations, a tetrodotoxin-resistant action potential has been observed in electrophysiological studies, which we believe is accounted for by the population of Na^+ channels with the low affinity STX/TTX receptor. (Supported by PHS NS 16183,RBR, PHS NS 17225, MW, and Core Grant HD06276.)

147.25 THE DEVELOPMENT OF SPONTANEOUS ACTIVITY AND ^{3}H-SAXITOXIN BINDING CORRELATES WITH IMPULSE-DEPENDENT NEURONAL SURVIVAL.
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Lab. Develop. Neurobiol., NICHD, and Lab. Neurobiol., NIMH, NIH, Bethesda, Md. 20205

Previous studies in our laboratory have shown that a critical period exists for the effect of tetrodotoxin(TTX) on neuronal survival in spinal cord (SC) cultures. TTX treatment of cultures between days 7-21 in culture results in a decrease in cell number and choline acetyltransferase activity. In order to study the development and TTX sensitivity of electrical activity in these cultures, patch electrodes were utilized to obtain intracellular recordings under current clamp from SC neurons during days 1-14 in culture. Ion channel development was studied with specific radioactive ligands on companion cultures during the same period.

All cells studied (n=83) showed regenerative action potentials (APs)to depolarizing current pulses(days 1-14). However, spontaneous APs were not seen until 4-5 days in culture. Small synaptic potentials(1-5 mV) were present in all cells by day 4. Large synaptic potentials(10-30 mV) and occasional spontaneous APs were observed in all cells by day 6. The intensity of spontaneous AP activity rapidly increased between days 6-10. Despite the lack of spontaneous APs on days 1-4, evoked APs with rise rates of 80-100 V/sec were blocked by miniperfusion with 1 μM TTX. AP rise rates above 100 V/sec were not seen until after day 6. There was no apparent shift in resting membrane potential during this period, but there was a downward trend in the measured input resistance from 0.5-2 gigaohms(days 1-4) to 50-250 megaohms (days 7-14).

^{3}H-Saxitoxin, ^{125}I-scorpion toxin, and ^{3}H-nitrendipine binding were compared during development. All ligands exhibited multiphasic increases in specific binding as the cultures matured. ^{3}H-Nitrendipine, a putative ligand for a class of calcium channels, exhibited greater binding (> 5 fold) than that obtained for the sodium channel ligands early in development (1-3 days in culture). The ratio of specific scorpion toxin to saxitoxin binding varied with development. Scorpion toxin was significantly greater than saxitoxin early in development (days 4-11). The onset (day 7) and duration of the critical period described above correlated best with a major increase in saxitoxin binding.

The presence of TTX-sensitive APs and low levels of saxitoxin binding precede the critical period. However, the onset of a critical period in impulse-dependent neuronal survival correlates closely with the development of spontaneous electrical activity and a period of major increase in ^{3}H-saxitoxin binding.

147.26 EXPRESSION OF THE SODIUM CHANNEL PROTEINS DURING RAT BRAIN DEVELOPMENT. J. Baumgold, I. Zimmerman* and J.R. Moskal*+. Lab. of Neurobiol., and +Lab. of Cell Biol. NIMH, Bethesda, MD. 20205

We have previously demonstrated that in the course of development of chick skeletal muscle, the sodium channel proteins first appear in an electrophysiologically non-functional form, capable of binding ^{125}I-scorpion toxin (^{125}I-ScTX) but not ^{3}H-saxitoxin (^{3}H-STX), followed by a change to an electrophysiologically functional form capable of binding both of these toxins (Baumgold, et al., J. Neurosci., 3:995-1003 and 1004-1013, 1983). We have extended these experiments using developing rat brain. ^{3}H-STX binding first became detectable in homogenates of rat brain two days before birth and reached maximal levels (1.1 ± 0.1 pmoles/ mg protein) by post-natal day twenty. The density of ^{125}I-ScTX binding sites, however, was maximal by post-natal day 7 (1.9 + 0.1 pmoles/ mg protein). ^{3}H-STX binding activity from mature and immature (7 day old) rat brain was solubilized (1% Triton X-100, 150 mM choline chloride, 10 mM $CaCl_2$, 0.02% phosphatidylcholine, 20 mM HEPES-TRIS pH 6.7) at a concentration of 6 mg protein/ml and chromatographed on DEAE-Sephadex ion exchange columns. Although the ^{3}H-STX binding activity from adult rat brain bound to the column and was eluted as a single peak at 200-300 mM choline chloride, two peaks of ^{3}H-STX binding activity were found from immature rat brain: 49% of the recoverable activity appeared in the void volume and the remaining 51% of the activity was eluted as above. We also compared mature and immature sodium channel proteins for their ability to bind to various lectin columns. While greater than 90% of the recoverable ^{3}H-STX binding activity from adult rat brain bound specifically to wheat germ agglutinin-Sepharose, less than 25% of the ^{3}H-STX activity from immature rat brain bound specifically to this column. ^{3}H-STX binding activity from mature and immature brain behaved alike on several other lectin columns. The behavior of ^{3}H-STX binding activity from 14 day old rat brain on on ion exchange and on wheat germ agglutinin columnns was found to be intermediate between that of 7 day and adult material. The behavior of the ^{3}H-STX binding activity on both the ion exchange and the wheat germ agglutinin columns was dependent upon the protein concentration of the solubilized material. When solubilized at 3 mg protein/ml, the ^{3}H-STX binding activity from 7 day old brains behaved like that of adult.

The binding studies and the behavior of the solubilized (at 6 mg/ml) ^{3}H-STX binding protein on ion-exchange and on lectin columns, suggest the existence of different molecular forms of the sodium channel protein. However, since different chromatographic profiles were obtained on ion-exchange and on lectin columns, depending on the solubilization conditions, it is also possible that differences in protein-to-lipid-to-detergent ratios could also account for these results.

147.27 ELECTROPHYSIOLOGICAL STUDY OF MAMMALIAN NEURONS GROWN IN PRIMARY DISSOCIATED CELL CULTURE FROM VENTRAL MESENCEPHALON. E.J. Heyer, M.D. Yahr. Dept. Neurology, Mount Sinai School of Medicine, New York, N.Y. 10029.

Neurons from ventral mesencephalon (VM) were grown in primary dissociated cell (PDC) culture, and characterized electrophysiologically in terms of resting membrane potential (RMP), input resistance (R_{in}), maximum rate of rise of the action potential (AP) ($\dot{V}_{max}$) and AP duration.

PDC cultures of dissected VM were prepared from 12 to 14 day old fetal mice. After 4 to 6 weeks in culture cells of various sizes could be seen. Using a modified glyoxylic acid method in which the cultures were preincubated with 10 uM α-methyl norepinephrine, the catecholaminergic nature of some of these neurons was established indicating that VM neurons were surviving in culture. The fluorescent cells were most probably dopaminergic because uptake blockers for dopamine (benztropine 5 uM) eliminated fluorescence, while uptake blockers for norepinephrine (desmethylimipramine 5 uM) did not. Without preincubating the cultures in α-methyl norepinephrine only occasional fluorescent neurites were seen, however, no cell bodies fluoresced.

Intracellular recordings of VM neurons bathed in Tris buffered saline solution with 5 mM calcium (TBS) were made with micropipettes filled with 4M KAc or 4M KAc plus 1% ethidium bromide (a red fluorescent dye). The larger cells (15-25 microns in diameter) could be readily impaled. In TBS solution the neurons generated APs spontaneously. However, spontaneously occurring APs were decreased in number when 12 mM $MgCl_2$ was added to TBS solution, a condition which reduces synaptic transmitter release. Therefore, this spontaneous activity was most probably synaptically mediated. Using a modified bridge circuit to inject current, APs could be elicited with depolarizing current pulses. Thus, RMP, $\dot{V}_{max}$, R_{in} and AP duration could be determined for cultured neurons. In addition, the catecholaminergic nature of electrophysiologically studied neurons was assessed as follows. The recorded neuron was identified by staining the cell intracellularly with ethidium bromide (micropipette filled with KAc and ethidium bromide), and its catecholaminergic nature determined by preparing the culture with the modified glyoxylic acid method. Since ethidium bromide fluoresced red and the catecholamines fluoresced yellow-green, with appropriate excitation wavelengths the concurrence of these two fluorescent products or their lack could be determined. Supported by NINCDS Teacher-Investigator Development Award (NS 00657) (E.J.H.), Research Grant from the American Parkinson's Disease Association (E.J.H.), NINCDS Clinical Center for Research in Parkinson's and Allied Diseases (NS 11631-10) (E.J.H. and M.D.Y.)

147.28 ACTION POTENTIALS OF MAMMALIAN CENTRAL SPINAL AXONS IN VITRO: DEPOLARIZING AFTER-POTENTIALS AND THE ROLE OF THE MYELIN SHEATH
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Depolarizing after-potentials (DAP) have been recorded from vertebrate peripheral axons and attributed to the discharge of axolemmal capacitance by Barrett and Barrett (J. Physiol., 323: 117, 1982). This requires the existence of a low-impedance pathway through the myelin sheath, which seems incompatible with the classically ascribed role of myelin.

Action potentials were recorded intra-axonally in vitro in tracts isolated from the thoracic spinal cord of rats. The falling phase of the action potential could be resolved into three exponential components. The time constant of the first component was less than 0.1 ms (interpreted as the relaxation of the nodal membrane), that of the second 1.6 ± 0.6 ms, and of the third 27.0 ± 9.2 ms. The amplitudes of the two late components, extrapolated to the peak of the action potential, were 16.0 ± 4.3 mV, and 4.7 ± 2.4 mV. The action potential amplitude was 80.9 ± 17.8 mV, and the conduction velocity 19 ± 8 m s^{-1}. (All values: mean ± S.D., N=32, t = 25°C.). Similar passive time constants were observed with injection of hyperpolarizing current pulses. The amplitude and duration of the late components decreased with depolarization, and DAP were rarely seen following action potentials less than 60 mV. The longer component of the DAP appears similar to (though smaller and briefer than) the DAP in peripheral axons (op. cit.), and is open to the same interpretation.

The DAP were simulated by a computer model of the passive electrical characteristics of the axon. The model required that the nodal leakage conductance be 5 to 10 times less than values measured from single fibers, that as much as 80% of the radial resistance of the internode be due to the axolemma, and that the electrode create an additional leakage through the myelin. The leak around the electrode accounts for the shorter component of the DAP and increases the amplitude of the longer component. The model predicts that under normal conditions the DAP are monotonic and much smaller in amplitude.

This analysis agrees with the previous study in indicating that the role of the myelin is chiefly to mask the capacitance of the axolemma, not to provide a resistive shield. We differ in attributing the prominence of the DAP to an artefact of recording. In future modeling of saltatory conduction and the effects of demyelination it will be important to take into account the distribution of internodal resistance.
(Supported by USPHS grants NS10164 and NS15590 from NINCDS)

147.29 DEVELOPMENT OF VOLTAGE-DEPENDENT CONDUCTANCES IN CULTURED AMPHIBIAN SPINAL NEURONS. Diane K. O'Dowd. Department of Biology, UCSD, La Jolla, CA 92093.

The ionic dependence of the action potential (AP) in spinal neurons of Xenopus laevis changes during development both in vivo and in vitro (Baccaglini and Spitzer, '77; Spitzer and Lamborghini, '76). Current clamp measurements reveal an AP that is initially long in duration and primarily Ca-dependent. The contribution of Na to the AP increases during development and the contribution of Ca decreases; the mature AP is primarily Na-dependent. This developmental progression could be due to changes in any of several neuronal properties, e.g. Na and Ca inward conductances and outward K-dependent conductances. To understand the molecular bases which underlie the expression of a series of developmentally changing phnotypes it is important to have a quantitative description of the differentiation of these properties. To this end the whole cell voltage clamp technique (Hamill et al., '81) has been used to identify and characterize the ionic conductances in developing spinal neurons.

Dissociated cell cultures are prepared from neural plate stage Xenopus embryos and grown in a defined medium. High resistance seals (5-20 gigohms) are formed between the patch electrode and the cell. A short pulse of suction ruptures the membrane separating the pipet and the cell interior, creating a low resistance pathway between the pipet and the cell. The cell is voltage-clamped and internally perfused in this manner.

Two inward currents have been isolated in these cells by blocking outward currents with Cs in the pipet and TEA in the external solution. Na and Ca currents are present at both early and late times in development, and may be separated by ionic substitutions or addition of blocking agents to the external medium. At early times in development (<10 hours in culture), the ratio of peak amplitude Na current to peak amplitude Ca current is 0.8 ± .2, as compared to 2.5 ± .7 at later times (>30 hours in culture). This data is consistent with the current clamp records previously obtained in which a developmental shift from a primarily Ca-dependent AP to a primarily Na-dependent AP has been documented. The voltage dependence for activation of the Na and Ca currents does not appear to change during this same period of development. The changes in the outward conductances as well as the inward conductances will be discussed as a function of developmental age. Early, chronic exposure of cultured neurons to inhibitors of RNA and protein synthesis prevents the maturation of the Na AP (Blair, '83; O'Dowd, '83). The identification of the normal changes in various conductances will facilitate evaluation of the effects of metabolic inhibitors. Supported by NSF predoctoral fellowship, and NIH grant NS15918 to N.C. Spitzer.

147.30 ELECTRICALLY EXCITABLE MEMBRANE PROPERTIES OF CULTURED MOUSE SPINAL AND SENSORY NEURONS ARE PRESENT EARLY IN DEVELOPMENT. A. MacDermott, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

The development of electrically excitable membrane properties in the mammalian CNS has proved difficult to study in vivo. Some of the technical problems have been obviated by culturing embryonic mammalian central neurons. However the small size of the cells (15 micron diameter cell bodies) has precluded easy application of conventional intracellular recording techniques, which often damage the cell irreversibly. Recently, another method of studying electrical activity in small-sized cells has evolved following the innovation of patch-clamp recordings (Hammill, et al., 1981). This "whole cell patch clamp" recording technique involves first isolating a small patch of surface membrane by forming a tight mechanical and electrical seal between microelectrode and cell, and then carefully rupturing the patch to gain intracellular entry. Such a technique produces a mechanically stable, low-resistance pathway for recording electrical activity in small cells.

This whole cell patch clamp method has been used to study the development of excitable membrane properties in spinal cord and sensory (dorsal root ganglion or DRG) neurons cultured from the embryonic (day 13 gestational age) mouse CNS and grown in monoculture and coculture . Under these experimental conditions embryonic SC and DRG neurons recorded at various times up to 11 days in culture had relatively high input resistances (0.2-5.0 Gohm) at the level of the resting potential, which ranged from -73mV to -45mV in the 27 cells studied. (The minimum acceptable seal resistance in these studies was 1 Gohm.) Most neurons studied within two days of culturing exhibited sizable outward currents in response to adequate depolarizing commands from holding potentials of -70mV. These currents were sustained for the duration of the 100msec command and were relatively insensitive to Mn^{2+}. Some of cells studies exhibited other currents, including transient types of outward current, long-lasting outward tail current, and slowly developing inward current with hyperpolarizing commands. All of these currents have been identified in neurons cultured for much longer periods. The results suggest that many electrically excitable membrane properties develop well before birth in the mouse CNS.

147.31 CURRENT NOISE GENERATED IN PATCH AND WHOLE CELL RECORDINGS OF CULTURED NEURONS DEPENDS ON THE VOLTAGE CLAMP APPLIED. G.D. Lange, A.B. MacDermott, and J.L. Barker, Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

The development of excitable membrane mechanisms in spinal and sensory cells cultured from the embryonic mouse CNS has been studied using several different electrophysiological recording techniques. We are now applying the patch clamp and whole cell clamp techniques (Hamill, et al, 1981), since they are quite useful for studying ion channel mechanisms in small cells. We have also applied the conventional two-electrode voltage clamp in order to compare the properties of current variance generated in patch membrane and whole cells. In addition we have applied these voltage clamp techniques to the pituitary clone GH3/6 since these cells are electrically excitable and constitute a relatively uniform population.

In a typical experiment, a substantial increase in current variance occurred when the patch membrane was ruptured. Whole cell current variance was minimum at resting potential and increased as the membrane potential was shifted in either direction. In both the embryonic neurons and the pituitary cells the spectra from one-electrode recordings were more complex than the spectra from two-electrode recordings. They were not the compound Lorentzian spectra expected from simple two-state channel theory. A broad interpretation of the spectra indicates that there are at least two processes activated on depolarization. Using gated channel terminology, one process has mean lifetimes of 1.5-2.5 msec., while the other is much slower (ca. 15 msec.). The faster component is consistent with the presence of a voltage activated outward current seen with the voltage step method. The slower component is the predominant component of the patch noise. We do not usually see two-state gated channels in the patch records. Therefore this low frequency component may have to be explained in a different way. Comparison of patch and whole cell current variance should contribute to a better understanding of the nature of the relationship between microscopic ion channel activities in patches of membrane and macroscopic conductance mechanisms recorded at the level of the whole cell.

147.32 NEURON LP1 OF HERMISSENDA: ELECTROPHYSIOLOGY AND EFFECTS OF DOPAMINE, 5-HT and cAMP. Jon Jacklet, Juan Acosta-Urquidi and Daniel L. Alkon. Section on Neural Systems, Lab. of Biophysics, NINCDS-NIH, Marine Biological Laboratory, Woods Hole, MA 02543.

The giant (200 µm) unpaired pedal neuron, LP1, (Jerussi and Alkon, 1981) has many of the voltage-dependent conductances found in the Type B photoreceptors. Thus, it may provide a model for analysis of biophysical (Acosta-Urquidi et al., Soc. Neurosci. Abstr., 1983) and biochemical (Neary et al., Soc. Neurosci. Abstr. 1983) mechanisms underlying long-term conditioning-induced conductance changes (Alkon et al., Science, 1982). It may serve as a model for long-term rhythmic changes also, because it shows two quasi-stable levels of membrane potential.

In normal sea water (SW), at its depolarized level (∿ -42 mV) spontaneous action potentials (AP), peak to peak amplitude ∿ 100 mV, occur at ∿ 0.5/S. At its hyperpolarized level (∿ -62 mV) the input resistance (R_{in}) is reduced ∿ 4X to ∿ 10 MΩ and it is silent. In many preparations, a positive current pulse (1 nA, 1S) will shift the membrane potential from the hyperpolarized to the depolarized level and negative current pulses will shift it to the hyperpolarized level, indicating a delicate balance between the currents responsible for maintaining LP1 at each level. It receives EPSP's and a biphasic PSP (E-ILD), which shifts the membrane to the hyperpolarized level. In 0.1 mM Ca^{++} -200 mM Mg^{++} ASW the PSP's are abolished but the shifts in potential may still be evoked and often occur spontaneously.

APs are largely eliminated in 0 Na^{+}SW and completely eliminated by blocking Ca^{++} influx with 1 mM Cd^{++}-0 Na^{+}-SW, indicating mixed Na^{+}/Ca^{++} APs. AP duration (∿ 4-5 ms at 1/2 amplitude) is broadened (∿ 30%) by conditioning depolarization. 5-HT (1 µM) and 8 BTcAMP (1 mM) cause broadening by 50% or more. Pronounced broadening (> 20 ms) is caused by 4-AP (5 mM), which blocks I_K, and RO-1724 (1 mM). Unlike 4-AP, which causes broadening starting at the peak of AP, RO-1724 adds an abrupt shoulder (blocked by 1 mM Cd^{++} SW) to the AP, which is followed by a large post-AP hyperpolarization, suggesting RO-1724 enhances $I_{Ca^{++}}$ and subsequently $I_{K(Ca^{++})}$. These currents may play a role in the observed shifts in membrane level noted above, because RO-1724 can often induce shifts of membrane potential between the depolarized and hyperpolarized levels, staying at each level for 30 S or more.

Dopamine (10 mM) hyperpolarizes LP1, reduces R_{in} in SW or Lo Ca^{++}-HiMg^{++}SW, and under voltage clamp in 0 Na^{+} SW causes an increase in I_A and $I_{K(late)}$. 5-HT (1 µM) and 8 BTcAMP (1 mM) depolarize LP1 in SW or LoCa^{++}-HiMg^{++}SW, and increase impulse activity. Under voltage clamp I_A and $I_{K(late)}$ are reduced by 1 µM 5-HT. At 10^{-5}M and higher doses more complex changes occur, probably involving Ca^{++} as well as K^{+} currents.

147.33 SINGLE CHANNEL RECORDING OF α-BUNGAROTOXIN RESISTANT ACETYLCHOLINE CHANNELS IN DISSOCIATED CNS NEURONS OF DROSOPHILA. C.-F. Wu, S.H. Young, and M.A. Tanouye. Dept. of Zoology, Univ. of Iowa, Iowa City, IA 52242, Dept. of Physiology, Univ. of California, Irvine, CA 92717, and Div. of Biology, California Institute of Technology, Pasadena, CA 91125.

High efficiency of acetylcholine synthesis from choline has been shown in larval CNS of Drosophila (Wu et al., 1983, J. Neurochem. 40:1386). We examined acetylcholine-induced currents in dissociated larval CNS cells in culture with the patch clamp technique. Using fire-polished patch electrode (1 μm opening), seal resistance of greater than 10 GΩ could be obtained with patches of membrane on intact Type I and Type III cells (Wu et al., 1983, J. Neurosci. in press). A class of channels could be activated only when the electrode was filled with saline containing 10-100 nM acetylcholine. The potential inside the patch electrode was held at different levels between -80 to +40 mV. In different patches, the current amplitude was 0.7 to 2.1 pA in outward direction when the membrane potential of the patch was held at 80 mV positive to the resting potential and it became 0.4 to 1.0 pA in inward direction when the membrane potential of the patch was held at 40 mV negative to the resting potential. The current voltage relation of the single channel currents was roughly linear in the voltage range tested, giving a single channel conductance of approximately 9 to 25 pS. Currents reversed at 5-15 mV positive to the resting potential.

These currents were observed only when the patch electrode contained acetylcholine and persisted when the saline in the bath and in the electrode contained 3 μM tetrodotoxin, 25 mM tetraethylammonium, 5 mM 4-aminopyridine and 1 mM Cd^{++}. Moreover, they were not blocked when the cells were pre-incubated with 50 μg/mℓ α-bungarotoxin.

The acetylcholine-activated single channel currents displayed relatively rapid kinetics. The mean channel opening time was 2 to 3.5 msec at 20°C, showing little voltage dependency. The opening frequency varied with acetylcholine concentration and exhibited strong tendency of bursting and clustering. These properties contrast with those of voltage sensitive channels such as the K^+ channels observed in these Drosophila neurons. The single K^+ channels showed strong voltage dependency in opening frequency and mean open time but the occurrence of channel opening was random at a constant holding potential.

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147.34 SINGLE MUSCIMOL-ACTIVATED ION CHANNELS SHOW VOLTAGE-SENSITIVE KINETICS IN CULTURED MOUSE SPINAL CORD NEURONS. G.A. Redmann, J.L. Barker and H. Lecar, Laboratories of Neurophysiology and Biophysics, NINCDS, National Institutes of Health, Bethesda, MD. 20205

Patch clamp recordings were made from the cell bodies of intact neurons cultured for 2-4 weeks from the embryonic mouse spinal cord. The medium in the patch pipette and bathing the cell consisted of Hank's Balanced Salt Solution. Micromolar concentrations of tetrodotoxin and 20mM tetraethylammonium were routinely included in the solution to suppress voltage-dependent Na^+ and K^+ channels. In most experiments 0.5-2.0 micromolar muscimol, an agonist at GABA receptors coupled to Cl^- ion channels, was included in the pipette solution to activate ion channels in the patch. Adequate signal-to-noise was obtained when the sealing resistance was at least 1 Gohm. Inward-going, all-or-none, pA-sized current jumps were recorded in 16 of 20 patches. Similar events were not seen when muscimol was omitted from the patch pipette solution. The inversion potential of the current jumps was in the range -40 to -60mV when the potential across the patch membrane was varied, and in the range -10mV to -20mV when the patch potential was altered in conjunction with an intracellular KCl-filled microelectrode used to inject Cl^- ions. Presumably, the ionic species carrying most of the inward current-signal is Cl^-. Kinetic analysis of current jumps recorded over -50mV to -120mV range of membrane potential revealed that in most cells the distribution of open times could be fitted by two exponentials. The two populations of current jumps had time constants of 1-3 msec and 30-120 msec. Both time constants decreased at hyperpolarized potentials. Closed times could be described by one or two exponentials in different patches. When two closed time constants were present, the amplitude ratio of the relative numbers of fast to slow events usually differed from that for the open times. The time constant(s) of closed times increased slightly at hyperpolarized levels.

These results show that the kinetics of muscimol-activated Cl^- ion currents are sensitive to membrane voltage in such a way that at hyperpolarized potentials less charge flows per unit time. This occurs because the rate of channel activation decreases and the rate of channel closure increases. Thus, on average, fewer channels open for less time at hyperpolarized levels. We conclude that the molecular efficacy with which muscimol activates Cl^- ion currents is sensitive to membrane voltage. How these results relate to synaptically activated Cl^- ion channels remains to be elucidated (Supported by F32NS07044-01).

147.35 IONIC CHANNEL DISTRIBUTIONS AND AXON-GLIA INTERACTIONS IN NERVE FIBERS OF THE CRAYFISH. P. Shrager, J.G. Starkus,* M.-V Lo,* and C. Peracchia.* Department of Physiology, University of Rochester Medical Center, Rochester, NY 14642.

We have studied the influence of the glial cell layer on the ionic microenvironment and on the distribution of ionic channels in giant axons of the crayfish. Dissected single fibers were voltage-clamped using an axial wire technique. The accumulation or depletion of ions in the periaxonal (Frankenhaeuser-Hodgkin) space was measured from zero-current (reversal) potentials following prepulses to activate ionic conductances. This technique quantitatively records concentration changes of the ion under study. During outward K^+ current flow excess K^+ ions accumulate in the periaxonal space, but at a rate far below that expected from the total ionic flux and the measured thickness of the space. At the conclusion of outward current flow the periaxonal K^+ concentration returns to the normal bulk value with a time constant of about 2 ms. The corresponding process in squid axons occurs with kinetics about 25x slower than in the crayfish. The rapid wash-out of periaxonal K^+ in crayfish axons represents a change in driving force that should be reflected in the shape of K^+ tail (repolarization) currents that are recorded at the conclusion of a depolarizing pulse. We found that at potentials negative to about -40 mV these tail currents generally exhibit two time constants, and may pass through a maximum before approaching a final asymptotic level. The initial rapid phase may in part reflect depletion of excess K^+.

In an attempt to compare the geography of Na^+ channels in the axon membrane with that of K^+ channels, we measured changes in periaxonal concentrations due to Na^+ ions moving through Na^+ channels. Axons were both internally perfused and voltage clamped. Inactivation of Na^+ channels was blocked by a brief exposure to tannic acid in the internal perfusate. After reversal of the Na^+ concentration gradient we were able to demonstrate accumulation and wash-out of excess Na^+ ions in the periaxonal space. Characteristics of these processes appeared similar to those of K^+.

We have examined crayfish glial ultrastructure both in thin sections and after freeze fracture. Layers of connective tissue and extracellular fluid alternate with thin layers of glial cytoplasm. A membranous tubular lattice in the adaxonal glial layer communicates with both the periaxonal space and with the first band of connective tissue. These tubules may provide a pathway for rapid diffusion of excess ions from the axon surface. In these neurons, Na^+ and K^+ channels may thus be located preferentially in regions of the axon membrane with relatively unrestricted access to bulk extracellular fluid, regions defined by glial cell structure. Supported by NIH Grant NS-17965.

147.36 SPONTANEOUS BURSTING PACEMAKER ACTIVITY IN AN IN VITRO PREPARATION OF THE ELECTROSENSORY LATERAL LINE LOBE (ELLL). W.B. Mathieson*, L. Maler, Dept. of Anatomy, University of Ottawa, Ottawa, Ontario K1H 8M5.

Tissue slices (300μM) through the ELLL and caudal lobes of weakly electric fish (gymnotidae) remained viable for up to 8 hrs in vitro. Extracellular recordings from the pyramidal cell layer revealed spontaneous activity of two characteristic patterns: 1) bursting pacemakers discharging very large bursts typically consisting of several hundred spikes, burst durations of 10 sec and interburst interval of 10 sec; 2) smaller, short interval bursting with 2-20 spikes per burst and variable interburst intervals of <1sec. Perfusion with Ca^{++}free CSF or Verapamil (100μM) abolished spontaneous activity presumably via blockade of chemical synaptic transmission or direct influence upon Ca^{++}flux across dendritic membranes. Atropine (150μM) also blocked spontaneous activity while eserine (5μM) caused a gradual increase in bursting frequency with intermittent paroxysmal discharges suggesting muscarinic, cholinergic input may contribute to the bursting behavior. Approximately 50% of bursting pyramidal cells could be driven monosynaptically by stimulation of their afferent fibers which evoked single action potentials of short latency (1-5msec). At higher frequency stimulation (>20Hz) the phase locked stimulus response was accompanied by inhibition of spontaneous bursting. The other class of neurons recorded from could not be stimulated but were inhibited from bursting by up to 100%. Nearly all of the units that responded monosynaptically also displayed the large long interval type burst pattern while the non-drivable units displayed small, short interval bursts. After recording bursting pacemaker activity, the slices were fixed for electron microscopy. Even after 5 hrs in vitro the ultrastructure of the ELLL was similar to in vivo preparations; occasional degenerating fibers and subtle changes in the disposition of some axon terminals were seen. These data suggest that basilar and non-basilar pyramidal cells described previously (Maler et al. '82, J. Comp. Neurol. 195: 87) are spontaneously active in vitro and display different patterns of bursting behavior. These patterns depend on cholinergic input to the ELLL which has been demonstrated in the dorsal molecular layer at the level of the pyramidal cells apical dendrites (Maler et al., '81, Brain Res. 226: 320). Supported by the Medical Research Council of Canada, Grant # MA 6027.

148.1 SINGLE CALCIUM-CHANNEL CURRENT MEASUREMENTS FROM BRAIN SYNAPTOSOMES IN PLANAR LIPID BILAYERS. Mark T. Nelson* (SPON: B.K. Krueger). Dept. of Physiology, University of Maryland School of Medicine, Baltimore, MD 21201

Calcium entering cells through voltage-dependent channels activates neurotransmitter release, muscle contraction, and many other physiological processes. I have been able to incorporate Ca^{2+}-channels (using Ba^{2+}, Ca^{2+}, and Sr^{2+} as the current carriers) from rat brain synaptosomes (a preparation enriched in pinched-off and resealed presynaptic nerve terminals) into planar lipid bilayers. Synaptosomes were made from rat whole brain and from rat median eminence, an area of the brain rich in nerve terminals. Both single channel currents and macroscopic (channel-ensemble) currents were strongly voltage-dependent.

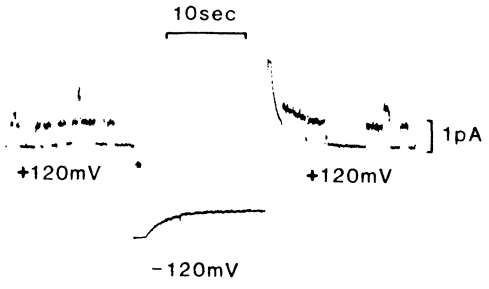

Figure: Voltage-dependence of single Ca^{2+}-channels from synaptosomes. Solution: symmetric 250 mM $SrCl_2$. At -120 mV, all channels were closed. Bilayer: PE:PS, 100 μm dia., 100 Hz.

The voltage across the membrane affected the open time of the channels. However, voltage did not appear to affect single channel conductance. In 250 mM $BaCl_2$, $CaCl_2$, and $SrCl_2$, the single channel conductances were about 10 pS, 5 pS, and 5 pS, respectively, suggesting that relative permeabilities of the channel to Ba:Ca:Sr are 2:1:1. These channels may contribute to voltage-dependent Ca^{2+}-influx into presynaptic nerve terminals.

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148.2 WHOLE-CELL PATCH CLAMP STUDY OF CALCIUM CURRENTS IN ADRENAL CHROMAFFIN CELLS. T. Hoshi*, J. Rothlein and S. Smith*. Dept. of Physiol. and Dept. of Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06510

Ca^{++} influx through voltage-dependent Ca^{++} channels regulates many important cellular events, including exocytosis of secretory vesicles. Ca^{++} current (I_{Ca}) in primary cultures of calf and adult bovine adrenal chromaffin cells was studied using the whole-cell patch clamp technique. I_{Ca} was separated from other ionic currents by using zero Na^+, TEA-Asp and/or Cs-Asp in the internal solution. The internal Ca^{++} concentration was buffered to $1x10^{-8}$ M with 11 mM EGTA. The peak current magnitudes ranged from 35 pA to 100 pA in 5 mM Ca^{++}. I_{Ca} shows the following four phenomena:

1) The internal K channel blockers affects the I-V relation of the peak I_{Ca}. When TEA^+ (140 mM) but not Cs^+ was present inside, the peak I_{Ca} occurred between -10 and -5 mV. When both TEA (20 mM) and Cs^+ (120 mM) were present inside, the peak I_{Ca} occurred between +5 and +10 mV.

2) I_{Ca} in different cells does not have the same time-course. I_{Ca} in some cells shows apparent inactivation: the current reached the peak value and then declined to 90 to 95% of the peak value during a 60 msec depolarizing pulse (inactivating type I_{Ca}). In other cells, however, no inactivation was observed: the current was still increasing at the end of a 60 msec pulse (non-inactivating type I_{Ca}).

3) The time-course of the I_{Ca} changes during wash-out in some cells. I_{Ca} in whole-cell patch-clamped cells has been found to disappear gradually in this preparation and in others (wash-out). In our experiments, I_{Ca} disappeared within 10 to 15 min after achieving the whole-cell configuration when pipettes with 1 μm opening were used. In those cells that originally had the non-inactivating type of I_{Ca}, the time-course of the current changed from the non-inactivating type to the inactivating type during the wash-out.

4) The non-inactivating type of I_{Ca} facilitates. Two step-depolarization pulses separated by 30 to 40 msec were applied. I_{Ca} magnitudes in the second pulse were greater than those without a prepulse when the first pulse potential was more positive than 0 mV. The facilitated I_{Ca} during the second pulse was of the inactivating type. Facilitation was not observed in cells that already had the inactivating type of I_{Ca}.

Facilitation in this preparation may represent an intrinsic property of Ca^{++} channels, and could be responsible for synaptic facilitation observed in neurons.

148.3 MUTATIONAL ALTERATIONS INDICATE CHANGES IN THE Ca^{2+}-CHANNEL STRUCTURE IN PARAMECIUM. Y. Saimi* and R. D. Hinrichsen* (SPON: A. Clark). Lab. of Molecular Biology, Univ. of Wisconsin, Madison, WI 53706

Two of the goals in the genetic dissection of the membrane-excitation process in Paramecium are to identify the channel proteins and to understand their molecular mechanisms. Of the seven ion channels recognized, the Ca^{2+} channel has been most extensively studied and is known to be inactivated by internal Ca^{2+} that enters after the channel is opened by depolarization. We have virtually isolated the Ca^{2+} current by inhibiting the K^+ currents with Cs^+ and TEA^+. In the wild type, this Ca^{2+} current peaks within $\sim$5 msec and decays to a steady level by $\sim$20 msec during a long voltage stimulation. This current inactivates exponentially in a voltage-dependent manner; the higher the step, the larger the time constant. The steady level after the inactivation is also dependent on the voltage. Because there is little inactivation of the Ba^{2+} current through this channel, there is little directly voltage-dependent inactivation.

We have recently isolated eight mutants which have stronger behavioral responses to certain stimuli and found that they fall into one complementation group. In such mutants, the inactivation of the Ca^{2+} current is much slower and less complete than that of the wild type. The mutant channels also have a shifted voltage sensitivity (up to 10 mV more negative). Above two changes in the inactivation are not simple parallel shifts along the voltage axis. Therefore, the mutations affect at least two properties of the Ca^{2+} channels: the inactivation and the voltage sensitivity.

We have also found that the slow Ca^{2+}-activated currents of the mutant are much larger than those of the wild type. These currents indicate that there is more internal Ca^{2+} in the mutant. Consequently, the poor inactivation of the mutant Ca^{2+} current cannot be attributed to a mutationally increased Ca^{2+}-removal mechanism. It appears that the mutation affects the Ca^{2+}-channel structure or its immediate environment.

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148.4 PROPERTIES OF CALCIUM CHANNELS IN DORSAL ROOT GANGLION CELLS. Martha C. Nowycky, Bruce P. Bean*, and Richard W. Tsien*. Depts. of Neuroanatomy and Physiology, Yale Univ. Sch. Med., New Haven, CT 06510, and Dept. of Physiology and Biophysics, Univ. of Iowa, Iowa City, IA 52242.

Dorsal root ganglion (DRG) cells were prepared from 8-12 day old chick embryos and grown in tissue culture conditions for a few days to several weeks. Calcium currents were studied using the whole-cell patch clamp technique combined with fluctuation analysis (Hamill et al., 1981; Sigworth, 1980). Non-Ca channel currents were suppressed by: (1) replacing external Na and K with tetraethylammonium, (2) replacing internal K with Cs, (3) including 10 mM EGTA in the internal solution. In the presence of 10 mM Ca_o, inward currents began to activate at -40 mV, and reached a maximum amplitude at +20 mV; since they were blocked by verapamil and not by TTX, they were taken as calcium channel currents.

Noise analysis was used to estimate the number of functional calcium channels (N), single channel current amplitude (i), and probability of channel opening (p). At 0 mV, i was 0.16 pA (n=9) which is slightly larger than the value of i we have obtained from rat heart cells in culture (i=0.11, n=12). N was estimated for eight cells. Measuring the cell capacitance and assuming 1 $\mu F/cm^2$, we calculated an average Ca channel density of 8.7±2.9 channels/μm^2 (mean ± s.e.m.). Near +20 mV, p reached values between 0.5 and 0.7 in 6 cells. These values are somewhat higher than our estimates of channel density and p in heart cells.

A further similarity between calcium channels in DRG cells and in heart cells is that low concentrations of external Ca blocked Ba currents. This suggests that the single-file, multi-ion model of calcium channels which has been developed for heart cells (Hess & Tsien, this volume) may be applicable to neurons as well.

Ca currents in heart cells and DRG cells were strikingly different in their rate of inactivation. In 10 mM Ca_o, neuronal inward currents did not decay to half during 500 msec pulses. The slow decline was unchanged when Ca_o was replaced by Ba_o. In heart cells, inward currents decayed to half within 50 msec; inactivation was greatly slowed by replacing Ca_o with Ba_o. These results raise questions about the importance of Ca channel inactivation in DRG neurons. It may be that Ca-dependent Ca channel inactivation plays a less important role than Ca-activated K current in terminating Ca action potentials in these cells.

In two preliminary experiments we found that Ca currents were decreased by (D-Ala^2,D-Leu^5)-enkephalin. (Supported by NIH grants NS17868 and HL13306.)

148.5 CALCIUM CHANNEL PERMEABILITY TO DIVALENT AND MONOVALENT CATIONS. A MODEL WITH TWO ION BINDING SITES AND ION-ION INTERACTION. P. Hess* and R. W. Tsien* (SPON: R. J. J. Benson). Department of Physiology, Yale University, New Haven, CT 06510.

Calcium channels select strongly for Ca^{2+} but are also capable of transferring ions at rates of millions/sec. To study the mechanism of ion permeation, we recorded Ca channel currents in single heart cells from adult guinea pig ventricles, with suction pipettes for voltage clamp and internal dialysis. Measurements of the reversal potential of the D600-sensitive current (E_{rev}) with 10 mM Ba outside and 150 mM monovalent cation inside gave the following selectivity sequence for the monovalents: Na > K > Cs. Similarly, with 150 mM Cs inside and various divalent cations outside, measurements of E_{rev} led to the following sequence: Ca > Sr > Ba >> Mg. External Ca (Ca_o) at submillimolar concentrations strongly inhibits Ba currents. In addition, an anomalous mole fraction effect was seen in mixtures where (Ca_o+Ba_o) = 10 mM: the current in the mixtures can be substantially smaller than that in either 10 mM Ca_o or 10 mM Ba_o. This is a property of open channels since it holds for unitary Ca channel current as well as for whole-cell current.

These results suggest that Ca channels contain more than one ion binding site. We formulated a single-file energy barrier model for the Ca channel with two internal wells and repulsion between ions in doubly occupied channels. The energy barriers and wells for each ion were adjusted to fit (1) measured reversal potentials, (2) measured values for elementary currents with only Ca_o or Ba_o, (3) behavior in mixtures of Ca_o and Ba_o. A satisfactory fit to the available data was obtained with the following energy profiles: equal entry and exit barriers for Ca, Sr and Ba but energy wells which increase in depth from Ba to Sr to Ca. (The well depth for Ca can be expressed as an equivalent equilibrium binding constant of 3×10^6 M^{-1}, quite comparable to that of known high-affinity Ca-binding sites on proteins). The model assigns uniformly shallow wells to all the monovalents (consistent with non-specific binding of these ions to the two sites) with barriers increasing in height from Na to K to Cs.

Thus, the model channel selects for monovalent ions by a conventional "selectivity filter" barrier mechanism. The situation for the divalent ions is quite different: selectivity occurs at the energy wells. The deeper the well the higher the selectivity (judged by E_{rev}) but the smaller the elementary current. Throughput rates are high, even for Ca, because of strong repulsion between ions. Under physiological ionic conditions the channel is occupied almost continually because of its high affinity for Ca; at 0 mV more than 90% of its current is carried by Ca despite the presence of Na and K at much higher concentrations.

148.6 β-ADRENERGIC MODULATION OF THE NUMBER OF FUNCTIONAL CALCIUM CHANNELS IN FROG HEART CELLS. B.P. Bean*, M.C. Nowycky and R.W. Tsien*. (SPON: T.G. Burrage). Departments of Physiology and Neuroanatomy, Yale University, New Haven, CT 06510, and Department of Physiology & Biophysics, Univ. Iowa, Iowa City, IA 52242.

Adrenergic neurotransmitters control the rhythm and strength of the heartbeat through a powerful modulatory effect on voltage-gated calcium channels. The mechanism of the increase in Ca conductance at the level of single channel molecules is not entirely clear. The most popular hypothesis, an increase in the number of functional channels (N), has not been demonstrated so far. Studies of unitary Ca channel activity have shown that isoproterenol increases p, the probability of a channel being open (Reuter et al.).

To look for possible changes in N, we studied Ca channel activity in frog ventricular cells with whole-cell recordings (Hamill et al.). K channel currents were eliminated by external TEA and internal Cs and EGTA. With depolarization to +20 mV in 10 mM Ba_o, 0.5 μM isoproterenol increased the Ca channel current by a factor of 6.2 ± 0.6 (mean ± s.e.m., n = 25 cells). Ensemble fluctuation analysis (Sigworth) allowed changes in N and p to be distinguished. Isoproterenol increased variance (var) as well as mean current (I). When var-I plots were fitted by the parabolic function $var=iI-I^2/N$, it appeared that isoproterenol increased N from 7,870 ± 2,410 to 44,480 ± 16,600 and p from 0.46 ± 0.04 to 0.51 ± 0.06 (n=4). Thus, at +20 mV, the predominant change is in N, not p. The unitary current (i) was not significantly changed, in agreement with earlier single channel recordings. Since E_m was under voltage clamp, and the reversal potential E_{rev} remained the same, we conclude that isoproterenol leaves single channel conductance $\gamma=i/(E_m-E_{rev})$ unchanged.

Single channel recordings from cell-attached patches gave results consistent with the fluctuation analysis. Isoproterenol greatly enhanced the mean current, and also strongly increased the maximal number of simultaneous unitary openings.

p increased only slightly at +20 mV. However, we found that the β-adrenergic enhancement of Ca channel current was more than twice as big at -40 mV than at +20 mV. Since the number of functional Ca channels is presumably voltage-independent, the implication is that p(-40 mV) more than doubled. One possibility is that the p(V) relationship is merely displaced to more negative voltages. A disproportionately large increase in Ca channel activity at more negative potentials might be especially important for sympathetic modulation of cardiac pacemaker activity.

148.7 ***NITRENDIPINE BINDING TO TWO CALCIUM CHANNEL SUBTYPES IN MOUSE BRAIN.** **A. de Bruyn Kops*, R.B. Rogart*, and V. Dzau*. Depts. of Neuroscience and Neuropathology, Children's Hospital and Dept. of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115 (SPON: A. Heller)**

Tritium-labeled nitrendipine (*NTP), a new 1,4-dihydropyridine derivative which has proved to be a potent calcium channel blocker, was used to label binding sites in mouse brain homogenates, and the results compared to those in rat cardiac and smooth muscle homogenates. Other groups have reported high-affinity binding of *NTP to brain membrane, but the relationship between the NTP binding site and the calcium channel has remained largely unestablished.

Receptor binding studies over the range of 1 - 5 nM yielded biphasic specific *NTP binding in brain membrane, indicating two distinct populations of receptors for nitrendipine. Non-linear least squares analysis indicated a high-affinity K_d value of 0.3 - 0.5 nM, with a corresponding uptake (B_{max}) of 3 - 10 fmoles/mg wet weight, in approximate agreement with other published reports.

However, in addition to high-affinity *NTP binding, we observed a substantial component of low-affinity binding sites in brain. As these sites could not be saturated over the *NTP concentration range (0 - 5 nM) studied, characterization of the low-affinity binding sites depended upon simultaneous analysis of competitive displacement of *NTP by unlabeled NTP, and yielded approximate parameters of K_d = 40 - 75 nM and B_{max} = 75 - 200 fmoles/mg wet.

From our studies in rat cardiac and smooth muscle, we have concluded that the high-affinity binding site (K_d = 0.25 - 0.5 nM) corresponded to calcium channels in smooth muscle, while the low-affinity channels (K_d = 40 - 75 nM) corresponded to channels in heart, since the K_d values were in good agreement with physiological estimates of nitrendipine dose-response effects in these tissues. Comparison with brain data suggests that the predominant calcium channel in brain membrane may correspond with the low-affinity cardiac *NTP binding site. Physiological studies and flux measurements of nitrendipine effects in nerve tissue will be required to firmly establish correlation between binding sites and physiologically relevant calcium channels.

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148.8 THE BINDING OF NIFEDIPINE TO THE SYNAPTOSOMAL [^{3}H]-NITRENIPINE RECEPTOR DOES NOT CORRELATE WITH ITS INTERACTION WITH SYNAPTOSOMAL Ca^{++} CHANNELS. T. Turner* and S.M. Goldin. Dept.of Pharmacology, Harvard Medical School, Boston, MA 02115.

Nifedipine and nitrendipine are dihydropyridine derivatives that are known to block the cardiac voltage-sensitive Ca^{++} channel. Saturable binding of [^{3}H]-nitrendipine to rat brain synaptosomes prepared by the method of Hajos (Brain Res. (1975) 93,485) was observed. The K_D value obtained was 0.66 nM, with a receptor concentration of 140 fmole/mg protein. By competition for this receptor, the K_i value for nifedipine was determined to be 5.2 nM. These results are in qualitative agreement with the findings of Gould, Murphy and Snyder (PNAS (1982)79,3656) as well as several other laboratories that have studied [^{3}H]-nitrendipine binding to brain tissue.

Under the same conditions as used in the above binding studies, $^{45}Ca^{++}$ flux into synaptosomes, apparently mediated by a voltage-sensitive Ca^{++} channel was detected by a modification of the method of Nachsen and Blaustein (Mol. Pharmacol. (1979)16,579). High levels of external K^+ in the incubation medium (75 mM vs. 5 mM for control) stimulated synaptosomal $^{45}Ca^{++}$ accumulation 2-3 fold. This flux could be blocked by the Ca^{++} channel blocker verapamil (IC_{50} ~ 100 μM) and by inorganic blockers such as Co^{++} (IC_{50} = 0.25 mM). However, nifedipine had no significant effect on K^+-stimulated $^{45}Ca^{++}$ uptake at concentrations as high as 0.3 mM.

These results indicate that the [^{3}H]-nitrendipine binding site is not the site that is capable of mediating the blocking effects of dihydropyridines towards this synaptosomal voltage-activated Ca^{++} channel. This synaptosomal Ca^{++} channel may be, by pharmacological criteria, distinct from the cardiac Ca^{++} channel.

Supported by grants to SMG by the NIH (NS 15236), the McKnight Foundation, and the Searle Scholars Program.

148.9 INACTIVATION OF PERSISTENT INWARD CURRENT MEDIATES POST-BURST HYPERPOLARIZATION IN APLYSIA BURSTING PACEMAKER NEURONS. Richard H. Kramer* and Robert S. Zucker
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The ionic conductances responsible for endogenous bursting activity in molluscan neurons have been extensively studied. A voltage-dependent Ca^{2+} current which is activated well below threshold leads to gradual membrane depolarization. It has been thought that accumulation of intracellular Ca^{2+} during the ensuing burst activates Ca^{2+}-dependent K^+ current ($I_{K(Ca)}$), which hyperpolarizes the cell and terminates the burst. We report here that the long post-burst hyperpolarization and corresponding slow outward current under voltage clamp are not due to increased $I_{K(Ca)}$, but probably result from a Ca^{2+}-induced decrease in a persistent Ca^{2+} conductance.

Aplysia abdominal ganglion bursting neurons L2-L6 which had been axotomized were used in all experiments. These cells exhibit slow inward and outward tail currents following voltage clamp depolarizations from various holding potentials (V_h) up to a test potential (V_t) of 0mv. These currents have similar time courses to the depolarizing afterpotential and after-hyperpolarization which follow spontaneous bursts. The slow outward tail current does not reverse at V_h between -35 to -90mv. Increasing extracellular $[K^+]$ does not decrease the slow outward tail current over this range, although it shifts the reversal potential of currents elicited by intracellular Ca^{2+} injection. When these cells are current-clamped below the measured E_K, a step depolarization elicits a burst of spikes, followed by a long post-burst hyperpolarization. The slow outward tail current is not decreased by 100mM TEA, although $I_{K(Ca)}$ elicited by Ca^{2+} injection is totally eliminated.

The slow outward tail current is supressed by values of V_t which result in minimal Ca^{2+} entry (near E_{Ca}) and is blocked by extracellular use of Ca^{2+} antagonists and by intracellular injection of EGTA. The best hypothesis consistent with these results is that the slow outward tail current and the post-burst hyperpolarization are due to a Ca^{2+}-induced decrease in a persistent Ca^{2+} current.

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148.10 TIMED APPEARANCE OF CALCIUM AND POTASSIUM CURRENTS IN STARFISH OOCYTES DURING OOGENESIS. W.J. Moody* and M.M. Bosma* (SPON: M.S. Letinsky). Jerry Lewis Research Center, UCLA, Los Angeles, CA, 90024 and Department of Zoology, University of Washington, Seattle, WA 98195.

Oocytes of the starfish, L. hexactis develop in a two-year oogenesis cycle. By July of the second year, oocytes have attained 90% of their full size and are held in the female for 6 - 8 months awaiting meiotic maturation and spawning, which occur immediately before fertilization. Oocytes which have completed oogenesis display action potentials in response to depolarization, and under voltage-clamp show three major ionic currents: an inward Ca current, a transient outward K current (A current), and an inwardly rectifying K current. It has been shown previously (Moody & Lansman, PNAS, in press) that when meiotic maturation is carried out in vitro in single oocytes by exposure to the normal hormone 1-methyladenine (1-MA), each of these currents is affected. During 1 hour of 1-MA exposure, the Ca current gradually increases in amplitude while both K currents are reduced in amplitude. In addition, 1-MA causes a substantial (60%) decrease in total membrane capacitance, probably indicating a decrease in surface area caused by retraction of microvilli.

We have now examined the electrical properties and hormone responses of oocytes during the final six months of oogenesis. Full size oocytes studied in July and August were found to have A currents and inwardly rectifying K currents of normal amplitude; membrane capacitance was also similar to that in oocytes which have completed oogenesis (apparent specific capacitance 3.5 uF/cm^2 However, Ca currents were small or completely absent in these oocytes, averaging about 15% of those found at the end of oogenesis. Furthermore, neither the ionic currents nor the membrane capacitance showed any detectible response to 1-methyladenine. In Sept. - Dec., Ca currents have attained their final amplitude and virtually all oocytes show the characteristic electrophysiological changes in response to 1-MA. The appearance of full-size Ca currents and normal hormone responses coincides in time with the migration of the nucleus from a central to a peripheral position within the oocyte. We have no evidence for a causal relationship between the appearance of hormone response, the appearance of Ca currents, and nucleus migration at this stage of oogenesis, but it is notable that the earliest detectible effect of hormone application in starfish oocytes is a transient increase in cytoplasmic Ca activity, and a major end point of hormone action is the breakdown of the nucleus and the resumption of meiosis.

148.11 CALCIUM ACTION POTENTIALS IN PRIMARY SENSORY NEURONS OF LAMPREY. J.P. Leonard and W.O. Wickelgren. Dept. of Physiology, University of Colorado Medical School, Denver, Colo. 80262.

Calcium action potentials (CaAPs) recorded from the somata of vertebrate neurons may provide a model for the less accessible calcium influx involved in transmitter release at the nerve terminal. Somatic CaAPs may thus be subject to modulations underlying presynaptic inhibition and facilitation. For these reasons we have pursued the characterization of CaAPs in dorsal cells, primary mechanosensory neurons of the lamprey. CaAPs revealed by K^+ channel blockers in dorsal cells were first described by Bookman and Selzer, 1980 (Soc. for Neurosci. Abstr.). We confirm that Ca^{2+} is the current carrier for these APs since they are: (1) elicited in 10^{-6} M TTX and when all Na^+ in the bath is replaced by choline or tetraethylammonium (TEA), (2) reversibly blocked by removing Ca^{2+} from the saline, (3) blocked by Cd^{2+} or Co^{2+}, and (4) elicited when Ca^{2+} is replaced by Ba^{2+}. In addition, the overshoot of these APs increases 18 mV for a 4 fold rise in external Ca^{2+}; the minimum value predicted on the basis of the Nernst equation is 17 mV. The effects of the K^+ channel blockers, TEA and 3,4-diaminopyridine (DAP), were examined. DAP (0.1 mM) caused an increase in AP duration (to 13.4 ± 3.4 ms) which was only slightly greater at 1.0 mM (15.2 ± 1.6) and 5.0 mM (17.9 ± 2.0). TEA caused a large additional increase in duration at 10.0 mM (91.1 ± 14.3 ms) and 15.0 mM (145.9 ± 13.8) reaching a maximum when all Na^+ is replaced by TEA (478.2 ± 70.3). It is clear that E_{K^+} is a major determinant of a hyperpolarizing afterpotential which follows CaAPs in these cells, since the amplitude of the undershoot varies linearly with the log of external K^+. Quite often the undershoot is obscured by "process spikes". These potentials resemble somatic CaAPs in the abruptness of repolarization but are only 1/10 as large in amplitude. Although their durations vary, they can be 2-3 times as long as the soma CaAP. In some cells "process spikes" can be elicited at a threshold below that for the somatic CaAP. Preliminary experiments on the neurotransmitter sensitivity of dorsal cell CaAPs suggest that at least one putative neurotransmitter in the lamprey central nervous system, GABA, prolongs the duration while lowering the overshoot of the CaAP. Supported by NIH Grant NS 09661.

148.12 CA^{2+} OVERLOAD: ALTERED ELECTRICAL ACTIVITY AND MITOCHONDRIAL ULTRASTRUCTURE IN CULTURED CARDIAC CELLS. M. C. Kitzes*, L.-H. Liaw* and M. W. Berns*. (SPON. H. Koopowitz). Dept. of Developmental and Cell Biology, University of California, Irvine, CA 92717.

Cultured neonatal myocardial cells underwent progressive membrane hyperpolarization during continuous exposure to isoproterenol (ISO). ISO mediates an increased Ca^{2+} influx into heart muscle. If excessive, high energy phosphate breakdown and known cellular ultrastructural alterations result. Cultured cells showed similar changes in mitochondrial ultrastructure during the period of altered electrical activity. Continuous exposure of neonatal cultured myocardial cells to ISO (5μM, 10μM, 20μM, 30μM) resulted in consistent, time dependent alterations of spontaneous electrical activity. These changes were: (1) a 30 to 40% increase in discharge frequency for a period of time that varied inversely with ISO concentration. The rate and strength of mechanical contraction increased during this period of augmented discharge frequency. Resting membrane potentials remained at normal levels (ISO 5um and 10μm) or at slightly hyperpolarized levels (ISO 20μM and 30μM). However, with continued exposure to ISO a subsequent phase of marked and consistent progressive hyperpolarization ensued. As the membrane polarized, the frequency of action potential discharge progressively decreased to below the control frequency. Action potentials showed a marked afterhyperpolarization, and interspike intervals became prolonged. In 45% of the cells the frequency of action potential discharge declined to nearly zero or zero when the resting membrane potential had stabilized at a hyperpolarized level. Spontaneous action potentials occurred occasionally and were accompanied by a strong mechanical contraction. In the remaining cells (55%), after the decline in the normal discharge rate, 20 to 25 min of additional exposure to ISO resulted in the development of a bursting pattern of action potential discharge. Discharge of spontaneous action potentials during interburst intervals ceased and interburst intervals became more prolonged as the cells stabilized at a hyperpolarized level. Eventually, discharge activity ceased entirely.

Our concurrent electrophysiological and electron microscopic findings with ISO indicate that prolonged mitochondrial exposure to increased $[Ca^{2+}]_i$ eventually diminishes their buffering capacity due to intramitochondrial Ca^{2+} overload and impairment of their normal metabolism. The loss of mitochondrial buffering capacity and the increase in $[Ca^{2+}]_i$ to greater than physiological levels lead to a significant cell hyperpolarization. We propose that the observed polarization may be due to a Ca^{2+} activated K^+ conductance. (Supported by NIH R01 HL 15740.)

149.1 MEMBRANE PROPERTIES OF PRIMARY AFFERENT AXONS: A STUDY BY INTRACELLULAR RECORDING. T. Hashiguchi and A.L. Padjen, Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec and Department of Physiology, Tokyo Medical College, Tokyo 160.

A method was developed to study intracellular potentials from large myelinated axons in dorsal roots of isolated frog spinal cord superfused with normal amphibian Ringer at 14°C (Padjen & Hashiguchi, Can. J. Physiol. Pharmacol. 61 (1983). Using KCl or K sulfate filled microelectrodes, it was found that these axons (conduction velocity > 10 m/s; n > 40) show an unexpected and unreported nonlinearity of their voltage-current curves (V-I; obtained by standard "current-clamp" technique). An outward rectification was present from -90 mV into the depolarizing range of membrane potential (resting membrane potential -75 mV). The V-I relationship was linear from -90 to -150 mV (effective resistance 20-200 M Ω). The longest time constant of a hyperpolarizing electrotonic potential often exceeded 50 ms. We have modelled these data using standard Hodgkin-Huxley equasions. The non-linear V-I relationship could be fairly well simulated by including parameters of a slow potassium current, recently described in frog nodes (Dubois, J. Physiol. 318 (1981) 297). Although a deactivation of the slow K conductance can contribute to slow charging phase of a hyperpolarizing electrotonic potential, the observed ETP is much slower than the calculated one. Other capacitative components, such as internodal membrane, may be responsible for the slow time course of an electrotonic potential in primary afferents axons (Barrett & Barrett, J. Physiol. 323 (1982) 117).

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149.2 BIOPHYSICAL PROPERTIES OF MEMBRANES FROM DIFFERENT AGE GROUPS OF MICE. W. G. Wood, R. Strong, H. J. Armbrecht* and R. W. Wise*. GRECC, VA Med. Ctr., and St. Louis Univ. Sch. of Med., St. Louis, MO 63125.

Changes in membranes (e.g., membrane order, lipid composition) have been suggested to be basic factors in the aging process. These age-related changes in membranes might affect functions such as enzymatic activity, release and uptake of neurotransmitters and receptor binding. The purpose of the experiments reported here was to determine if aging was associated with changes in membrane order as measured by electron spin resonance (ESR) and changes in lipid composition. Synaptic plasma membranes (SPM), brain microsomes and erythrocyte membranes were examined. Membranes were prepared from three different age groups of C57BL/6NNIA male mice (3-5 mo; 11-13 mo; 22-24 mo). For the ESR experiments, membranes were spin labeled with the 5-nitroxide stearic acid spin label. The 5-nitroxide spin label reports the motion of the acyl chains of phospholipids near each surface of the membrane lipid bilayer. SPM and erythrocytes also were labeled with the 16-nitroxide stearic acid spin label that reports motion deeper in the membrane. ESR spectra were recorded at 37°C. Lipid extracts from whole brain homogenate, SPM, brain microsomes and erythrocyte membranes were used for analysis of cholesterol and phospholipid content. Membrane order as measured by the 5-nitroxide spin label did not differ significantly among membranes from the three different age groups. Erythrocyte membranes were the most ordered followed by SPM and brain microsomes. Age differences were not observed with the 16-nitroxide spin label. There was a significant increase in cholesterol content with increasing age. This increase was observed for SPM, brain microsomes and whole brain homogenate. Phospholipid content also increased with age for microsomes and SPM. Membrane order did not differ with age using the 5- and 16-nitroxide spin labels. These spin labels report on the membrane order of the bulk lipid environment and represents an average of the fluid and gel phase lipids. Age differences in membrane order may be present in specific areas of the membrane, e.g., the lipids surrounding membrane proteins but not be detected by these spin labels. Lipid composition did significantly differ with age and may influence the phase transition of the membrane which, in turn, could affect membrane-bound proteins.

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149.3 TWO POOLS OF CHOLESTEROL IN ACETYLCHOLINE RECEPTOR-RICH MEMBRANES FROM TORPEDO. W. Liebel*, L. Firestone*, L.M. Braswell*, K.W. Miller* (SPON: Roger Brett). Departments of Pharmacology and Anaesthesia, Harvard Medical School and Massachusetts General Hospital, Boston, MA 02114.

The local membrane environment of the acetylcholine (ACh) receptor-channel complex may be critical to its physiological function. Since cholesterol is a major constituent of electroplax membrane isolated from Torpedo californica, the role of cholesterol in modulating the functional properties of the receptor was examined by liposome-mediated depletion of receptor-rich membrane fragments. Using small unilamellar phosphotidylcholine (PC) vesicles, incubation conditions were established which allowed cholesterol depletion without phospholipid back exchange or fusion with Torpedo membranes. This was verified using egg PC vesicles with trace algal ^{14}C-PC(Sp. Act. 1.5 Ci/mmol). Following incubation, cholesterol-depleted membranes or suitable controls were separated from liposomes by velocity sedimentation centrifugation on sucrose step gradients. The optimal membrane-liposome incubation conditions (3-hr. with 10-fold excess of liposomes) achieved a maximum of 40% (C:P molar ratio) cholesterol depletion. The remaining cholesterol could not be depleted either by longer incubations or multiple sequential depletions, as confirmed both directly by chemical assay and by use of ^{3}H-cholesterol as a radiotracer. This limiting cholesterol depletion was accompanied by a significant alteration in membrane fluidity as measured by electron spin resonance spectroscopy. For example, in one preparation of membranes which had an order parameter (S) of 0.683 ± 0.002, depletion of 29% of the cholesterol reduced S by 4.7%. This marked structural change was not accompanied by change in any of the measured receptor equilibrium binding parameters ($K_{\frac{1}{2}}$, n_{Hill}) or kinetics. Recently, Ellena et al. (Biophys. J. 41:205a, 1983) have shown that cholesterol has a four-fold preference for the lipid annulus surrounding the ACh receptor, relative to the bulk membrane where our spin probe "reported" from. This is consistant with our observation of two pools of cholesterol, and suggests that there exists a cholesterol-rich region of lipid around the receptor which may serve to buffer it from changes in bulk fluidity.

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149.4 DIRECT SPECTROPHOTOMETRIC DETECTION OF CESIUM FLUX IN MEMBRANE VESICLES. STOPPED-FLOW MEASUREMENTS OF ACETYLCHOLINE RECEPTOR-MEDIATED ION FLUX. Jeffrey W. Karpen* and Elena B. Pasquale* (SPON:G.P. Hess) Section of Biochemistry, Molecular and Cell Biology, Cornell University, 270 Clark Hall, Ithaca New York 14853

A spectrophotometric stopped-flow method to measure ion flux in membrane vesicles in the msec to min time region has been developed. The technique is based on fluorescence quenching of an entrapped fluorophore (anthracene-1,5-disulfonic acid) by Cs^{+}. The method has been applied to the measurement of acetylcholine receptor-mediated ion flux in membrane vesicles prepared from the electric organ of both Electrophorus electricus and Torpedo californica. The method is applicable to any vesicle system in which Cs^{+} can substitute for either Na^{+} or K^{+}. Loading of the vesicles with the fluorescent dye is accomplished using the routine procedure for making the vesicles. Neither the dye-loading procedure nor the presence of Cs^{+} changes the permeability of the membrane to ions, allowing ion translocation measurements to be made in the msec to min time region. The stopped-flow design allows two sequential mixings of solutions. Data obtained with stopped-flow and Cs^{+} is identical to data obtained previously using a quench flow technique and $^{86}Rb^{+}$ (Hess, G.P., Cash, D.J. & Aoshima, H. Nature (London) 282:329 (1979)).

The method represents an improvement over two existing techniques for measuring rapid ion flux in membrane vesicles, the quench flow technique (using $^{86}Rb^{+}$) and a stopped-flow technique developed previously which utilizes Tl^{+} (Moore, H-P.H. & Raftery, M.A. Proc. Natl.Acad.Sci.U.S.A. 77:4509 (1980)). The advantages of the present method over quench flow are as follows: (1) Stopped-flow allows measurement of an entire influx curve in one mixing event whereas quench flow allows measurement of only one time point per mixing event. Stopped-flow is therefore more rapid and requires less vesicle preparation. (2) Stopped-flow does not utilize radioactive isotopes and is therefore safer. (3) Stopped-flow data can be recorded and analyzed directly by computer. The advantages of Cs^{+} over Tl^{+} are that Tl^{+} moves through vesicle membranes in a few seconds, has a toxic effect on membrane systems (e.g. Tl^{+} makes membranes more permeable to other ions), and TlCl is only sparingly soluble in water. Cs^{+} has none of these disadvantages. (Karpen, J.W., Sachs, A.B., Cash, D.J., Pasquale, E.B. & Hess, G.P. (1983) Analyt.Biochem.(in press).

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149.5 PROPERTIES OF EXCITABLE ARTIFICIAL CELLS FROM VARIED POLYAMINO ACIDS

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Objectives in studying artificial excitable membranes in microspheres assembled from thermal copolyamino acids (proteinoids) are (a) to determine the minimal molecular requirements for electrical behavior and (b) to explore the relationships between compositions and patterns of the discharges. The question of whether artificial cells can yield electrical properties had been answered affirmatively for thermal copolyamino acids complexed with vegetable lecithin (Ishima et al., BioSystems 13 243, 1981). The spherules display electrical polarization across the membrane (membrane potential), and electrical discharges (oscillations and spiking resembling all-or-none action potentials). Examination by the Hodgkin-Huxley equation shows much similarity to natural cells (Stratten, 12th Ann. Mtg. Soc. for Neurosc., Abstr. 66.12). More recently, similar activities have been observed for proteinoid microspheres devoid of lecithin, some at decreased amplitude (Przybylski, et al., Naturwissenschaften 69 561, 1982). The proteinoid itself is known to have lipid quality due to nonpolar sidechains in amino acid residues (Lehninger, Biochemistry, 2nd ed., 1975, p. 1047). Potentials are observed during impalement by microelectrodes containing KCl, and by metallic microelectrodes as well. Electrical activities cease on drying of microspheres, but are recovered on rehydration.

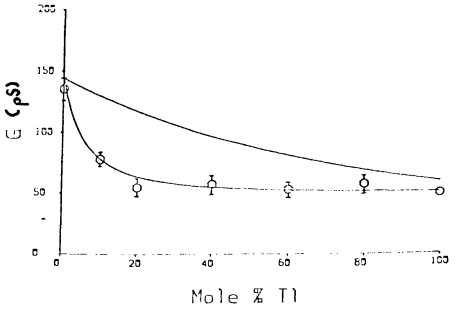
The microspheres of proteinoid-only are more uniform in size and have more regular patterns than those containing lecithin. Since the proteinoid contains flavin and pterin pigments formed by the heating of amino acids (Heinz and Ried, BioSystems 14 33, 1982), the microspheres are sensitive to white or ultraviolet light. A number of characteristic patterns of generation and shape of spike have been found to be repeatable within microspheres from a single type of copolyamino acid, and appear to be distinctive between types. These are being tested to differentiate effects due to pigment or polymer, different polymers, aging, size of spherule, etc.

149.6 THALLOUS ION INTERACTIONS WITH THE CATION SELECTIVE CHANNEL OF THE SR. J.Fox* (SPON: S.Hagiwara). Neuroscience Program, B.R.I., U.C.L.A., Los Angeles CA 90024.

Thallous ion was found to permeate the cation selective channel of the sarcoplasmic reticulum (SR channel), and to block current when present in mixtures with other permeant ions. Channel conductance in pure thallium acetate saturates with increasing concentration with a single dissociation constant of 18 mM and a maximum conductance of 60 pS. The permeability ratio P_K/P_{Tl} is near 2 over the concentration range .01 M to 1M. These results indicate that only one thallium ion may occupy an SR channel at a time in symmetric thallium salt solutions.

When thallium is present in mixtures with K^+ or NH_4^+ it blocks current through the SR channel in a mole fraction dependent manner. In mixtures with Li^+ more current is found than is predicted by the model of Lauger (B.B.A. 311:423-441,1973) using the constants found from single salt solutions. The thallium block can be fit by the equation of Neher (B.B.A. 401:540-544,1975) for thallium block in gramicidin channel.

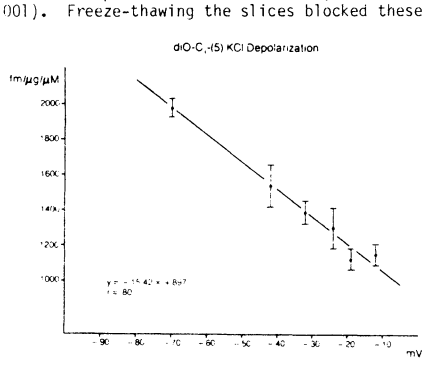
The figure shows conductance in mixtures of K^+ and Tl^+ at .1 M total salt concentration. The upper curve shows the conductance expected in this mixture without blocking, the lower that expected with thallium block. The data points are mean single channel conductance $\pm$ one standard deviation.



149.7 CYANINE VOLTAGE-SENSITIVE DYES RESPOND TO CELLULAR DEPOLARIZATION IN RAT HIPPOCAMPAL SLICES. R.M. Dasheiff and M. Hirvonen*. Neurology Dept., Univ. of Wisconsin and VAH, Madison, WI 53705.

Voltage-sensitive dyes (VSD) provide optical signals to changes in membrane potential. The cyanine VSD are permeant and work by a mechanism involving potential-dependent redistribution of the charged dye between the extracellular medium and the inside of the cell. Their response is rapid (sec) and biphasic (opposite signals for depolarization and hyperpolarization). The amount of dye entry into rat hippocampal slices maintained in vitro is linearly proportional to incubation time, dye concentration in the artificial CSF, and protein content per slice. The rate of dye entry is expressed as femtomoles of dye per microgram of protein per micromolar concentration of dye in bath per 10-min incubation (the 10-min period is standard and thus dropped: fm/µg/µM). The dye is extracted from each hippocampal slice by vortexing in 95% EtOH, and quantitated in a spectrophotofluorometer.

The RATE of entry of dye into slices depolarized with KCl is linearly related to the calculated Goldman membrane potential (Fig.). Since the cyanine VSD are positively charged, less dye enters a depolarized cell. Independent confirmation of the dye's voltage sensitivity was obtained by depolarizing with 100 µM veratrine (V), blocking this with 1 µM tetrodotoxin (TTX) and causing hyperpolarization with TTX alone: fm/µg/µM ± SEM (n=21), nl 1561±33, V 830±28, V + TTX 1724±37, TTX 1860±90. Valinomycin (1 µM) increased the effect of KCl depolarization. Ouabain induced depolarization: nl 2176±96, ouabain 1686±43 (n=13, p<.001). Freeze-thawing the slices blocked these effects.



149.8 CATION DISTRIBUTION IN NONMYELINATED NERVE AFFECTED BY LICL AND PHLORETIN. E.L. Roberts, Jr.* and D.M. Easton (SPON: P. Cancalon). Department of Biology, Florida State University, Tallahassee, FL 32306.

Li^+ affects nerve excitability as well as synaptic transmission. This research examines the distribution of Li^+ in Li^+-treated nerves exposed to phloretin, which reportedly blocks Na^+-Li^+ countertransport. The distribution of Na^+ and K^+ was also noted. The olfactory nerve of the gar (Lepisosteus osseus) was used in these experiments. Cation content in the nerve was measured with flame emission spectrophotometry (FES). The total Li^+ content of nerves soaked in 10 mM LiCl rose from 0 to 14 mM during 2 h. The same nerves gained Na^+ and lost K^+ to the extent of about 15% when compared to control levels. Increases in Na^+ and decreases in K^+ were also seen when 0.1 mM or more phloretin was present in the absence of Li^+. While phloretin concentrations of less than 0.1 mM did not greatly affect Na^+ and K^+ distributions, they did increase the nerve's Li^+ content when 10 mM LiCl was present. In the presence of 0.05 mM phloretin average total nerve Li^+ content increased about 10% from the control level. This result suggests that phloretin blocks Na^+-Li^+ countertransport in the nerve. However, the rate of efflux of Li^+ from Li^+-loaded nerves into Na^+-free solutions was indistinguishable from efflux into control solutions. That result does not support Na^+-dependent Li^+ efflux from the nerve. Thus, phloretin may block an Li^+ exclusion mechanism other than Na^+-Li^+ countertransport. (Aided in part by a Grant-in-Aid of Research from Sigma Xi.)

149.9 INTRACELLULAR FREE MAGNESIUM IN HELIX ASPERSA NEURONES. S.M. Gamiño* and F.J. Alvarez-Leefmans (SPON: J. Villarreal). Department of Neuroscience. CINVESTAV del IPN Apartado Postal 14-740, México 07000,D.F.

Cytoplasmic Mg^{2+} influence important cellular functions. Mg^{2+} is an essential co-factor for many intracellular enzymes eg. those concerned with glycolisis, respiration and membrane transport. In spite of this, direct measurements of cytoplasmic free Mg concentration ($[Mg^{2+}]_i$) have been reported for only very few cell types and only recently such measurements have been performed in neurones (Alvarez-Leefmans, F.J., Gamiño, S.M. and Rink, T.J. J. Physiol. in the press, 1983). Furthermore, there is evidence in the literature suggesting Mg^{2+} entry during nervous activity in squid axons. Therefore we thought it would be interesting to see if there is any difference in the basal levels of $[Mg^{2+}]_i$ between neurones undergoing spontaneous spike activity and those which are not spontaneously active.

Mg^{2+}- sensitive microelectrodes (MgSE) having tips $\leq 1\mu m$ were prepared with a liquid sensor based on the neutral ligand ETH-1117 (Lanter, F. et al, Anal. Chem. 52: 2400, 1980). This sensor shows significant interference from K^+. Therefore, measurement of $[K^+]_i$ is mandatory for adequate calibration of MgSE. Since $[K^+]_i$ was 91 ± 2.2 mM, MgSE were calibrated in solutions containing (mM): K^+, 91; Na^+, 7.5; HEPES, 5 (pH=7.5) and variable concentrations of Mg^{2+} (0,0.5,1,2,5,10).

The bathing solution contained (mM); NaCl, 80; KCl, 4; $CaCl_2$, 4.5; $MgCl_2$, 5; Na-HEPES, 5; pH 7.5 (21-25°C). The largest cells of the right or the left parietal ganglion were impaled first with a 3M-KCl microelectrode (5-20 MΩ) and then the MgSE (15-40 GΩ). In 7 neurones which fulfilled stringent criteria for cell viability and electrode performance, $[Mg^{2+}]_i$ was 0.66 ± .05 mM (mean ± S.E). No distinction was made between spiking and quiescent cells. (Alvarez-Leefmans et al, J.Physiol. in the press). In the present experiments the basal $[Mg^{2+}]_i$ in 6 quiescent cells was 0.71 ± 0.12 mM (mean ± S.E.; range 0.50-1.29 mM), while that of cells showing sustained bursting activity was 0.80 ± .07 mM (mean ± S.E.; range 0.58 - 1.15 mM; n=8). The difference between the means was not statistically significant. Our data suggests that, even if there is a Mg^{2+} entry, these neurones keep their $[Mg^{2+}]_i$ quite stable.

149.10 CO_2 INDUCED IRREVERSIBLE LOSS OF VOLTAGE-DEPENDENCE AT A RECTIFYING ELECTROTONIC SYNAPSE. C. Giaume and H. Korn,(SPON: L. Jacobs) INSERM U261, Institut Pasteur, Paris, France.

At the crayfish lateral giant axon (LGA) and giant motor fiber (GMF) rectifying synapse, conductance is increased and transmission is bidirectional while the LGA is more positive than the postsynaptic GMF (Furshpan, E. and Potter, D., J. Physiol., 145: 289, 1959; Giaume, C. and Korn, H., Science, 220: 84, 1983). We have examined if this voltage-dependence persists under conditions of reduced junctional permeability by lowering internal pH, (pH_i), with short CO_2 applications. As expected, this procedure produced complete uncoupling. However, when the cells had recoupled, the synapse had become bidirectional at all levels of polarization. Methods were the same as previously (see ref. above); two microelectrodes, one for current injections and one for voltage recordings, were inserted at each side of the synapse. Resting potentials, which normally were ≃ -90 mV and -75 mV in the pre and post fibers, respectively, (ΔV = -18 ± 6 mV in the five complete experiments used for this report) were monitored continuously and coupling parameters were assessed using classical equations. A 2 to 3 minutes exposure of the preparation to CO_2 saturated perfusate induced rapid depolarizations of 7 ± 3 mV in the LGA and 31 ± 19 mV in the GMF, and the junctional resistance (measured with positive pulses injected presynaptically) increased from 1365 ± 180 KΩ in the control, to infinity; i.e. the coupling ratio, k, was totally abolished. After wash, resting potentials gradually recovered to their original values, in about 15 min. for the LGA, and 40 min. for the GMF, leading to a final ΔV of -14 ± 5 mV. At this time, both positive and negative current pulses in the prefiber spread to the postsynaptic element, with k(+) and k(-) reaching 0.19 ± 0.03 and 0.21 ± 0.03, respectively. This new state of bidirectionality was accompanied by a fall of the junctional resistances to steady state values of 372 ± 53 KΩ for $R_J(+)$ and 370 ± 37 KΩ for $R_J(-)$, a modification which persisted for over an hour, until the experiment was discontinued. Symmetrical transmission was associated with a nearly complete loss of transjunctional voltage-dependent properties: with reversal of ΔV to ≃ +35 mV, $R_J(+)$ and $R_J(-)$ were 358 ± 97 KΩ and 342 ± 58 KΩ, respectively, while in the controls they were 128 ± 16 KΩ and 129 ± 29 KΩ . Acidification due to the well known effect of CO_2 could be linked to depletion of internal Cl^- in the motor fiber: during exposures, i) high frequency of IPSPs occur spontaneously in this cell and ii) the large CO_2-induced depolarization seems to be Cl^--dependent. This study shows that prolonged loss of rectification can be induced by brief tissue acidification and that in this system, voltage and pH_i may share a common site of action.

149.11 AFTEREFFECTS OF NERVE IMPULSES ON THRESHOLD OF FROG SCIATIC FIBERS DEPEND ON pH (pCO_2). Stephen A. Raymond, Richard Roscoe*. Brigham and Women's Hospital, Dept. of Anesthesia Research Laboratories, Harvard Medical School, Boston, MA 02115.

The dependence of threshold and conduction velocity on impulse activity was studied in excised frog sciatic nerve axons as pH was varied from 6.8 to 8.0. Aftereffects were examined in 2 solutions: Boyle Conway (BC) Ringer buffered with bicarbonate, and frog Ringer (FR) buffered with Hepes. Threshold was measured by adjusting the duration of current pulses delivered through Ag/AgCl electrodes. Individual axons were teased from the nerve and recorded (DC) using Ag/AgCl contacts and suction electrodes. The electrodes were located at least 6 cm from the region of stimulation. All experiments were done at 18°C. Axons at pH 7.2 are known to show characteristic variations of threshold and conduction velocity following single impulses, short bursts, and maintained repetitive activity. These aftereffects include the relative refractory period, a "superexcitable" phase that reaches its peak in 20 ms and diminishes over 1 sec, and a phase of "depression" that peaks in several seconds and diminishes over several min (Raymond, J. Physiol. 290:273-303, 1979).

Resting Threshold: The firing threshold at "rest", measured by stimulating every 2 seconds, was stable (<1%) for a day or more at control pH 7.2. Lowering pCO_2 in air bubbled through BC solutions raised pH to 8.0. This lowered the resting threshold to as little as 50% of the control level. At pH 6.8, threshold rose to as high as 130% of control. Resting threshold did not change (<1%) with pH in FR buffered with 10mM Hepes adjusted between pH 6 and pH 8 using 1 M NaOH. However, bubbling FR-Hepes with CO_2 restored dependence of resting threshold on pH.

Aftereffects: In BC Ringer's the extent of depression depended on pCO_2. For any given rate of firing, depression was less as pCO_2 was reduced (alkalization). No depression occurred in fibers bathed in unbubbled FR solution with Hepes (pH 6.4 to 8.0). Depression was also absent in BC solutions near pH 8.0.

As pCO_2 was reduced, superexcitability also diminished. In FR with Hepes (over the entire range of pH), and in BC solution (only at high pH), some fibers showed a transient subexcitability in place of the superexcitable phase.

A pH dependence existed only when pH was varied by changing pCO_2. In the CO_2-free FR with impermeable Hepes, CO_2 can leave the axon, increasing the intra-axonal pH. Axons at high pH in BC gave results similar to axons in FR at high or low pH. This suggests that intracellular pH, influenced by CO_2/bicarbonate, governs both resting threshold and the dependence of impulse conduction on the history of firing in nerve axons.

This work was supported by USPHS grant 30160.

150.1 DIFFERENTIAL PROJECTIONS FROM LOCUS COERULEUS TO OLFACTORY BULB AND OLFACTORY TUBERCLE: AN HRP STUDY. R. Guevara-Aguilar, L. P. Solano-Flores*, O. A. Donatti-Albarrán and H. U. Aguilar Baturoni. Departamento de Fisiología, División de Investigación, Facultad de Medicina, U.N.A.M., Apdo.Postal 70250, 04510-México, D.F.

The microiontophoretic administration of horseradish peroxidase (HRP) to the olfactory bulb (OB) or olfactory tubercle (OT) in cats and rats yielded similar results in both species. After an OB HRP-injection ipsilateral and contralateral labelled neurons were seen in the piriform cortex, polymorphic layer of OT,magnocellular preoptic region, lateral hypothalamus, ventromedial hypothalamic nucleus and locus coeruleus (LC). In both species more labelled structures were found after an OT HRP-injection than after an OB HRP-injection. The substantia nigra in rats was more abundantly labelled after an OT injection than after an OB one. In cats the dorsal and the ventral raphe were also labelled. In either species, OT HRP-injections resulted in a higher frequency of LC labelled neurons than after OB injections. These results favor the hypothesis that the OT plays an important role as a relay station for efferent inflow from the brain stem en route to the OB.

150.2 LARGE AND SMALL GROUPS OF NEURONS WITH MESENCEPHALIC PROJECTIONS IN THE VENTROMEDIAL HYPOTHALAMUS OF THE RAT. Y. Sakuma and T. Akaishi*. Dept. of Physiology II, Niigata Univ. Sch. of Med., Niigata 951, Japan.

The spatial spread of the extracellular antidromic action potentials was measured in 83 neurons in the ventromedial hypothalamus (VMH) of the ovariectomized, estrogen-treated female rats under urethane anesthesia, following electrical stimulation of the mesencephalic central gray (CG). The positive-negative configuration of the antidromic action potentials throughout the extracellular field suggested that the potentials were generated predominantly by neuronal soma with simple geometries. The spatial spread of the extracellular field along electrode tracks ranged 25-174 μm at half the maximum spike amplitude. The frequency distribution for the field size was distinctively bimodal and could be divided into large and small groups at 85 μm. Antidromic action potentials with larger extracellular fields had significantly larger maximum spike amplitude with shorter duration. The pattern of changes in the amplitude of isolated initial segment spikes of the antidromic response across the field were quite different from either of the two types of the extracellular field spread. Therefore, differences in the field size could be associated with neuronal size.

A full 55% of the CG projection of the VMH originated from small neurons, which scattered through the ventromedial nucleus (VMN), the retrochiasmatic area and the cell-poor zone between the VMN and the arcuate nucleus. Large neurons were located in the core of the VMN. Taking into account the sampling bias, a much greater proportion of the CG projection may arise from small neurons in the VMH.

Transsynaptic facilitation from the corticomedial amygdala (AMY) was observed in some cells of either size. Three large neurons were driven antidromically from the AMY as well as from the CG, and were concluded to have simultaneous projections to both sites. The lack of spontaneous activity in nearly all cells prevented analysis of inhibitory effects of AMY stimulation.

The lack of systematic difference in the antidromic spike latencies between large and small cells indicated that axonal thickness, which varies directly with the neuronal size and critically affects the conduction velocity, is not the major factor in determining the latency of the responses of VMH neurons to CG stimulation. Thus, the conduction distance, i.e. the projection path, may be the contributing factor to the different sizes of the neuronal population in the VMH between the male and female rats (Sakuma & Pfaff, Brain Res. 225, 184), which responded to CG stimulation at a particular range of antidromic spike latency.

150.3 THETA BURSTING NEURONS IN THE NUCLEUS OF THE HORIZONTAL LIMB OF THE DIAGONAL BAND, J.E. Marchand, F. Macrides and Wm. B. Forbes. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The bursting characteristics of single units in the nucleus of the horizontal limb of the diagonal band (HDB) were studied in paralyzed hamsters which had their normally cyclic nasal airflow interrupted so as to prevent odor-evoked rhythmic activity. The relationship of this bursting activity to rhythmic slow wave activity (RSA) in the main olfactory bulb (MOB) and dorsal hippocampal field CA1, and the responses of HDB units to electrical stimulation of the MOB and hippocampus, were analyzed. Units which project to the MOB were identified using antidromic stimulation (constant latency, high frequency following, and collision tests) and unit recording sites were verified using iontophoretic injections of pontamine sky blue. Antidromically driven units in the HDB, as well as units which could not be driven antidromically by MOB stimulation, exhibited periods of rhythmic bursting in the theta band (4 to 12 Hz). During such periods, RSA tended to be present in both the MOB and hippocampal recordings. The rhythmic bursting in the HDB was well correlated with individual cycles of the RSA in the MOB. This rhythmic bursting and olfactory RSA were not invariably correlated with the hippocampal RSA (e.g., could exhibit a different dominant frequency). Hippocampal stimulation did not elicit antidromic responses in the HDB but often produced suppression of firing and occasionally elicited excitatory responses of variable latency. Thus, theta bursting neurons in the HDB may drive the theta rhythm in the MOB, and this rhythm can be distinguished from the hippocampal theta rhythm but could be modulated by the septo-hippocampal system.

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150.4 LATERAL HYPOTHALAMIC - MEDIAL FOREBRAIN BUNDLE NEURONS: RESPONSES TO OLFACTORY BULB, TONGUE, AND SCIATIC NERVE STIMULATION. Craig A. Velozo* and C. Robert Almli. Washington University School of Medicine, St. Louis, Mo. 63110.

The lateral hypothalamic-medial forebrain bundle (LH-MFB) area has been implicated in the regulation of feeding and drinking behaviors via brain damage, stimulation, and electrophysiological research. Electrophysiological studies have revealed that LH-MFB neuronal activity can be modulated by changes in body fluid composition (e.g., osmotic and glucose) and external sensory stimulation (e.g., olfactory, gustatory, tactile, visual). In previous studies (Fisher, R.S. and Almli, C.R. Neuroscience Abstracts, 1979, 5, 159.), we have shown that single neurons (LH-MFB) of neonatal rats are responsive to multimodal sensory stimulation (e.g., olfactory, gustatory, tactile, and osmotic). However, as the rat ages, there appears to be a developmental "fine-tuning" of LH-MFB neurons. That is, the proportion of "multimodal" neurons decreases with stimultaneous increases in the proportion of neurons more selectively responsive to a particular sensory system.

The purpose of the present study was to systematically evaluate the degree of sensory convergence-divergence on LH-MFB neurons of adult rats using electrical stimulation of the olfactory bulb, tongue, and sciatic nerve.

Adult male albino rats were anesthelized (Dial-Urethane or Urethane) and surgically prepared. Stimulation electrodes were placed in the olfactory bulb, on the tongue and on the sciatic nerve. Single pulses and pulse trains were used for electrical stimulation. Extracellular single neuron activity was recorded with glass micropipettes (2-3 M NaCl).

In response to stimulation of individual sites (bulb, tongue or nerve), LH-MFB neurons showed simple and complex changes in activity patterns. In some neurons the response (spikes) was time-locked to the stimulus, while for other neurons, variable forms of spike inhibition were seen. There was no obvious relation between the sensory modality stimulated and the type of LH-MFB neuronal activity change. Analysis of sensory convergence revealed that many LH-MFB neurons were responsive to one or two forms of sensory stimulation, with olfactory bulb and sciatic nerve stimulation being the most effective stimulation sites. These results indicate that many individual LH-MFB neurons of adult rats have their activity modulated by one or more sensory systems, and these results do not appear to be due to the general "activation" qualities of sensory stimulation per se. (Supported by BRSG Grant 5389).

150.5 CYTOARCHITECTURE OF THE MAMMALIAN HYPOTHALAMUS: A REINTERPRETATION
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Traditional descriptions of the anatomical organization of the mammalian hypothalamus have emphasized the apparent diffuse nature of the cytoarchitecture of this important brain region. Whereas a number of hypothalamic functions, both neuroendocrine and autonomic, have been shown to be regulated and controlled precisely, an anatomical basis for this precise regulation has been lacking. The development and exploitation of immunohistochemical methods to characterize selected populations of hypothalamic neurons has provided evidence for anatomically and functionally related neuron groupings that are not apparent in material (e.g., Nissl stains) used for traditional cytoarchitectural analysis. Such studies have suggested that the hypothalamus is more highly organized anatomically than had been suspected.

In the present study, we have used a variety of neurohistological methods to re-examine the cytoarchitecture of the hypothalamus of adult female Sprague-Dawley rats. Immunohistochemical localization of the protease Cathepsin D (Whitaker et al., Brain Research, 216 (1981) 109) has been of particular value in defining specific neuronal populations in hypothalamus. Series of adjacent sections have been processed by a number of traditional neurohistological and immunohistochemical methods, as well as several variants of these methods developed in this laboratory. Using these procedures, it is evident in our material than an extremely large number of groups of neurons may be defined in hypothalamus using standard cytoarchitectural criteria. Our results to date indicate that the apparent lack of cytoarchitectural organization in the hypothalamus is due in part to the extreme complexity of these different neuronal groups and that many of the cytoarchitecturally defined neuronal groups overlap with other groups, making identification of these groups difficult.

These results suggest that a reinterpretation of the anatomical organization of hypothalamus may be warranted. In particular, the view that the neurons of the hypothalamus are organized diffusely and are dependent on stochastic processes, rather than specific and highly organized anatomical relationships, may be questioned. Examples of this approach to defining hypothalamic cytoarchitecture will be presented, with particular emphasis on the cytoarchitecture of the mediobasal hypothalamus.

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150.6 AFFERENT PROJECTIONS OF THE MEDIODORSAL THALAMUS: COMBINED FLUORESCENT RETROGRADE TRACERS AND IMMUNOHISTOFLUORESCENCE.
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The concept of a ventral striatal and pallidal system in parallel to the classic striatal-pallidal system has recently been proposed and studied (Heimer and Wilson, in Golgi Centennial Symposium: Perspectives in Neurobiology, 1975; Heimer, et al., Neuroscience 7:1891, 1982). The mediodorsal n. of the thalamus (MD) is envisioned as a ventral pallidal outflow target. This possible projection to the MD as well as others have been studied using fluorescent retrograde tracers in combination with immunohistochemistry. Male Sprague-Dawley rats (110-160g) had injections of 50 nl of Fast Blue (FB), Granular Blue (GB), or a red "SITS" (courtesy Dr. Swanson; by mass spectroscopy, no disodium SITS is present. Authentic yellow disodium SITS (Schmued and Swanson, Brain Res. 249:137, 1982) does not transport.) into the MD or adjacent tissues. Some animals received 50-100µg colchicine intraventricularly 48 hrs. prior to sacrifice. Antisera directed against glutamic acid decarboxylase (GAD) was used to define ventral pallidal regions in FITC staining. Other antisera directed against met-enkephalin (ME), substance P(SP), neurotensin(NT), and cholecystokinin(CCK) were also used in this study.

Tracer-positive cells in the frontal pole split into two groups caudally: one capping the claustrum and one in the dorsomedial cingulate cortex. Approximately 30-40% were found contralaterally. Frequent cells were seen in the ipsilateral deep piriform cortex. Some of these were NT-positive. In the tubercle, cells occurred in areas of GAD-positivity (ventral pallidum) only, both in association with Islands of Calleja and in deep polymorph layer. Rarely, cells were also GAD-positive. More frequently, simultaneous GAD- and tracer-positive fibers were observed. Less often, ME costained fibers were seen. Tracer-positive cells were also found in the globus pallidus, both limbs of the diagonal band, medial forebrain bundle, lateral hypothalamic area, amygdala, and thalamic reticular n. Occasional cells were observed in these contralateral areas (except amygdala). No CCK or SP double-labelled cells or processes were observed. NT double-labelled cells and processes also were seen in the vertical limb of the diagonal band and medial ventral pallidum.

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150.7 A RE-INVESTIGATION OF MAMMILLARY NUCLEI AXON COLLATERALS BY MEANS OF FLUORESCENT TRACERS AND TRANSNUCLEAR TRANSPORT OF WGA-HRP.
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Considerable effort has been directed towards understanding the organization of mammillary nuclei projections to the thalamus and brain stem. In addition, the mammillary nuclei have frequently been used as a model system to study lesion effects and axonal transport. However, because of the small size of the mammillary nuclei and the existence of ascending and descending axon collaterals, many details of the connectivity of the nuclei remain unclear and it is uncertain to what extent this lack of information affects their use as a model system.

In the present study the connections of the mammillary nuclei have been re-investigated using fluorescent tracers (Fast Blue, True Blue, Nuclear Yellow) and the transnuclear transport of wheat germ agglutinin-horseradish peroxidase conjugate (WGA-HRP) in the rat. Stereotaxic injections of fluorescent tracers were made singly or in combination into the thalamus and tegmentum and WGA-HRP was injected into the thalamus or tegmentum. After survival periods of 20 hrs to 3 days, the animals were perfused and the brains were processed according to standard procedures.

The most striking finding was that after either thalamic or tegmental injections of each of the fluorescent tracers and WGA-HRP, all ipsilateral subdivisions of the mammillary nuclei were heavily retrogradely labeled. After tegmental injections the labeling in the medial and lateral magnocellular parts of the mammillary nuclei was somewhat stronger than that in the subdivisions containing small cells. Double labeling after combined thalamic and tegmental injections was most evident in the magnocellular subdivisions. Double labeling could also be discerned in the small cells but the intense nuclear labeling by transported Nuclear Yellow tended to obscure Fast Blue labeling of the scanty cytoplasm of the small cells. The transnuclear transport of WGA-HRP demonstrated that not only do all subdivisions of the mammillary nuclei send collaterals to both thalamus and tegmentum but also that these collaterals have an extremely well-organized topographical projection. The medial mammillary nuclei project topographically with very little overlap to the ventral tegmental nucleus. The present results also suggest that under certain conditions double labeling experiments with fluorescent tracers may not account for all of the collaterals of a given nucleus.

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150.8 DIRECT PROJECTIONS FROM THE BRAINSTEM INTO ANTERIOR THALAMIC NUCLEI OF THE RAT.
R.W. Sikes and B.A. Vogt. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The anterior thalamic nuclei(ATN), a prominent group in the mammalian dorsal thalamus, projects to much of limbic cortex, particularly the medial cingulate cortex. It has long been recognized that afferents to the ATN originate in the mamillary nucleus of the hypothalamus. While direct afferents from the brainstem have been reported, no thorough investigation has examined the origins of the midbrain input.

To clarify these projections, small injections(0.02 µl) of horseradish peroxidase conjugated to wheat germ agglutinin (HRP; 5%) were placed into the ATN in albino and hooded rats. In several animals, the overlying somatosensory and hippocampal cortices were removed for direct visualization and injection of the ATN. This approach obviated the problem of diffusion of HRP into these cortical regions. The injections involved all subdivisions of the ATN with limited spread into adjacent structures such as the stria medullaris, intralaminar nuclei and rostral lateral nuclei.

In addition to previously reported afferents from the mamillary nuclei and layer VI of cingulate cortex (areas 24 and 29), labeled neurons were found in several nuclei of the brainstem. These nuclei included the pretectum, central gray, laterodorsal tegmentum, raphé, locus coeruleus, and the cuniform and lateral divisions of the mesencephalic reticular formation.

Although the function of these projections is not known, it is interesting that several of these nuclei receive sensory visceral projections from the caudal medulla (solitary and vagal nuclei) through the dorsal longitudinal fasciculus. Therefore, these projections may provide a direct route through which visceral sensations reach the ATN in addition to the projection relayed in the mamillary nucleus. Since the cingulate cortex contains many neurons which are responsive to autonomic activity, the projections described in this study may provide the morphological basis for these responses. This study was supported by NIH grants NS18745 and T32NO7152.

150.9 SEX DIFFERENCES OF OPIATE RECEPTORS IN THE PREOPTIC SEXUALLY DIMORPHIC NUCLEUS. Ronald P. Hammer, Jr., Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205

The sexually dimorphic nucleus of the medial preoptic area (SDN-POA) in the rat is a cell-dense region which is larger in males than in females. Recent neurochemical studies have shown a catecholamine innervation in the SDN-POA, particularly in the female (Anat. Rec. 1983, 205: 187A), as well as neurotensin, substance P, and cholecystokinin immunoreactive cells and fibers in both sexes (Anat. Rec. 1983, 205: 210A). The present autoradiographic study examines the density of opiate receptors in the region of the SDN-POA.

Cryostat-cut, slide-mounted sections through the region from six male and nine female (three females each in proestrus, estrus, and diestrus) brains were incubated in [^{3}H]naloxone, fixed in paraformaldehyde vapors, defatted in xylene, and exposed to [^{3}H] sensitive x-ray film. The autoradiographs were digitized and analyzed using a computer-assisted image processing system. The optical densities of the left and right receptor-dense SDN-POA regions and the adjacent portion of POA located laterally were determined, as were the areas of receptor-dense SDN-POA regions on both sides. The ratio of the optical density of SDN-POA to adjacent POA was calculated for each side in every section.

Comparison of optical density ratios failed to show any difference between sides in any group. In addition, no significant difference was observed between optical density ratios obtained from female brains in various estrous states. However, the SDN-POA/POA optical density ratio in males was significantly less than in females (1.06 vs. 1.20, $p < 0.001$). Mean of areal measurements of the receptor-dense SDN-POA region in sections from males was significantly greater than that of females (0.119 mm^2 vs. 0.091 mm^2, $p < 0.001$). The size of the receptor-dense region appeared to be larger than the cell-dense SDN-POA nuclear region in both sexes. Within the receptor-dense region, a gradient of labeling seems to exist, especially in females. Naloxone binding appears denser medially in the central core of the nucleus, and less dense laterally. The receptor-dense nuclear region is surrounded by a zone of low receptor density.

In summary, the SDN-POA in the female rat contains a greater density of opiate receptors than the SDN-POA in the male rat. This receptor-rich region is smaller in the female. The sex difference of opiate receptor density in the SDN-POA could relate to functional differences in this region between the sexes. Endogenous opiates acting in the SDN-POA could be involved in the regulation of lordosis in the female rat via opiatergic pathways from this region to the mesencephalic central gray substance.

150.10 THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA (SDN-POA): ULTRASTRUCTURE. H.-Dieter Dellmann* and Carol D. Jacobson Dept. Vet. Anatomy, Iowa State Univ., Ames, IA 50011.

The SDN-POA is a morphological marker of the process of central nervous system sexual differentiation. Since the fine structure of SDN-POA neurons and surrounding neurons in the medial preoptic area (MPOA) has not been described, the present study was conducted. The fine structure of SDN-POA and MPOA neurons in the adult male and female Sprague-Dawley rat are similar. Using 5% glutaraldehyde fixation, three basic neuron types are present: TYPE I, II and III. 74.7% of the neurons are TYPE I (mean area ($\bar{x}$), 131.26μm^2). Their nuclei ($\bar{x}$, 53.18μm^2) have sparse amounts of chromatin. The cytoplasm has few polyribosomes, and rER composed of long, branching, isolated or grouped cisternae. Either elongated, flat or short, curved stacks of cisternae associated with electron-lucent, dense core and coated vesicles comprise the Golgi complex. Lysosomes, multivesicular bodies (MVB) and autophagic vacuoles (AV) together with mitochondria may form massive accumulations in the perikaryon or at the site of dendrite origin. Synapses may contain oblong electron-lucent vesicles, synaptic vesicles and/or dense core vesicles in varying proportions. 22.5% of the neurons are TYPE II ($\bar{x}$, 104.18μm^2) with densely packed polyribosomes located between short, stacked, parallel and dilated cisterne of rER. The electron-dense nuclei ($\bar{x}$, 39.84μm^2) have multiple deep indentations. These neurons also possess Golgi complexes in various configurations and associated electron-lucent, dense core and coated vesicles, lysosomes, MVB, AV and mitochondria. In contrast to TYPE I NEURONS, TYPE II NEURONS are often deeply indented by synapses, large dendrites or glial perikarya or processes. 2.7% of the neurons are TYPE III (somal $\bar{x}$, 77.64μm^2; nuclear $\bar{x}$, 29.20μm^2). They possess the same organelles as TYPE II NEURONS. However, the density is decreased. These neurons lack surface indentations as well as dendritic and glial contacts. There is a statistically significant difference in both the area of the neuronal cell body and of the nucleus for the three types of neurons. The size of TYPE I > TYPE II > TYPE III neurons. Furthermore, there is a significant difference in the percentage (%) of neurons which fall within each category such that the % of TYPE I > TYPE II > TYPE III. These results were consistent for males and females as well as for SDN-POA and MPOA neurons. Thus, no significant sex or location differences were observed in the characterization or size of the neurons in this area. Studies are in progress to determine the functional and developmental meaning of the various neuronal types. Supported by HD16148 & the Basil O'Connor Starter Research Grant No. 5-367 from March of Dimes Birth Defects Foundation (CDJ).

150.11 ESTROGEN-CONCENTRATING NEURONS IN THE MEDIAL PREOPTIC AREA SEND AXONS TO THE VENTRAL TEGMENTAL AREA AND AMYGDALA. S.E. Fahrbach, J.I. Morrell and D.W. Pfaff. Neurobiology and Behavior, The Rockefeller University, New York, NY 10021.

The medial preoptic area (MPOA) has been shown by steroid autoradiography to contain large numbers of estradiol (E_2)-concentrating neurons. Estrogen action on the MPOA facilitates maternal behavior in the rat, and knife cuts placed lateral to MPOA abolish this behavior. Using a method combining steroid autoradiography and fluorescent dye retrograde tracing, we asked if estrogen acts directly on any long-projection neurons of the MPOA. Microinjections of the fluorescent dyes DAPI, true blue, or granular blue were made into the ventral tegmental area (n=6), into the VTA and the midbrain central gray (n=2), or into the amygdala (n=6), known targets of MPOA neurons. After 2-3 days the rats (ovariectomized, adrenalectomized, 3 month old females) were given an i.p. injection of a nuclear-saturating dose of 2,4,6,7-[^{3}H]-estradiol (0.8 μg/250 g body weight; specific activity 151 Ci/mM) and after 2 hr perfused. Brains were frozen onto cryostat chucks; 6 and 12 μm sections were picked up onto dry emulsion-coated slides. The autoradiograms were developed after 2-6 months, and examined under standard bright-field conditions for nuclear silver grains and with UV light for somal fluorescent dye label.

The pattern of cells labeled after retrograde transport from the VTA and amygdala was similar to that reported using HRP, but labeled cells were more numerous than previously described. The combined method demonstrated that some cells in MPOA which send fibers to VTA also concentrate E_2. The greatest number of double labeled cells was found in the caudal MPOA/anterior hypothalamic region at the level of the anterior pole of suprachiasmatic nucleus. Scattered dye-labeled, E_2-concentrating cells were also found in the lateral preoptic area and lateral hypothalamus, regions which project strongly to the VTA but contain few E_2-concentrating neurons. By contrast, very few double labeled cells were seen in the bed nucleus of the stria terminalis, a region which concentrates E_2 heavily and sends fibers to VTA. After amygdala injections, double labeled cells were also seen but the pattern was not the same, as most of the dye-labeled, E_2-concentrating cells seen were located more rostrally in the MPOA. Thus, a subset of the E_2-concentrating cells of the MPOA have long axonal projections, and a subset of the MPOA neurons which project to VTA and to the amygdala are E_2-concentrating. We hypothesize that these neurons, which may be under genomic control by E_2, are involved in the control of estrogen-dependent maternal behavior in the rat. This does not exclude a role for the many E_2-concentrating neurons in the MPOA which have specific connections not revealed in these studies.

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150.12 EFFERENT CONNECTIONS OF THE DORSAL TEGMENTAL REGION IN THE RAT. H.J. Groenewegen, N.W. Kowall* and W.J.H. Nauta. Dept. of Anatomy, Vrije Universiteit, Amsterdam, and Dept. of Psychology, M.I.T., Cambridge, MA 02139.

The term dorsal tegmental region (DTR) here denotes the caudal central grey substance comprising the dorsal tegmental nucleus of Gudden and the nucleus tegmenti dorsalis lateralis. Earlier studies have shown that DTR receives massive projections from lateral habenular (HL), interpeduncular (IP) and mammillary nuclei. This abstract summarizes efferent connections of DTR demonstrated by autoradiography or immunohistochemistry, using, respectively, tritiated amino acids and the lectin phaseolus vulgaris.

Unilateral injections in DTR (not involving locus coeruleus) elicit dense fiber labeling in contralateral DTR. Farther rostrally, the aqueduct is flanked by labeled fibers which extend to the posterior hypothalamic nucleus and deep layers of the superior colliculus (SC). However, the main projection ascending from DTR is represented by two other fiber systems: one (a) tracing a largely contralateral, paramedian course rostroventrally through the median raphe nucleus, the other (b) composed of slender, mainly ipsilateral arcuate fascicles curving widely through the tegmentum, then returning to a paramedian position at the base as main component of the mammillary peduncle (MP). Midbrain structures invaded by b include the peripeduncular region, deep layers of SC, and both lateral-geniculate nuclei. Both a and b innervate the ventral tegmental area and IP. In IP, DTR efferents distribute most densely to contralateral subnuclei dorsalis parvocellularis, dorsalis granulocellularis, and lateralis. Rostral to IP, fiber labeling is mainly ipsilateral. Very dense terminal label fills the lateral mammillary nucleus (MB), but other labeled MP fibers bypass MB as components of the medial forebrain bundle (MFB) to the supra- and premammillary nuclei, lateral preoptico-hypothalamic region, dorsal hypothalamic area, medial amygdaloid nucleus, nucleus of the diagonal band, medial and lateral septal nuclei, hippocampus, and frontocingulate cortex. Further labeled DTR efferents deviate from MFB dorsalward, 1. alongside the fasciculus retroflexus to the HL, parafascicular nucleus, and pretectal area, and 2. along the mammillothalamic tract to the thalamic nuclei reuniens, gelatinosus, centralis medius, centralis lateralis, periventricularis anterior, anteroventralis and lateralis dorsalis.

These findings indicate that DTR, caudal pole of the 'limbic midbrain area', can affect widespread forebrain regions either directly, or indirectly through the supramammillary and diagonal-band nuclei which both project to extensive neo- and allocortical regions (Saper, 1981). Supported by the Dutch Organization ZWO and NSF grant BNS80-07905.

150.13 INTRACELLULAR RECORDINGS FROM TEMPERATURE-SENSITIVE SEPTAL AND HYPOTHALAMIC NEURONS. M.N. Perlmutter and J.A. Boulant (SPON: J. Curry). Dept. of Physiology, Ohio State University, Columbus, Ohio 43210.

Certain neurons within the diencephalon are notably sensitive to their own local temperature. These cells are believed to function as central thermoreceptors which are responsible for the control of body temperature. To investigate the mechanisms by which individual neurons may act as central thermodetectors, intracellular potentials were recorded from temperature-sensitive and temperature-insensitive neurons in the septal, preoptic and anterior hypothalamic nuclei of anesthetized male Sprague Dawley rats. Conventional intracellular recording techniques were employed using electrodes filled with 2M potassium citrate. Brain temperature was manipulated between 32-42°C by a water perfused thermode and was monitored by a fine thermocouple positioned near the recording site.

Previous extracellular studies suggest that neuronal warm-sensitivity can be maintained in the absence of synaptic input, whereas neuronal cold-sensitivity was dependent on excitatory and inhibitory synaptic input. The results of this study tend to support these findings. Warm-sensitive neurons were characterized by a significant increase in the number of subthreshold and threshold depolarizations at warmer local brain temperatures. Eighty percent of these warm cells displayed long summated depolarizations with trains of action potentials; and there was little evidence of inhibitory synaptic input at any temperature. Cold-sensitive neurons were characterized by a significant decrease in the amount of subthreshold and threshold depolarizations at warmer local brain temperatures; and inhibitory synaptic input was more prevalent at warmer temperatures. In most cold-sensitive cells the action potential amplitude varied inversely with brain temperature. A prominent depolarizing after-potential was common in 40% of the cold-sensitive cells. Temperature-insensitive neurons displayed a variety of electrical activity, however, in no case did the frequency or amplitude of either subthreshold or threshold activity change as a function of local brain temperature. (Supported in part by NIH grant NS - 14644 and by a Grant-in-Aid from the American Heart Association.)

150.14 CORTICAL AND SUBCORTICAL AFFERENTS TO THE PERIRHINAL CORTEX (AREA 35) IN THE RAT. K. C. Kosel, G. W. Van Hoesen and D. L. Rosene. Dept. of Anatomy, Boston University, Boston, MA 02118 and Dept. of Anatomy and Neurology, University of Iowa, Iowa City, IA 52242.

In the rat, the perirhinal cortex (area 35) begins in the depths of the rhinal sulcus and expands ventrolaterally for a short distance forming a narrow strip of cortex along the lateral bank of the sulcus. Previous studies in the rat have tended to focus solely on the efferent connections of the area, and have demonstrated a limited series of projections to the molecular layer of the subiculum, to all lamina and subdivisions of the entorhinal cortex, and to layer I of the contralateral perirhinal cortex. Despite the fact that considerable information now exits regarding these projections, little information is available in the literature on the afferent input to area 35. Most of the findings that have been reported to date have come from studies involving primates.

In an attempt to demonstrate the source of afferent input to the perirhinal cortex, small injections of horseradish peroxidase (HRP: 0.02-0.06µl of a 10% solution), were made throughout area 35. Following a 24-48 hour survival, the animals were sacrificed and the tissue prepared for HRP histochemistry using conventional histological methods and tetramethyl benzidine as chromagen.

In the cortex, retrogradely labeled cells were observed in the anterior most parts of the ipsilateral frontal sulcal (insular) cortex following injections in the ventral part of area 35 and in both the anterior and posterior portions of the ipsilateral prepiriform cortex (layer II). Labeled cells were also present in the contralateral perirhinal cortex and indicated the existence of a topographically organized commissural connection between the cortices of the two hemispheres. Subcortically, retrogradely labeled cells were present ipsilaterally in the nucleus reuniens of the thalamus, the ventral claustrum and in the lateral and laterobasal nuclei of the amygdala. Both the nucleus basalis and the septal nuclei were negative. Finally, labeled cells were also observed in layer II of the medial, and layers II and III of the lateral entorhinal cortices, with a lesser number in the ventral subiculum and parasubiculum.

The results of the present study demonstrate the existence of a number of cortical and subcortical projections to the perirhinal cortex in the rat. In view of the strong efferent projections of area 35 to the entorhinal cortex and subiculum, it is plausible that the perirhinal cortex may represent an alternative route for information transfer to the hippocampal formation. (Supported by NIH grants NS 14944 to G.W.VH. and NS 19416 to D.L.R.)

150.15 POSTERIOR CORTICAL ABLATIONS IN THE RAT AT 1, 7, 30, or 70 DAYS AFTER BIRTH AND PERFORMANCE ON THE MAIER 3-TABLE REASONING PROBLEM. J. M. Stahl, V. L. Stroud*, R. A. Wingate*, and G. C. Daniels*. Dept. of Psychology, Morris Brown College, Atlanta University Center, Atlanta, GA 30314.

Ninety six male and female Long-Evans hooded rats received unilateral or bilateral posterior cortical ablations or merely had the skull lifted (operated control) at either 1, 7, 30, or 70 days following birth. At 90 days of age subjects were tested on the Maier 3-table reasoning problem which was developed to test the ability of the rat to combine the essentials of 2 isolated past experiences to reach a goal (Maier, 1929). Studies have indicated that the process involved in solving this problem is different than that involved in learning tasks which depend upon the association of contiguous events (Campbell, 1935; Maier, 1932, 1940) and is one that is not demonstrated in rats until they reach about 60 days of age (Stahl and Ellen, 1979). Performance on this task is impaired by cortical (Maier, 1932), septal (Stahl and Ellen, 1973), and hippocampal damage (Rabe and Haddad, 1969). Thus, the purpose of this study was to investigate the age-dependency of recovery of function on a task which requires an ability which is age-dependent in Long-Evans hooded rats. Results clearly showed that rats receiving large cortical ablations (usually bilateral) at any age made significantly fewer correct choices on the Maier 3-table problem than did animals which received sham surgery at the same age or smaller lesions (usually unilateral) at 1, 7, or 30 days of age. Histological data showed that a significant amount of hippocampal damage occurred in animals which received the larger ablations at 1 or 7 days of age and subsequently did not perform well on the task. Thus, results indicated that the amount of cortical tissue damaged at any age is the determining factor in functional recovery and that no functional sparing follows even minor hippocampal damage at any age. Supported by an NIMH grant through the Minority Biomedical Research Support Grant Program.

150.16 ORIGIN OF PROJECTIONS TO THE SUPRACALLOSAL ANTERIOR CINGULATE CORTEX (AREA 24) OF THE RABBIT. M. L. Woodruff and R. H. Baisden. Dept. of Anatomy, Quillen-Dishner Col. of Med., East Tenn. State Univ., Johnson City, TN 37614.

As an adjunct to studies of the function of the cingulatecortex in behavior, the subcortical and cortical projections to area 24 were studied in the New Zealand rabbit using retrograde HRP labeling. Thalamic projections to the retrosplenial-cingular (areas 23 and 29) regions in the rabbit have been demonstrated using both retrograde cell body degeneration (J. Rose and Woolsey, J. Comp. Neurol., 89:279, 1948) and retrograde enzyme transport approaches (Berger et al., Brain Res., 201:411, 1980), but apparently only the retrograde degeneration approach has been employed to study the source of afferents to area 24 in the rabbit (Rose and Woolsey, 1948). Horseradish peroxidase was injected into area 24 in 5 rabbits. Approximately 48 hr after the injection the brains were removed, sectioned at 50u, and the sections were processed following the cobalt chloride modification of Adams (Neurosci., 2:141, 1977) which produces an enhanced HRP reaction product. Microscopic examination of the reacted sections revealed that, with the exception of contralateral homotypic sites, labeled neuronal soma were found only ipsilateral to the injection. Labeled cortical cells were found predominately in layer 3, (although some were present in layer 5), of areas 32, 4, 29d and the dorsal part of 29c. Of the thalamic nuclei containing labeled cells, the heaviest concentration was observed in the ventral anterior and anteromedial nuclei. Many labeled cells were also present in the centromedial nucleus and in the rhomboid and reuniens nuclei. Fewer labeled cells were found in the dorsomedial nucleus. Additional labeled cells were also observed in the nucleus of the diagonal band, the lateral part of the medial hypothalamus, the midbrain tegmentum medial to the cerebral peduncle, and in the central tegmentum of the pons.

These results agree with the above cited degeneration studies, but extend them to include cortical and non-thalamic subcortical areas projecting to area 24. Comparison of these results to similar studies in other species (e.g. cat, Robertson and Kaitz, J. Comp. Neurol., 195:501, 1981; monkey, Baleydier and Mauguiere, Brain, 103:525, 1980) indicate some species differences in origin of afferents to area 24.

150.17 SEPTAL UNIT ACTIVITY IN PAVLOVIAN CONDITIONING: A REGIONAL COMPARISON. E. Thomas and E. Yadin. Department of Psychology, Bryn Mawr College, Bryn Mawr, PA 19010 and Isotope Department, The Weizmann Institute of Science, Rehovot 76100, Israel.

Unit activity was recorded from the ventral medial septal region of the rat during Pavlovian conditioning and was compared to our previous data obtained from the dorsal lateral septum. Unit activity was assessed under three Pavlovian paradigms, 1) food US with the animals satiated, 2) food US with the animals deprived and 3) shock US.

Male Wistar rats were implanted with fine nickel chromium wires 62.5 micrometers in diameter, enamel insulated to the tip. Recordings were made either via a monopolar derivation with the indifferent electrode attached to a stainless steel screw on the skull or via a bipolar derivation with both electrodes in the active site. All of the paradigms were differential conditioning in which one stimulus (either a tone or a light) was paired with the US and the other was presented unpaired with the US. The CS-US interval was 10 sec. For appetitive conditioning the US was sweet chocolate milk, for aversive conditioning a 1 mA footshock. Unit activity was evaluated separately for CS+ and CS-. The results were as follows:

1. Good appetitive conditioning was obtained both under the deprived and the satiated conditions and the sweet chocolate milk was also able to maintain good rates of lever-pressing in the satiated animals suggesting that the hedonic properties of the US could support both Pavlovian conditioning and instrumental performance in the absence of drive.
2. In the appetitive paradigm unit activity was suppressed in the presence of CS+ compared to an equivalent baseline period. There was no consistent change in unit activity in the presence of CS-. This effect was seen both under the deprived condition and under the satiated condition, suggesting that the activity change is not related to drive, but more likely to the hedonic properties of the US.
3. In the aversive paradigm, increased activity was seen in the presence of CS+ and slightly decreased activity in the presence of CS-.

These results are virtually the opposite of those we have seen in previous experiments where the electrodes were in the anterior dorsal lateral septum. We propose a possible division within the septal region regarding the mediation of positive and negative hedonic states.

This research was performed at The Weizmann Institute of Science.

150.18 MECHANISMS SUBSERVING SEPTAL AREA MODULATION OF AGGRESSIVE BEHAVIOR. M.Brutus, M.B.Shaikh, H.E. Siegel* and A. Siegel. Dept. of Neuroscience, University of Medicine & Dentistry of N.J.-New Jersey Medical School, Newark, New Jersey 07103.

We previously demonstrated that the septal area is capable of modulating hypothalamically-elicited quiet biting attack behavior in the cat (Siegel & Skog, Br. Res., 23: 371, 1970). The present study was undertaken as part of our ongoing analysis of the mechanisms underlying limbic system control of aggressive behavior. We specifically wished to determine: (1) whether stimulation of septal sites previously shown to modulate attack could modify the trigeminal sensory fields which are essential for its occurrence, and (2) the neural pathways that are activated following stimulation of septal area modulating sites utilizing ^{14}C-2-deoxyglucose autoradiography.

Under aseptic conditions, moveable stimulating electrodes were stereotaxically implanted into the hypothalamus from which quiet attack could be elicited and into the septal area and adjacent regions from which modulation of this response could be achieved. Following identification of a modulating site, we determined the extent of the lipline, which after probing, could elicit a jaw-opening response during stimulation of the hypothalamus alone or during concurrent stimulation of the hypothalamus plus septal area. In the final aspect of the experiment, ^{14}C-2-DG was injected systemically and septal stimulation was applied for 45 min., applying a regimen of 30 sec. on and 30 sec. off. Brains were removed and processed for autoradiography.

We observed that stimulation of 18 septal area sites which significantly suppressed quiet attack also constricted either the ipsilateral or contralateral trigeminal sensory fields. However, septal stimulation did not alter the latency for jaw-opening responses. The autoradiography indicates that septal stimulation results in intense labeling of the diagonal band nuclei and hippocampal formation and less intense labeling of the preoptico-hypothalamus and prelimbic cortex.

The results suggest that the septal area modulates attack behavior, in part, by regulating the effective trigeminal sensory fields, utilizing a pathway which supplies the nuclei of the diagonal band.

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150.19 PERFORMANCE ON A LATERALIZED VISUAL DISCRIMINATION FOLLOWING NEGLECT PRODUCING UNILATERAL LATERAL HYPOTHALAMIC LESIONS IN RATS. J. E. Ackil* and G. P. Frommer. Dept. of Psychology, Western Illinois Univ., Macomb, IL 61455 and Indiana Univ., Bloomington, IN 47405.

Several behavioral mechanisms have been proposed to account for the contralateral "neglect" that follows unilateral lateral hypothalamic lesions (LH). Hoyman et al. (Physiol. Behav., 1979, 22, 139) used a lateralized tactile discrimination to show that neglect is associated with a deficit in making responses contralateral to the lesion. We here use that design with visual discriminative stimuli (SD), which permit accurate measurement of duration of observing and latency of responding.

Twelve adult male albino rats were trained to make an observing nose poke response at a hole in the front of a small, flat black painted chamber. This turned on a green LED located just below the rat's left or right eye level. Half the rats learned to make instrumental nose poke responses in a side hole on the same side as the SD (TT group) to get water reinforcement there; half had to respond at the side hole on the side opposite to the SD (TA Group). They were also tested for turning and visual and tactile orienting. When a rat learned the discrimination ($\leq$ 4 errors on two consecutive 32-trial days), it was anesthetized with ether and received 20 sec of 2 mA anodal current through a stainless steel electrode previously implanted in LH. The rats were retested for orienting and turning and were retrained on the discrimination. Terminal anatomical verification showed that the lesions were centered in LH at the level of the ventromedial nucleus.

Both TT and TA Groups showed deficits in visual and tactile orienting and turning contralateral to the lesion. They also showed a 3X decrease in correct responses contralateral to the lesion and a 7X increase in ipsilateral incorrect responses, regardless of whether the SD was contralateral (TT) or ipsilateral (TA) to the lesion. Both groups observed the SD for contralateral responding longer than the SD for ipsilateral responding, regardless of whether the SD was also contralateral (TT) or was ipsilateral (TA) to the lesion. The TT Group made ipsilateral responses faster than the infrequent contralateral responses. The TA Group made both ipsilateral and (rare) contralateral correct responses faster than incorrect responses. The TT Group sometimes made correct contralateral responses and then turned to the ipsilateral hole before the programmed water reinforcement arrived. For technical reasons the TA Group could not show this behavior.

Almost all the data can be interpreted as a deficit in initiating instrumental responses directed contralateral to the lesion. The remaining data suggest that the SD could trigger automatic responses organized low in the neuraxis.

150.20 OLFACTORY CONTEXT, SPONTANEOUS ALTERNATION, SOCIAL COHESION AND SEPTAL LESIONS IN MICE. M. Widmayer-York*, C.R. Goodlett, T. Moy*, R.G. Burright*, and P.J. Donovick. Dept. of Psychology and Center for Neurobehavioral Sciences, SUNY Binghamton, N.Y. 13901.

A number of behavioral changes are associated with lesions of septal nuclei. Some of these changes have been reduced or even eliminated as a result of specific manipulations in either the training or testing environment. In a T-maze over clean bedding, group housed rats with septal lesions typically fail to spontaneously alternate between the two choice arms, preferring one arm far more than the other. However, when the testing environment is altered by placing the T-maze over the rat's home cage litter shavings, lesioned animals show spontaneous alternation comparable to that of controls. The cues from the shavings apparently minimize the perseverative deficit related to the septal damage. Familiarity and social stimuli provided by the home cage shavings have been suggested as mediators of this effect.

Experiment 1 was designed to demonstrate the septal spontaneous alternation effect in mice. Heterogeneous male mice 90-120 days of age were given either bilateral septal lesions or control surgery. Half of the mice from each surgical condition were given 5 consecutive trials in a T-maze over clean shavings and the remaining half over home shavings. These mice displayed the same effects noted for rats; that is, there was a lesion x test condition interaction, with controls alternating more frequently than septals when tested over clean shavings, but with no difference over home shavings. If the effect of home cage shavings on rats with septal lesions is mediated by the social aspects of the home shavings, one would expect differential responses of septals and controls to situations involving direct social contact. Several investigators have shown an increase in social cohesion in rats with septal lesions, with fewer reports for mice.

Experiment 2 examined social cohesiveness in mice. Intact male mice, individually housed 1 week prior to and throughout testing were placed in pairs in an open field and social behavior was recorded. Half of the pairs then received bilateral septal lesions and half control surgery. The same pairs were retested again in the open field, pairing septals with septals and controls with controls. While the social interactions of controls remained relatively stable from pre- to post-surgical testing, septally damaged mice initially showed a marked increase in social contact post operatively. These results suggest that social cues have a different impact on lesioned animals and may be responsible for the lesion x environment effects seen in spontaneous alternation testing over clean or soiled shavings.

This research was supported in part by NSF (DAR7911233) to PJD and RGB.

150.21 LABELING KNIFE CUTS USED TO LOCATE THE CELLS OF ORIGIN OF AXONS NECESSARY FOR LORDOSIS BEHAVIOR. C. W. Scouten and C. W. Malsbury Dept. of Psych., Mem. Univ. of Newfoundland, St. Johns, NF, A1B3X9

One of the oost serious difficulties with using lesions or knife cuts to study localization of function is that, while it is possible to describe the size and location of the zone of necrosis around the lesion, this may be only the tip of the iceberg in terms of the total damage done. Most regions of the brain, especially the diencephalon and brainstem, are crossed by axons from many other parts of the brain. When some lesions in an area are effective and some are not, as is usually the case, it is frequently impossible to distinguish consistent differences in the lesion size or location that would explain the behavioral outcome.

We have dried a small amount of HRP on the .005" blade of a retractable wire knife (Labeling knife cut technique: Scouten et al, Br. Res. Bull, 1982) prior to making unilateral hypothalamic cuts about 2.5 mm long in a sagittal plane lateral and adjacent to the MFB at the level of the supraoptic commissures. In female hamsters, these cuts usually cause a severe deficit in lordosis in response to stroking of the contralateral but not the ipsilateral flank (Ostrowski et al., Physiol. Behav., 1981). Each female was tested for such a lateralized deficit and sacrificed 5 days post surgery. The brains were reacted with TMB to visualize HRP transported by the cut axons.

Damage was even more extensive than expected. An average of 80 cell groups per brain contained cells labeled with HRP reaction product. However, all but two of these cell groups can be ruled out as the source of the axons whose destruction produces the lateralized deficit. This is either because they were not labeled in the brains of some females which displayed the deficit, or were labeled as densely in the brains of some females which did not display a lateralized deficit as in the brains of females which did. The 2 areas in which cell labeling was related to the behavioral deficit were the posterior pretectal area and the deep layers of the superior colliculus. The posterior pretectal area has not previously been implicated in the control of lordosis. Lesions of the deep layers of the superior colliculus block lordosis in hamsters (Muntz et al, 1980). This area contains numerous cells that respond to lordosis-inducing stimuli (Rose, 1982).

The ventromedial nucleus (VMN) of the hypothalamus mediates a facilitation of lordosis by gonadal steroids. The VMN sends axons laterally through the plane of our cuts, and contained HRP labeled cells in all of our animals with behavioral deficits. However, the VMN contained similar numbers of labeled cells in the brains of 5 of the 7 females which showed no lordosis deficits. We conclude that cutting the VMN lateral efferents alone is not sufficient to produce the lateralized lordosis deficits observed.

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NUTRITIONAL AND PRENATAL FACTORS IN DEVELOPMENT I

151.1 EFFECTS OF DIETARY PHOSPHATIDYLCHOLINE SUPPLEMENTATION ON SENSORIMOTOR BEHAVIOR AND BRAIN BIOCHEMISTRY IN THE RAT, J.M. Bell*, and P.Lundberg. (SPON: T.Slotkin) Dept. of Psychology, Washington Univ., St. Louis, Mo. 63130

Research has shown that dietary administration of the acetylcholine (Ach) precursor, choline, produces elevations in Ach. This precursor approach to elevate brain Ach levels has largely used massive acute doses of either choline chloride or phosphatidylcholine (PC) in the adult. Thus, the purpose of the present investigation was to examine the effects of low level chronic PC exposure on choline acetyltransferase (CAT) activity levels, as well as a variety of behavioral and sensorimotor tasks in the developing and young rat.

Prior to breeding, during gestation and lactation dams were maintained on control (C), 2% PC enriched (2% PC), or 5% PC enriched (5% PC) diets. After weaning, offspring were maintained on their respective diets (control, 2% PC PRE/POST, 5% PC PRE/POST), or placed on the control diet (2% PC PRE, 5% PC PRE). Pups were sacrificed at various ages and either whole brain or forebrain sections were frozen for biochemical analysis.

Preweaning results indicated that animals receiving the 5% PC enriched diet weighed more, took longer to right, and showed delays in swimming development. Postweaning activity measures showed that animals exposed to PC enrichment the longest (2% PC PRE/POST, 5% PC PRE/POST) reared less in an open field situation, and emitted fewer stabilimeter counts than other animals. Rats exposed to 5% PC (PRE/POST) also had weaker neurological responses (placing and hopping) than other dietary conditions. Animals receiving long term 2% PC (PRE/POST) enrichment showed attenuated analgesia in a tail flick test, 60 and 90 minutes following morphine injection. Brain/body weight ratios were significantly reduced in animals receiving both 2% and 5% PC enrichment during preweaning life. CAT activity levels were also elevated in all animals receiving dietary PC enrichment by 42 days of age.

These data suggest dietary PC enrichment does influence offspring reflexive and sensorimotor behavior. Moreover, the extent of the effect was greater in those animals who received either high or long term dietary supplementation. These early effects may not be persistent. Although the question remains as to whether chronic low level dietary PC enrichment can modify later emerging cholinergic deficits, the evidence presented here implies that long term, low level PC supplementation may be necessary to effect such changes, and that there are some immediate consequences of this enrichment if begun in early life.

(Supported by NINCD Grant NS-11002)

151.2 DIFFERENCES IN PERINATAL SODIUM CHLORIDE INTAKE PRODUCE PERSISTENT CHANGES IN FLUID CONSUMPTION OF ADULT RATS. Edythe Bird* & Robert J. Contreras (SPON: Christie Sahley). Yale Univ., Dept. of Psychol., New Haven, CT 06520

To examine further the effects of early salt intake on body weight and fluid balance, nine adult female rats were maintained on a diet containing either 0.12% (low), 1.0% (mid), or 3.0% (high) sodium chloride (NaCl) throughout pregnancy and lactation. At parturition, litters were culled to eight pups (approximately 4 male and 4 female) per mother; the remaining pups were decapitated to obtain blood samples. This resulted in 15 males and 9 females in the low salt group, 14 males and 9 females in the mid salt group, and 13 males and 11 females in the high salt group. The offspring were weaned on day 22 but were continued on these same diets until 30 days postpartum. Thereafter for the duration of the experiment all the offspring were maintained on the mid (1%) salt diet.

At birth, the body weights and serum sodium levels of the offspring were linearly related to dietary sodium content (males: low-6.11±.10g, mid-6.42±.17g, high-6.69±.14g; females: low-5.39 ±.15g, mid-5.90±.17g, high-6.08±.14g). At weaning and at 30 days of age, both the low and high salt animals were lighter than the mids. Despite these differences, dietary salt had only an immediate effect on body weight; during maintenance on the control diet between 30 and 200 days of age, neither the body weights nor the rates of weight gain of the animals differed.

Several assessments of fluid intake were made beginning at 90 days of age, including (1) measurements of 24-hr water intake, urine volume, and urine electrolyte levels, and (2) two-bottle preference tests between deionized distilled water and one of two test solutions, 0.1M glucose and 0.3M NaCl. The low salt males showed greater fluid intakes and urinary sodium levels, and lower preferences for NaCl. The high salt males showed lower fluid intakes, greater urinary sodium levels, and greater preferences for NaCl. These effects were not, however, evident in females.

We suggest that early consumption of a diet either low or high in NaCl causes the male offspring to respond as if the set point for osmoregulation has changed. Although this was not investigated, the sex differences may be accounted for by (1) hormonal influences on fluid intake of female rats, (2) the greater susceptibility of male rats to environmental influences, or (3) sexual dimorphism of brain areas thought to be involved in water regulation.

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151.3 NUTRITIONAL TAURINE DEFICIENCY IN THE CAT RESULTS IN RETARDED DEVELOPMENT OF THE OFFSPRING. J. A. Sturman, H. S. Rowe*, and R. C. Moretz*. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314.

Taurine is an essential amino acid for cats, and if not supplied in the diet, they become taurine depleted and suffer retinal and tapetum degeneration. Nursing cats supply the kittens with all of their nutrition, including taurine, which comprises the major portion of the free amino acid pool of cat's milk, presumably to supply this great need during development. We have examined the effect of nutritional taurine on the development of kittens in the following ways: depriving the mother of taurine for a long period of time (prior to conception); depriving the mother for a few days prior to birth; and orally supplementing the kittens with taurine after birth. These procedures allow the effects of taurine depletion to be examined during the whole developmental period (prenatal plus postnatal), and the prenatal and postnatal effects to be separately dissected out.

The prenatal effects are considerable, resulting in reduced growth and development of the fetuses, including malformations, and a high incidence of abortions and stillbirths. All of these consequences are associated with severely reduced tissue taurine concentrations. Postnatally, taurine deficiency results in reduced overall growth including reduced brain growth. The cerebellum is particularly affected by nutritional taurine deficiency, having a greater growth retardation than other areas of the brain. This growth retardation appears to be associated with a delayed migration of cerebellar granule cells. These kittens have also decreased hind limb muscle control, and easily visible kyphoscoliosis. Taurine-depleted kittens also suffer from degenerating and disorganized photoreceptors, retinal ganglion cells, and tapetal cells.

These preliminary results suggest that taurine is involved in the normal migration of cerebellar granule cells, and that a deficiency during development may retard this process and lead to a cascade of abnormalities. This constellation of signs and symptoms in taurine deficient kittens resembles many of those seen in the human disease, Freidreich's Ataxia.

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151.4 PRENATAL EXPOSURE TO DRUGS OF ABUSE IN HUMANS: EFFECTS ON PLACENTAL NEUROTRANSMITTER RECEPTORS V. May, B.D. Perry, P.H. Kussie*, D.J. Pesavento*, S.H. Schnoll*, and D.C. U'Prichard
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Recently the concept of behavioral teratology has evolved (e.g. Vorhees et al, Science 205: 1220, 1979). In animal models, prenatal exposure to low doses of psychotropics (e.g., methadone, diazepam) results in abnormal development of behavior. Furthermore, in animals, pre- and perinatal exposure to psychoactive drugs results in altered brain neurochemistry (e.g., Slotkin et al, Life Sci., 24: 1223, 1979). In humans, similar behavioral and neurological disruptions have been reported (e.g., Dinges et al, Science 209: 620, 1979). The mechanisms responsible for these effects are unknown; however, all of these psychotropics have high affinity, specific interactions with various neurotransmitter receptors (NRs). Furthermore, normal development of nervous tissue appears to be mediated through NRs. Disruption of normal, receptor-mediated "signals" by psychoactive agents would be expected to alter development of brain. In order to examine the regulation of NRs by centrally-acting agents, we have compared placental NRs from "control" (C) and substance-abusing (SA) women, hypothesizing that 1) placental NR regulation may mirror fetal brain NR regulation and 2) placental NRs themselves may play a role in development. Standard radioligand binding methods were employed; 5-7 concentration saturation studies resulted in the following values (receptor: radioligand, and K_D (nM), B_{max} (fmol/mg prot) values for C, caesarian, no labor (CS), opiate (O), ethanol (E), amphetamine (A), and phencyclidine (P) placentas). α_1: ^{3}H-prazosin, C: 0.43, 30.5; CS: 0.58, 65.5; O: 0.48, 12.6; E: 1.0, 12.1 α_2: ^{3}H-rauwolscine, C: 2.4, 24.5; CS: 1.9, 37.6; O: 1.6, 42.0; E: 2.2, 18.0; A: 2.0, 180; P: 1.1, 61.5. β: ^{125}I-iodocyanopindolol, C, 0.065, 132; CS: 0.05, 181; O: 0.13, 51.3, A: 0.14, 106. μ-opiate: ^{3}H-naloxone, C: 1.7, 26.9; CS: 0.66, 38.1; O: 1.8, 57.0; E: 1.8, 33.5, A: 0.7, 90.9; P: 1.6, 51.3. Control values were relatively consistent while SA values were inconsistent with wide variation in each SA group, possibly indicative of "regulation" of NRs yet certainly complicated by other variables surrounding gestation and birth (e.g., drug dosage and schedule, length of labor). These preliminary results demonstrate 1) endogenous agonist exposure during labor may "regulate" NRs in placenta (fetal brain as well) 2) psychoactive agents may regulate placental NRs and developing fetal NR-neurotransmitter systems. More extensive characterization of C and SA placental NRs are required and are in progress.
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151.5 PRENATAL STRESS REDUCES HIPPOCAMPAL BENZODIAZEPINE BINDING SITES AND ABILITY TO FUNCTION IN STRESSFUL CONDITIONS AT ADULTHOOD.

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Prenatal stress has been found to influence several behaviors of the offspring as adults. Exposure of adult rats to a stressful situation has been shown to reduce the number of benzodiazepine (Bz) binding sites. In the present study we investigated the hypothesis, that prenatal stress may interfere with the development of the septo-hippocampal system, thereby impairing the ability to cope with stressful situations in adult life. Rats were exposed to noise (100 db) and flashing lights on a random basis, for 4 hour periods, 3 times a week throughout pregnancy. Pregnant controls were left undisturbed. The female offspring were mated at the age of 6-8 weeks and maternal behavior was assessed when their pups were 4-5 days old. 7 pups of each litter were separated from the mother in the home cage by a barrier and alley of 60x10 cm. The time taken for each mother to return all pups to the home cage was determined twice: under normal conditions and during stressful conditions in which the mother had to pass through an air stream in order to retrieve the pups. Under control conditions, both prenatally stressed (PS) and control (C) mothers retrieved all pups within 5 min. However, when stressed, only 50% (11/21) of the PS mothers retrieved their pups within 15 min, as opposed to 96% (26/27) of the C mothers (χ^2=12.9; p<0.001).

The no. of specific binding sites for ^{3}H-flunitrazepam in the hippocampus of the female PS and C rats was measured at the age of 3 months. The PS offspring exhibited a significant decrease in the Bmax (from 660 to 385 fmol/mg protein) with no change in k_d (1.5 nM). We conclude from these results that prenatal stress reduces the number of Bz binding sites and causes a permanent alteration in hippocampal function in the female offspring. This is associated with an impaired ability to function under stressful conditions.

151.6 DIETARY REHABILITATION OF MALNOURISHED RATS: EFFECTS ON BRAIN LIPID CONCENTRATIONS. Paula K. Lundberg, C. Robert Almli, Peter J. Morgane, Michel Motamedi* and Daniel Gattegno.* Univ. of Texas, Tyler, Texas 75701, Washington Univ. Med. School, St. Louis, MO 63110 and Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Various methods for producing malnutrition in rats have been shown to alter the concentrations of brain cholesterol (CHOL) and phospholipid (PHOS). Although early protein restriction appears to reduce the total amounts of CHOL and PHOS present in brain, it does not consistently reduce the concentrations of these brain lipids. As part of our effort to develop animal models of the marasmic child and the small-for-gestational-age infant, we have been investigating the impact of early protein restriction upon telencephalon (TEL), CHOL and PHOS concentrations. Previously, we have reported that combined prenatal and postnatal (PN) exposure to a 6% protein diet resulted in reduced TEL CHOL concentrations on PN day 35, but not on PN day 21 whereas TEL PHOS concentrations were reduced on PN day 21 only. The purpose of the present study was twofold; to replicate our previous findings, and more importantly, to assess the effect of PN dietary rehabilitation upon TEL CHOL and PHOS concentrations.

Prior to breeding, during gestation and lactation dams were maintained on normal (N) (25% casein) or protein-restricted (PR) (6% casein) isocaloric (4.3 kcal/g) diets. After weaning pups were maintained on their respective diets. Dietary rehabilitation (R) was effected by cross-fostering, prenatally, PR pups to N dams at birth. Pups were sacrificed on PN days 1, 21, and 35, their brains removed, dissected, and frozen for lipid analysis. Lipids were extracted during homogenization as described by Folch. CHOL was analyzed enzymatically and PHOS was analyzed using the method of Fiske and Subbarow.

Body weights and TEL weights of the PR group were reduced relative to the N group at all time points ($p<.001$). CHOL concentrations were actually higher in the PR group at birth ($p<.001$) but significantly reduced on PN21 ($p<.001$) and PN35 ($p<.01$). PHOS concentrations were also higher in the PR pups at birth ($p<.01$) but no different from the N group on PN21 or PN35. While dietary rehabilitation consistently improved the body weights ($p<.001$) and TEL weights ($p<.001$), relative to PR pups, body weights were still less ($p<.001$) than those of the N group at both time points. TEL weights of the R group, however, were no different from N pups. Yet, in spite of ameliorating TEL weights, dietary rehabilitation did not reverse the deficits in brain CHOL or PHOS concentrations due to prenatal protein restriction. Thus, prenatal protein restriction appears to result in significant reductions in TEL CHOL and PHOS concentrations that cannot be reversed by PN days 21 and 35 even when rehabilitation is instituted at birth.

151.7 SEX AND STRAIN DIFFERENCES IN THE DEVELOPMENTAL ACTIVITY PROFILE OF RATS PRENATALLY EXPOSED TO SODIUM SALICYLATE. J. Buelke-Sam, C.A. Kimmel*, C.J. Nelson* and P.A. Sullivan*, Divisions of Teratogenesis Research and Biometry, National Center for Toxicological Research (FDA), Jefferson, AR 72079.

Administration of large doses of salicylates (>250 mg/kg) to pregnant animals during organogenesis has been shown to result in malformations of the CNS and skeleton as well as several internal organs. At lower doses, it is possible that the functional integrity of these systems may be altered in the absence of gross malformations. Perinatal exposure to a variety of agents has been shown to result in enhanced and/or prolonged juvenile hyperactivity in rodents. Therefore, as an indicator of CNS function, the developmental pattern of locomotor activity was monitored in the offspring of Sprague-Dawley (CD) and Long-Evans (LE) rats treated by gavage on days 8-10 of gestation with either 0, 125 or 175 mg/kg/day sodium salicylate (NaS). No malformations were observed in any of the offspring usea in this study. A total of 51 CD litters were tested in 3 replicates and 42 LE litters in 4 later replicates. On postnatal day (PND) 1, litters were culled to 8 pups (4+2 of each sex) following normal delivery. The apparatus and general procedure used for activity testing has been described (Buelke-Sam and Kimmel, Neurosci. Abst., 6:631, 1980). Offspring were tested for 30 min on PND 12, 16, 20, 24, 30, 60, 90 and 120. Half of each litter was tested over clean bedding (C) and half over shavings removed from each pup's home cage (HC) through PND 24 in CD rats. At later ages all CD offspring were tested over C. The dichotomy of bedding condition (C vs HC) was maintained at all ages for LE offspring. A repeated measures ANOVA on log transformed data showed main effects on activity levels due to strain at > PND 20 (CD > LE), sex (males > females at < PND 16; females > males at > PND 60) and bedding condition at < PND 20 (HC generally > C). Significant overall effects due to prenatal dose of NaS were found only at PND 24 and 30 with NaS-treated LE rats tending to show decreased activity and prenatally-exposed CD rats tending to be more active than controls at these ages. When separate analyses were conducted for each strain, sex and bedding condition, highly significant age effects were found for all groups. A main effect due to prenatal dose of NaS was found only in LE males tested over C, with treated rats being less active than controls at > PND 30. Significant dose by age interactions were found for LE males tested over HC and also LE females and CD males tested over C, suggesting that prenatal exposure to low doses of NaS may produce subtle alterations in developmental activity, the pattern of which is dependent on strain, sex and bedding condition during testing.

151.8 THE EFFECTS OF PROTEIN MALNUTRITION ON SLEEP STAGE RESPONSE TO LIGHT-DARK CYCLES IN RATS OF THREE AGE GROUPS. L. Cintra, W. Forbes, S. Diaz-Cintra and P.J. Morgane. Worcester Found. for Expt. Biol., Shrewsbury, MA 01545 and Instituto de Invest. Bio-Medicas. UNAM, Dpto. de Fisiologia, C.P. 04510 Mexico, D.F.

In these studies we used a 12/12 light/dark cycle in rats at 60, 120 and 220 days of age fed either an 8% or 25% casein diet. We found that the vigilance states follow a clear developmental trend during this ontogenetic period, i.e., show a reduction of waking and REM sleep activity and an increase in slow-wave-sleep as a function of age. Malnutrition in the rat produces significant changes in the pattern of age related changes in various vigilance parameters and also affects responses of the animals to changes in the light-dark cycle. In order to obtain information on the effects of malnutrition on the distribution of the vigilance states, we analyzed several periods during 24 hours of continuous recording, i.e., 12 hours light, 12 hours dark analyzed in 4 hour blocks. We found most significant changes in the vigilance states in 8% rats in the lights-on period only at the older ages (220 days). The most striking finding in the malnourished animals is a significant reduction in waking and a significant increase in slow-wave-sleep and REM sleep at 220 days of age during the lights-on period. In the lights-off period the malnourished animals show a significant decrease in REM sleep at 220 days of age but no changes at 60 and 120 days. We also found that 8% rats at 220 days of age, studied in 4 hour blocks,show significant changes in vigilance cycles only in the first and last 4-hour block of the lights-off period indicating that malnourished rats react differently to light-dark transitions (or anticipated transitions). Light/dark ratios in 8% animals showed significant differences in slow-wave-sleep at 120 days and waking at 220 days suggesting that the amplitude of the circadian rhythms of slow-wave-sleep and waking is different in 8% versus 25% rats at these two age periods. These findings indicate that malnutrition may be having an effect on the endogeneous generator mechanism of the vigilance states since the changes observed in normals vs. malnourished in the three vigilance states in the first and last 4-hours of lights-on period (when lights go on or are anticipated to go off) and in the last 4-hours of lights off period (when animal is predicting lights will go on). The changes observed in malnourished animals to entrainment of lights-on and lights-off periods suggest that malnutrition affects structures that control vigilance transitions in response to light-dark cycles seemingly by delaying their maturational processes in the 8% diet group of animals. Further studies, aimed at revealing some of the underlying mechanisms of these findings, are in progress on the effects of malnutrition on sleep cyclicity. (Supported by NIH Grant HD-06364).

151.9 THE EFFECT OF PROTEIN MALNUTRITION ON THE HIPPOCAMPAL EEG OF THE DEVELOPING RAT. K.B. Austin*, C.J. Siok*, J.D. Bronzino, O. Resnick and P.J. Morgane. (SPON: W.L. McFarland). Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

Protein malnourished pups were born to dams fed a 6% or 8% casein diet initiated 5 weeks prior to mating. Pups born to dams fed the 8% casein diet had brain and body weights at birth that were no different from the control (25% casein) group (Forbes et al., Brain Res. Bull., 2:131-135, 1977). Dams subjected to the 6% casein diet yielded pups with significantly lower brain (P<.05) and body weights (P<.001) than the controls (Resnick et al., Neurosci. Biobehav. Rev., 6:55-75, 1982). In order to study the effects of these levels of protein malnutrition on hippocampal development, power spectral analysis was used to analyse the hippocampal EEG of animals in the three diet groups at 14, 18, 22, 30 and 45 days of age. Two indices derived from the power spectrum of the hippocampal EEG proved to be sensitive indicators of maturation. Peak-Theta frequency represents the frequency within the Theta band (4-11 Hz.) at which the power spectrum exhibits a local maximum. Total spectral power represents the sum of power spectral values within a frequency band. The spectrum was divided into 4 frequency bands: 1- Delta band (0.5-3.5 Hz.), 2- Tonic or low Theta band (4.0-7.0 Hz.), 3- Phasic or high Theta band (7.5-11.0 Hz.) and 4- a high frequency band (11.5-32.0 Hz.). We have previously shown that, as the normal rat develops from 14 to 45 days of age, the power spectrum of the hippocampal EEG during REM sleep becomes concentrated in the Theta band (indicated by a greater total spectral power in the 4-11 Hz. range). This is accompanied by a highly significant (P<.001) shift in peak Theta frequency from 4.4 Hz. at 14 days of age to 6.6 Hz. at 45 days of age. The concentration of power in the Theta band is marked by an increase in total power within the phasic component (7-11 Hz.). Animals reared on 6% and 8% casein diets show similar spectral concentrations during maturation, but peak Theta frequency lags behind that of control animals, with the greatest lag being that of the animals reared on a 6% casein diet. Experiments are underway to examine the brainstem-septo-hippocampal system to determine how protein malnutrition alters its functional development during the vigilance states. (Supported by NIH Grant HD-06364).

151.10 THE EFFECT OF PRENATAL PROTEIN MALNUTRITION ON THE DEVELOPMENT OF THE SLEEP-WAKING CYCLES IN THE RAT. Chester J. Siok*, Joseph D. Bronzino, Kevin Austin* and Peter J. Morgane, (SPON: E. Shaskan). Trinity College, Hartford CT, 06106, and Worcester Foundation for Experimental Biology, Shrewsbury, MA, 01545.

In order to understand the implications of prenatal protein malnutrition on the developing rat brain, we have studied sleep behavior in normal and protein deprived rats at various ages. The present study examines the sleep-waking profile in rat pups born to dams fed either a normal diet (25% casein) or a protein deficient diet (6% or 8% casein) throughout a 5 week pregravid period, pregnancy and lactation. After weaning (22 days of age) offspring were maintained on the same diet as the mother. Animals were tested at the ages of 14, 18, 22, 30 and 45 days when chronic EEG recordings from the hippocampus and frontal cortex were monitored and used in determining the sleep-waking behavior of the animal. Our results indicate that the most significant effects of prenatal protein malnutrition occur during REM sleep. Slow-wave sleep, on the other hand, is not affected. It was found that the 8% animals (when compared to the 25% animals): (1) spent significantly less time in REM sleep (p<.01), (2) had fewer REM episodes (p<.01), (3) had shorter REM episodes (significant only at 18 days, p<.01), (4) spent more time between REM episodes (significant only at 18 days; p<.01) and (5) spent more time in the waking state (active and quiet combined) (p<.05) at 14 and 18 days of age. After weaning no statistically significant differences were noted. In the case of the 6% animals, tests were conducted after weaning. At 22 days of age, it was found that the 6% animals (when compared to the 25% animals and 8% animals): (1) spent less time in REM sleep (p<.08), (2) had shorter REM episodes (significant only vs. the 25% animal, p<.02) and (3) spent more time in the waking state (p<.01). By 45 days of age, no statistically significant differences were noted. These results indicate that prenatal protein malnutrition alters the vigilance profile of the rat primarily during the preweaning stages of development. From this data it can be postulated that derangments in those neuronal structures involved in the REM sleep control system may be effected by prenatal protein malnutrition.

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151.11 COMBINED PRENATAL EFFECTS OF ETHANOL AND NICOTINE: BRAIN MYELIN AND BEHAVIOR, F. E. Lancaster, M. F. da Cunha* and C. Johnson*. Biology Department, Texas Woman's University, Houston, Texas 77030

Pregnant Long Evans rats were received on day 5 of gestation and divided into four groups. Animals were group fed to control for nutritional influence as follows: (1) Nicotine-Ethanol (NE) received 27% calories as ethanol from Sandra Weiner ethanol diet (1226-PR-A, Bio Serve) (days 5-15 of gestation) mixed with Dutch Chocolate Sego, and 2 mg nicotine/kg/day via Alzet minipumps (days 4-19 of gestation); (2) Nicotine (N) received Sandra Weiner Control diet (1226-PR-C, Bio Serve) mixed with Dutch Chocolate Sego (days 5-15 of gestation) and 2 mg nicotine/kg/day via Alzet minipumps (days 4-19 gestation); (3) Ethanol (ET) received 27% of calories as ethanol in ethanol diet and were implanted with saline filled minipumps; (4) Controls (LQ) received control diet and saline filled minipumps. Nicotine-Ethanol animals were allowed ad libitum access to the diet; all other groups were fed diet isocaloric to NE consumption. Following day 19 of gestation, all animals received lab chow and water ad libitum. animals were observed for gross anomalies at birth; time of eye opening was recorded on days 14-16. Groups of offspring were sacrificed at 16, 21, 30 and 52 days of age. Organ weights, myelin accumulation and levels of spermatozoa and testicular DNA were measured. A battery of behavioral tests was administered days 7-20. Brain myelination in female ethanol and nicotine offspring was significantly delayed. Eye opening and development of several behavioral parameters were delayed in offspring of nicotine and ethanol dams. Levels of spermatozoa and testicular DNA were not affected.

151.22 SYNAPTIC MEMBRANE PHOSPHOLIPIDS IN DEVELOPING CONTROL & ETHANOL RATS. J. H. Hofteig, A.B. Noronha*, M.J. Druse-Manteuffel and C. Keresztes-Nagy. Depts. of Biochemistry & Biophysics and Medicine, Loyola Univ. Med. Ctr., Maywood, IL. 60153.

In the present study the phospholipid composition of synaptic plasma membranes (SPM) was determined in the developing offspring of rats that were pair-fed on a chronic basis (prior to and during gestation) control or isocaloric 6.6% (v/v) ethanol liquid diets, containing 21% protein (Noronha & Druse, 1982). At 17,24 and 31 days of age SPM were isolated from control and ethanol pups (Cotman & Matthews, 1973). Lipids were extracted from SPM (Folch et al., 1957) and separated into neutral, glyco- and phospholipids (Rouser et al., 1967). Total phospholipids were quantitated (Gottfried, 1967) and separated by 2-dimensional TLC (Horrocks & Sun, 1972, as modified by de Sousa & Horrocks, 1979).

In both control and ethanol pups the major SPM phospholipid was phosphatidyl choline (PC) which accounted for > 40% of the phospholipid phosphorus. A small but significant developmentally-related decrease in PC concentration was observed. Choline plasmalogens were not detected. The ethanolamine phosphoglycerides comprised the second most prominent group of SPM phospholipids. Phosphatidyl ethanolamine (PE) accounted for approximately 10% of the phospholipids at all ages and ethanolamine plasmalogen accounted for 5% (17 days) to 15% (31 days) of the phospholipids. Phosphatidyl serine (PS) + phoshatidyl inositol (PI) comprised 12-16%. Small quantities of sphingomyelin (4-7%) and phosphatidic acid (< 2%) were also found.

Although the yield of SPM (as assessed by protein content) was comparable in ethanol and control pups, the composition of the SPM was affected in the ethanol pups. SPM from 17 day ethanol pups had a lower concentration of total phospholipids than SPM from control rats (0.80 vs 0.96 µg phospholipid / µg protein). In addition, the normal developmentally-related increase in the proportion of ethanolamine plasmalogen was delayed in ethanol pups. Whereas the control animals demonstrated an increase in the proportion of ethanolamine plasmalogen between 17 and 24 days of age, the ethanol pups did not demonstrate an increase until 31 days. The results of the present study suggest that there may be a delay in the maturation of synaptic membrane phospholipids in ethanol pups.

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VESTIBULAR COMPENSATION AND BRAINSTEM PATHWAYS

152.1 THE EFFECT OF SPINAL CORD TRANSECTION ON MEDIAL VESTIBULAR NEURONS OF COMPENSATED HEMILABYRINTHECTOMIZED GERBILS. T.J. Clegg* and A.A. Perachio, Depts. Otolaryngol. and Physiol. & Biophys., Univ. of Texas Medical Branch, Galveston, TX 77550

Unilateral labyrinthectomy results in characteristic acute symptoms. Following vestibular compensation, the reappearance of those symptoms (decompensation) can be produced by spinal cord transection (Azzena, Arch. Ital. Biol. 107: 1969). Neurons of the medial vestibular nuclei (MVN) on the injured side exhibit activity changes during compensation (Precht, et al., J. Neurophysiol. 29: 1966). Decompensation is paralleled by changes in neuronal activity in the lateral (Azzena, et al., Experientia 3: 1977) and descending (Jensen, Neurosci. 4: 1979) vestibular nuclei. The purposes of this study were to measure in compensated hemilabyrinthectomized gerbils with or without cord damage the activity of MVN neurons and their responses to sinusoidal angular acceleration. Recordings were made from MVN neurons bilaterally in 24 decerebrate gerbils from each of the following groups: 1) acute labyrinthectomized , 2) compensated , 3) compensated and acute spinal cord transected , 4) non-labyrinthectomized, and 5) non-labyrinthectomized spinal cord transected. As reported previously, acute labyrinthectomy eliminated type I neurons on the injured side. No other significant changes in spontaneous activity were observed in type I neurons on the intact side or type II neurons bilaterally. The influence of the spinal cord on type I and II MVN neurons differed for measures of both spontaneous activity and dynamic responses to yaw angular acceleration. Sample sizes were not sufficiently large for statistical comparison in all groups. The gains of the responses of type I neurons were significantly lower following cord damage in compensated animals than in the non-labyrinthectomized spinal cord transected group even though spontaneous activity was statistically equivalent in both samples. Type II neurons on the intact side exhibited significant gain increases following cord damage or acute labyrinthectomy. In compensated animals, cord damage resulted in a gain decrease on the intact side and a gain increase on the injured side. Cord transection in non-labyrinthectomized animals produced a significant decrease in the firing activity of type II neurons but did not influence type I neurons. In compensated animals transection of the spinal cord resulted in an increase in the firing rate of type II neurons on the intact side but no statistical change in the activity of type I neurons. These results suggest a significant contribution of type II neurons to vestibular compensation.

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152.2 QUANTITATIVE 2-DG STUDY OF THE BRAINSTEM OF THE RAT DURING NYSTAGMUS PRODUCED BY LESION OF THE LATERAL SEMICIRCULAR AMPULLA. F.A. Kutyna, J.W. Patrickson*, H.J. Bryant, M. Kadekaro and J.B. Clark*. Depts. of Physiology and Neurosurgery, Uniformed Services University, Bethesda, MD 20814 and Lab Cerebral Metabolism, NIMH, Bethesda, MD 20205.

Brain functional activity of male Sprague-Dawley rats was studied using the quantitative 2-deoxy-D-glucose (2-DG) autoradiographic method. Under halothane anesthesia the left horizontal canal was exposed and a fine wire was inserted to destroy the crista ampullaris. In sham-operated controls the canal was not opened. The bone was sealed with wax and the animals allowed to recover 2 hrs before 2-DG injection. The lesioned rats showed nystagmus with the fast phase to the right side. The animals were unrestrained during the 45 minutes following 2-DG injection and exhibited postural signs and circling movements consistent with this lesion.

Glucose Utilization (µM/100 g/min)

	Experimental		Control	
Structure	Left	Right	Left	Right
Abducens N.	85.9±1.9*†	116.3±1.9	113.4±1.9	112.7±1.9
Ambiguus N.	52.6±0.7*†	49.2±0.7§	73.8±0.7	73.7±0.7
Cerebral Cortex	49.2±1.6	48.6±1.6	54.6±1.4	53.7±1.4
Cerebellar Nodulus	127.8±2.2*†	85.9±1.2§	100.3±1.2	101.3±1.2
Cerebellar Vermis	98.6±0.8	99.8±0.8	92.3±0.8	92.7±0.8
Dentate N.	84.4±2.0	82.4±2.0	95.1±2.0	97.8±2.0
Fastigial N.	75.3±0.5†	77.7±0.5§	86.4±0.5	88.2±0.5
Medial Longitudinal Fasciculus	93.6±1.8*	77.2±1.8§	94.7±1.8	95.9±1.8
Interpositus N.	84.4±5.6	81.1±5.6	92.9±6.3	93.6±6.3
Vestibular Complex	89.8±1.6*†	122.2±1.6§	116.6±1.6	116.1±1.6
Oculomotor N.	90.4±0.9*†	85.4±0.9§	94.8±0.9	94.7±0.9
Substantia Nigra	61.0±0.7	60.2±0.7	63.9±0.7	64.8±0.7
Interstitial N. Cajal	91.0±1.7*†	75.2±1.7	76.9±1.7	77.4±0.7
Trochlear N.	87.4±1.3	87.8±1.3	88.1±1.3	87.5±1.3
Olivary N.	70.4±1.3	71.4±1.3	66.0±1.3	69.4±1.3
Lateral Reticular N.	42.8±0.9	43.1±0.9	48.0±0.9	47.7±0.9
Prepositus N.	99.4±3.4	107.4±3.4	82.5±3.2	81.9±3.2
Ventral Posterior Lat. Thalamic N.	91.8±1.9*	83.3±1.9§	91.7±1.9	92.6±1.9
Anterior Ventral Thalamic N.	108.3±1.9	105.6±1.9	120.9±1.7	118.8±1.9

Significantly different at the $p<0.05$ level; * Experimental left vs. experimental right; † Experimental left vs. control left; § Experimental right vs. control right.

These changes are consistent with the neuroanatomy of the vesitbular system.

152.3 FURTHER STUDIES OF OTOLITH-SPINAL REFLEX ADAPTATION TO ALTERED GRAVITY: VISUAL CONTRIBUTIONS AND EFFECTS OF HEAD ORIENTATION. D.G.D. Watt and L.M. Tomi*, Aviation Medical Research Unit, Dept. of Physiology, McGill Univ., Montreal, Quebec, Canada H3G 1Y6.

In previous studies, it has been shown that(1) gastrocnemius-soleus emg activity occurring 50 to 150 msec after the onset of a sudden fall is predominantly otolith-spinal in origin, (2) the size of this response is reduced by rotating the gravity vector 90° relative to the body, and (3) the response steadily increases during prolonged exposure to the supine position. The present experiments were designed to test the contribution of vision to this otolith-spinal reflex adaptation and to measure the effects of different head angles during testing.

Eight subjects were exposed to sudden, unexpected vertical falls of 15 cm while surface emg activity was recorded from their left calf muscles. Each was then tested in the supine position every 60 mins for 8 hrs, substituting for gravity with elastic cords running from a torso harness to the wall. Finally, vertical testing was repeated immediately after leaving the supine position and every 60 mins for 4 hrs. The control, first and last horizontal, and first and last vertical test sessions consisted of 45 drops (1 drop eyes open, 1 drop eyes closed, 1 drop neck flexed forward 45° and looking 'down', all repeated 15 times). All other test sessions consisted of 15 drops with eyes open only. EMG activity was rectified, averaged for each set of 15 drops (eyes open, eyes closed or neck flexed), and the area under the resulting curve was measured from 50-150 msec after release. All data were then normalized with respect to the eyes open controls.

All subjects demonstrated a significant ($P<.001$) reduction of the response to sudden falls on assuming the supine position (average response reduced to 28%). After 8 hrs, the response was significantly larger ($P<.05$), averaging 74%. Contradicting the previous, more limited experiments, the reflex was found to return to its control value immediately after re-assuming the vertical position ($P>.7$). Closing the eyes, or flexing the neck and looking down, had no effect on any aspect of these findings (P always $<.05$).

These results confirm the previous finding of adaptation of an otolith-spinal reflex to prolonged exposure to the supine position, but do not demonstrate any residual effects on returning to the vertical position. They suggest that the increased reaction to falls after 8 hrs supine is not due to an enhanced visuo-spinal reflex, or increased visual facilitation of the otolith-spinal response. The reaction to sudden falls occurs irrespective of head orientation relative to the test acceleration, and this is maintained even during adaptation, which therefore must involve all parts of the otolith system.

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152.4 CROSS-MIDLINE COUPLING OF THE VESTIBULAR NUCLEI: A PUTATIVE SITE FOR VESTIBULAR COMPENSATION. H. Flohr*, Dept. Neurobiology, Univ. Bremen, Bremen, F.R.G., H.L. Galiana and G. Melvill Jones, Aviat. Med. Res. Unit, Dept. Physiology, McGill Univ., Montreal, Quebec, Canada H3G 1Y6 (SPON: R. Capek).

Compensation following unilateral labyrinthectomy is associated with restoration of symmetry in the activity of the vestibular nuclei (VN), and with synaptic changes along commissural pathways (e.g. 1,2). However, seen in the usual context of push-pull, feedforward, commissural connections, these findings cannot alone explain observations in various species following a second peripheral loss (the Bechterew phenomenon), or the decompensatory effect of commissurotomy (3) in frog. Thus other processes are often postulated as being also involved, though none can satisfactorily reconcile all observations. For example, general supersensitivity of the primarily deafferented VN is often proposed as an explanation for the Bechterew phenomenon, but it does not explain the decompensatory effect of commissurotomy.

Recent neuroneurophysiological and neuroanatomical observations indicate that some commissural pathways linking the bilateral VN form positive feedback loops for signals between the two sides (4,5). As a result, we will show that observed synaptic changes in commissural pathways, if placed at the level of such closed loops, are quite sufficient (though not necessarily exclusive) to explain acute and compensated stages after unilateral labyrinthectomy, the Bechterew phenomenon and its subsequent compensation, and the loss of compensation after commissurotomy. Such a site for vestibular compensation would also be compatible with the fact that balance recovery is still possible after vestibulocerebellectomy (6). This is relevant to clinical diagnosis in man, since the observation of spontaneous nystagmus could result either from a central lesion, or from a peripheral lesion/recovery such that central commissural gains are no longer appropriate for the new input levels.

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152.5 A MODEL RECONCILING CENTRAL OBSERVATIONS DURING PLASTICITY OF THE VESTIBULO-OCULAR REFLEX (VOR). H.L. Galiana, Aviation Medical Research Unit, McGill Univ., Montreal, Quebec, Canada H3G 1Y6

There is evidence that some commissural pathways linking the bilateral vestibular nuclei (VN) provide a positive loop for signals between 2° vestibular cells on both sides of the brainstem (1,2). A bilateral model of the VOR has been developed, showing that such commissural loops would contribute to the realization of the VOR's central integrator during compensatory slow phases (3). Hence any changes in intervestibular coupling strength should modify both the gain and phase of the VOR. Changes in commissural efficacy have been reported after compensation for unilateral vestibular lesions (4), associated with parallel improvement in the VOR. It follows that commissural changes should be investigated as a possible site for long-term (days) VOR plasticity in the healthy system, e.g. induced with optical devices.

Analytic studies are applied to a model of the VOR including the postulated commissural loop, feedback of an efference copy of eye position to 2° VN cells, and cerebellar Purkinje cell (PJ) pathways. Results indicate that modifications at the commissural level would cause changes in the responses of PJ and VN cells fully compatible with published observations: i) VOR gain, and Type I PJ cell modulation during VOR suppression, vary in parallel but not by the same factor (5); ii) a greatly reduced VOR gain can be associated with deteriorated central integration (6); (iii) measured changes in the modulation of 2° VN cells can be much smaller than expected from the VOR gain changes (6,7). Thus cross-midline coupling of the VN could be the site of the modifiable brainstem element, postulated by Miles & Lisberger (5) to account for their observations after long-term VOR plasticity. Short-term (hours) plasticity of the VOR is more likely related to synaptic changes on PJ cells, according to the observations of Ito (8): here, the model predicts (as observed) that the resulting changes in VN responses would be similar to those expected from commissural modifications, but that Type I PJ cell sensitivity to head velocity would vary inversely with the VOR gain.

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152.6 EFFECTS OF SEMICIRCULAR CANAL LESIONS ON SPATIAL ABILITY H.Cohen*and M.Potegal. (SPON: L. Marco). Teachers College, Columbia Univ., N.Y.,N.Y. 10027 and N.Y.State Psychiatric Inst., N.Y., N.Y. 10032

We have developed procedures for testing the hypothesis that, as an animal moves through space, it can use vestibular information to monitor its distance and direction from the starting point (Potegal, Spatial Abilities: Development and Physiological Foundations, Academic Press, 1982; see Mittelstaedt and Mittelstaedt, Naturwissen., 1980,67,566 for behavioral evidence of a particular role of the semicircular canals in "path integration"). In earlier studies we developed a passive-transport-and-return task (PTR) in which water-deprived rats are given a brief drink of water from one of eight identical spouts bordering a visually homogenous room. The rats are then passively transported along successively longer right angle trajectories in an opaque vehicle. To obtain water upon release the rats must return to the spout from which they were transported on that trial. We found that both normal and blinded rats can perform PTR but rats with lesions of the vestibular nuclei were significantly impaired on acquisition and post-operative retention of the task (Miller et al., Neuroscience Abstracts, 1981, 7, 484; Physiol. Psych., 1983, 11, 1-10). Vestibular nuclear-lesioned rats, however, have motor deficits (e.g.,head nystagmus) and increased emotionality which could have nonspecifically impaired task performance.

We now report the results of a more selective vestibular lesion: destruction of the lateral semicircular canals. Rats previously trained on PTR received either sham operations or had both lateral canals drilled open and their ampullae reamed out with a 0.1mm diameter wire matching the curvature of the canal. On post-operative retention testing sham rats reached criterion in 14% fewer trials than pre-operatively; canal-lesioned rats took 19% more trials than pre-operatively. There were no differences between canal- and sham-operated rats in PTR return latencies or on subsequent response-to-handling tests, indicating that neither motor impairment nor emotionality accounted for the PTR deficit. By contrast, high-speed cinematographic analysis of response to whole body oscillation in the horizontal plane revealed an increased head movement phase lag in canal-operated rats, independently confirming the canal dysfunction. These data are congruent with the Mittelstaedts' observations and support the vestibular navigation hypothesis.

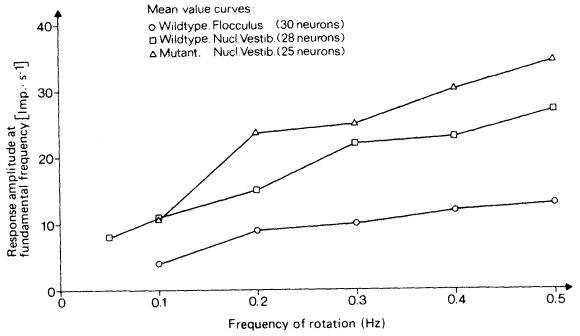
152.7 MODIFICATION OF THE VESTIBULOOCULAR REFLEX IN AROUSED CATS BEFORE AND AFTER 6-OHDA INDUCED NOREPINEPHRINE DEPLETION. J.G. McElligott and W.F. Freedman. Department of Pharmacology, Temple U. Sch. of Medicine, Phila., Pa. 19140 and Electrical and Computer Engineering Dept. Drexel U., Phila., Pa.

Norepinephrine (NE) has been implicated in learning and neuroplasticity, i.e., the ability of the nervous system to adapt to changing conditions by forming new or enhancing existing neural connections. Modification of the vestibuloocular reflex (VOR) has been presented as a model system for investigating neuroplasticity and adaptive motor behavior. Much work has centered around the cerebellum's role in the modifiability of the VOR. Cerebellar NE has been shown to be important for the learning but not the performance of specific locomotor tasks. Previous work has also shown that VOR modification (decrease in gain) is hindered in cats whose norepinephrine was reduced by intra-cisternal injection of 6-OHDA treatment. Since an animal's arousal level during modification and testing of the VOR is of critical importance, we decided to replicate this other work but to use an increase rather than a decrease in the VOR gain. Reduction in VOR gain can be due to a reduced level of alterness in the animal brought about by the 6-OHDA lesion. Modification was accomplished by oscillating head restrained cats, fitted with preformed ocular search coils, in the horizontal plane while an optokinetic drum was oscillated 180 deg out of phase with the animal. Tests of VOR gain were made every 16 min by oscillating the animal in the dark at 0.25 Hz (+/-10 deg). During the modification period, the animal's arousal was enhanced by auditory and tactile stimulation. In addition, modification of the VOR was also carried out in a heightened arousal condition following an injection of amphetamine (5mg s.c.). Assays of arousal were made by counting the number of saccades made by the animal during the modification period. The animals in both the behaviorally as well as the amphetamine aroused condition increased their gain equally to an average of 1.5x over the 4 hour period. The animals were then injected with 6-OHDA intracisternally to deplete CNS norepinephrine. Following a two week recovery period, the animals again underwent modification and testing. In behaviorally aroused animals, there was no change in the modifiability of the VOR. However, in the amphetamine aroused situation little or no modification of the VOR took place. A possible explanation for these results is that after the 6-OHDA treatment there was insufficient depletion to effect the modifiability of the VOR. However, treatment with amphetamine caused a further release and functional inactivation of NE that rendered the animal incapable of modifying its VOR.

152.8 RESPONSES OF VESTIBULAR NEURONS FROM THE NUCLEUS VESTIBULARIS AND THE FLOCCULUS IN WILDTYPE MICE AND MUTANTS. U. Grüsser-Cornehls*(Spon: R.F. Schmidt), Department of Physiology, Freie Universität, Berlin, Germany.

Neurons were recorded from the N. vestibularis and the flocculus of wildtype mice and WEAVER mutants (B6CBA) by means of glass micropipettes in the horizontal plane in the dark at different frequencies and amplitudes. The recording sites were localized by means of small iontophoretically applied HRP markings.

The neuronal response amplitude increased with frequency and amplitude of the sinusoidal rotation. The average slope of the amplitude frequency response characteristics of N. vestibularis and flocculus neurons did not differ significantly between wildtype mice and mutants, but the response amplitude at a given frequency has been shifted along the ordinate. The lowest response amplitude was displayed by wildtype flocculus neurons and the highest response amplitude by mutant N. vestibularis neurons. A possible explanation for this finding would be that the flocculus exerts inhibitory influences on the N. vestibularis neurons. The mean value curve for the relative phase relationship (normalized at o.2 Hz to 0) for floccular neurons of wildtype mice showed a strong increase in phase lead by increasing frequency of the sinusoidal rotation, while the mean value curve for the N. vestibular neurons demonstrated that the relative phase relationship was practically independent of the frequency between o.2 and o.5 Hz.



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152.9 RETROGRADE DOUBLE-LABELING OF VESTIBULAR NEURONS THAT PROJECT TO THE OCULOMOTOR NUCLEUS AND CERVICAL SPINAL CORD. F. Fang*, B.S. Phipps, R. Maciewicz and S.H. Wray, The Stanley Cobb Lab. and the Depts. of Neurology and Psychiatry, Mass. General Hospital and Harvard Med. School, Boston MA 02114.

Using the retrograde tracers horseradish peroxidase (HRP) and nuclear yellow (NY), a double retrograde labeling study was performed in the cat to determine the extent of overlap of cells that give rise to vestibulo-ocular and vestibulospinal projections. Following a posterior craniotomy and aspiration of the anterior cerebellar vermis, the tip of a 1ul syringe was advanced through the floor of the exposed aqueduct into the region of the oculomotor nucleus, and 50nl of 20% HRP was pressure injected. In the same animals 1% NY was injected bilaterally at C2 and C3 levels (six 1ul injections) following a partial C1 and C2 laminectomy. After a two day survival, animals were sacrificed and processed with tetramethylbenzidine to demonstrate HRP labeling; sections were viewed using brightfield illumination and fluorescent epiillumination.

Consistent with previous studies, injection of HRP into the oculomotor complex resulted in a large number of retrogradely-labeled neurons in the vestibular complex. In rostral pontine sections, densely labeled neurons were found throughout the superior nucleus. More caudally, HRP-positive cells were found in the medial nucleus and also in the border region between the ventral part of the lateral nucleus and the rostral descending nucleus. At more caudal medullary levels a smaller number of HRP-positive cells were found scattered in the medial and descending nuclei.

In the same material, cervical injections of NY retrogradely labeled numerous vestibulospinal neurons in the medial and descending nuclei. NY-positive cells were also found in the dorsal and ventral parts of the lateral nucleus; no vestibulospinal cells were found in the superior nucleus. The major area of overlap between vestibulospinal and vestibulo-oculomotor neurons in the cat occurred in the rostral descending nucleus as it borders on the ventral part of the lateral nucleus. Within this border region, many small neurons (<24um) were found to contain both HRP and NY. The presence of such double-labeling suggests that some vestibular neurons may have axons that bifurcate to the cervical cord and the region of the oculomotor nucleus. Vestibular cells with direct projections to both of these areas may function in head-eye coordination; the pattern of termination and functional linkage of such cells at cervical and mesencephalic levels remains, however, to be determined.

152.10 CENTRAL DISTRIBUTION OF THE VESTIBULAR NERVE IN THE BULLFROG, RANA CATESBEIANA. A. Kuruvilla*, V. Honrubia*, S. Sitko* and I. R. Schwartz (SPON: D. Strelioff). Div. of Head and Neck Surgery, UCLA Sch. of Med., Los Angeles, CA 90024.

Previously we demonstrated that the thick- and thin-caliber ampullary nerve fibers in the bullfrog have different physiological characteristics and innervate different regions in the cristae. In the present study we have evaluated their central projections using intra- and extracellular horseradish peroxidase labeling of the primary vestibular afferents. We first analyzed the central pathway of the whole VIIIth nerve by labeling it extracellularly. In order to identify the central projections of the various vestibular components of the VIIIth nerve we made separate extracellular injections of the whole anterior branch, the whole posterior branch, the ampullary nerve of each of the three semicircular canals and the branch to the sacculus. Individual thick-caliber fibers from the anterior and horizontal ampullae and the sacculus were labeled intracellularly in separate animals.

Fibers of the vestibular nerve innervate an ipsilateral area which extends caudally from the rhombencephalon at the level of the XIIth nerve nucleus and rostrally up to and including the nucleus cerebelli and the cerebellum. We have identified the following vestibular nuclei by the fact that they receive primary vestibular input: the nuclei ventralis, medialis, descendens, superioris and cerebelli. The nucleus dorsalis, which receives primary auditory input, also receives afferents from the sacculus. A few vestibular fibers project to the ipsilateral superior olivary nucleus, the motor nuclei of the Vth, accessory VIth, VIIth, and IXth cranial nerves and the reticular formation. The primary vestibulo-cerebellar fibers terminate mainly among the granular cells of the lobus auricularis and of the corpus cerebelli in the caudal part of the cerebellum. Fibers from each of the three ampullary nerves project into the cerebellum. From the presently available data it appears that at the single neuron level individually labeled thick-caliber fibers project to all of the vestibular nuclei. Heavily labeled large fusiform neurons, presumably vestibular efferents, are seen in the ipsilateral reticular formation, adjacent to the facial motor nucleus.

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152.11 SPONTANEOUS AND EVOKED NEURAL ACTIVITY CHANGES IN SEMICIRCULAR CANAL AFFERENTS DURING TRANSIENT ISCHEMIA. S. Sitko* and V. Honrubia* (SPON: E. Decima). Div. of Head and Neck Surgery, UCLA Sch. of Med., Los Angeles, CA 90024.

In the bullfrog Rana catesbeiana we have studied the effect of impairing the blood flow in the vestibular artery on the spontaneous and evoked activity of single horizontal and anterior semicircular canal afferents. Interruption of blood flow was produced by clamping the vestibular artery using fine forceps under microscopic visual control. The spontaneous activity, evoked response to sinusoidal rotations (typically 0.2 Hz, 60 deg/sec), and, when possible, membrane potential, action potential size, and electrical threshold for depolarizing currents were monitored continuously throughout the experiments: before, during, and after removing the arterial blockage.

In most cases we observed the spontaneous activity to increase during the initial 1-5 minutes following the occlusion, usually by 10-30% above its resting value. In all cases there was a subsequent reduction of the activity to a level between 80% and less than 1% of the baseline over a period of 2-20 minutes. The physiological sensitivity of the fibers followed a similar pattern with a short period of increased sensitivity in the first 1-5 minutes after clamping, followed by a progressive loss in sensitivity and compressed dynamic range.

The changes in both spontaneous activity and physiological sensitivity were partially and in many cases totally reversible upon removal of the occlusion and resumption of arterial blood flow. In those fibers of which we were able to maintain intracellular records throughout the experiment, we observed no change in membrane potential, action potential size, or electrical threshold which could be related to the changes in spontaneous and evoked nerve impulse activity. We presume, therefore, that the observed effects originate within the receptor organs at the nerve hair cell synaptic complex. These results demonstrate the importance of maintaining a normal blood supply to the labyrinth when evaluating physiological experiments in the vestibular nerve of cold-blooded animals.

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152.12 PRIMARY VESTIBULAR FIBER CONNECTIONS IN THE RAT. W. R. Mehler NASA-Ames Res. Ctr., Moffett Field, CA 94035

Trans-bullar vestibular ganglionectomies were attempted on 54 Long-Evans rats. Out of 24 that survived the required 5 to 7 days 15 were excluded because of incomplete lesions or excessive pontine tegmental infarctions. Six verified complete and 3 partial ganglionectomy experiments were serially sectioned in either the coronal, sagittal or horizontal plane. Alternate sets of uranyl nitrate (Nauta and Ebbessen, '70) and Fink and Heimer ('67) method impregnations were prepared with and without cresylectviolet counterstaining.

Analysis of complete lesion series revealed massive vestibular fiber degeneration which enveloped the interstitial nucleus and entered the lateral vestibular nucleus. A small group of fibers seperates from the caudal part of the incoming nerve and without apparent bifraction terminates in cell group Y of Brodal. The degenerating 8th nerve fibers penetrate the lateral vestibular nucleus and bifracate into ascending and descending limbs made up of many secondary fascicles. The descending limb traverses the length and breadth of the spinal vestibular nucleus issuing large numbers of small degenerating collateral bundles medially into the medial vestibular nucleus where they diffuse and terminate profusely right up to the ependyma of the IVth ventricle. No terminals appear in cell group X, f-like nuc. or the prepositus hypoglossi or parasolatary nuclei. Fiber and connections were consistently found in the solitary tract and nucleus but no reticular or contralateral vestibular endings were observed. Limited terminal degeneration was encountered in parts of the spinal and in the doral part of the lateral vestibular nuclei.

Fascicles of the ascending limb ascend chiefly in the superior nuc., which exhibits terminals throughout and these fibers issue collaterals that terminate in the oral-third of the medial nucleus. Multiple small fascicles splay out dorsal to the superior nuc., penetrate the brachium conjunction and then deflect caudalwards through or ventral to the interposed and fastigial nuclei. A minor component of these vestibulocerebellar fibers project laterally into the flocculus and a few medially into the lingula. The majority of these fibers loop diffusely caudalwards and then assemble into intermitent concentrations restricted to the white matter in the nodulus and the ventral half of the uvula from which they enter and terminate in the granular layer. Some direct 8th nerve connections with the caudal ventral fastigial cells were evident but no ventral, parvocellular dentate connection were suggested.

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152.13 SPONTANEOUS ACTIVITY AND DRIVEN RESPONSES OF SEMICIRCULAR CANAL PRIMARY AFFERENT NEURONS IN THE ALERT PIGEON. T.J. Anastasio*, M.J. Correia and A.A. Perachio. Departments of Otolaryngology and Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550-2778.

The properties of pooled anterior, posterior and horizontal semicircular canal (SC) primary afferents were studied in a chronic, alert pigeon preparation. The average spontaneous mean firing rate (MFR) reported for a sample of 124 SC primary afferents from eight alert pigeons was 168 impulses per second (I/S), range 8 - 396 I/S. In contrast, the average MFR reported for a sample of 124 horizontal canal primary afferents from anesthetized pigeons was 45% less (92 I/S, range 10 - 200 I/S, Lifschitz, W.S, Brain Research, 63: 43-57; 1973). Coefficient of variation (CV) of the mean interspike interval (ISI) was classified as follows: regular - CV < 0.1, intermediate - CV = 0.1 to 0.4 and irregular - CV > 0.4. The percentage of SC primary afferents in each class is similar for both alert and anesthetized pigeons. In alert pigeons, there is a linear relationship between mean ISI and the standard deviation (SD) as described by the following equation and coefficient of correlation (r):
SD = 1.271 (ISI) - 6.683, r = 0.9803.

Four alert pigeons were subjected to sum of sines rotational stimuli at two separate bandwidths: low bandwidth - 0.029 to 0.615 Hz and high bandwidth - 0.293 to 6.152 Hz. Best-fit transfer functions, and associated mean square error (MSE) values, were determined for ten intermediate and five irregular SC primary afferents:

$$\text{int. - } H(s) = Gs^{1.14}(9.9s+1)^{-1}(0.01s+1), \text{MSE} = 0.11;$$

$$\text{irr. - } H(s) = Gs^{1.10}(9.2s+1)^{-1}(0.02s+1), \text{MSE} = 0.12.$$

Best - fit transfer function for 14 anterior ampullary afferents in the anesthetized pigeon across CV classes over the bandwidth - 0.01 to 2.0 Hz was found to be (Landolt, J.P. and Correia, M.J., J. Neurophysiol., 43: 1746-1770; 1980):

$$H(s) = Gs^{1.24}(10.2s + 1)^{-1}$$

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153.1 SPINAL CORD POTENTIALS EVOKED BY CUTANEOUS AFFERENTS IN MAN
A.M. Sherwood, M.R. Dimitrijevic, J. Faganel*, J.A. Halter*, L.D. Lehmkuhl*, Department of Clinical Neurophysiology, The Institute for Rehabilitation and Research, and Department of Rehabilitation, Baylor College of Medicine, Houston, TX, 77030.

We recorded spinal cord potentials from epidural electrodes in a group of patients with spinal cord injury or lumbosacral radiculopathy. The patients were undergoing evaluation of the effectiveness of spinal cord stimulation on spasticity or pain. The electrodes were located over the dorsal aspect of the midline of the cord, from T9 to L1 vertebral levels. Mechanical stimuli supplied by an electrodynamic hammer were applied to dermatomes from T11 to S1 and tendons of the triceps surae and quadriceps muscles. Electrical stimuli were applied to sural, peroneal, tibial, and femoral nerves with 0.5 mS duration, and intensities ranging from 50 to 150 volts, at a rate defined by the patient's EKG. 16 to 128 responses were averaged for each recording. Characteristics of spinal potentials evoked by mechanical taps have been compared with those evoked by electrical stimulation of peripheral nerves in the lower extremity. Tapping the toenail and skin areas within the same dermatome elicited a spinal potential with initial negative and following positive components similar to those evoked by electrical stimulation of the sural nerve when recorded from an epidural electrode at T12. Tapping cutaneous regions of more proximal dermatomes resulted in a reduction in amplitude of the negative potential which corresponded to the distance of the segmental input from the recording electrode. In addition, the positive component was substantially reduced or eliminated. Taps applied to skin overlying the Achilles and patellar tendons elicited spinal potentials of dispersed waveform even when applied at a strength below that which was necessary to elicit a stretch reflex. Furthermore, no new features were revealed when the stimulus intensity was increased above the threshold for the stretch reflex. These findings correspond to the results of animal studies which show that the potentials recorded from the dorsal aspect of the spinal cord following stimulation of a peripheral nerve trunk reflect primarily sensory input, which has been found to be distributed over a number of adjacent spinal cord segments.

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153.2 SPINAL CORD POTENTIALS ELICITED CONCURRENTLY WITH MONOSYNAPTIC REFLEXES IN MAN,
J. Faganel*, M.R. Dimitrijevic, J.A. Halter*, L.D. Lehmkuhl*, A.M. Sherwood*, Department of Clinical Neurophysiology, The Institute for Rehabilitation and Research, and Department of Rehabilitation, Baylor College of Medicine, Houston, TX, 77030.

Evoked potentials from the lumbosacral spinal cord (SC) were recorded with epidurally placed electrodes in response to concurrently elicited myotatic reflexes or H reflexes in triceps surae muscles. This study was done in patients who had epidurally placed electrodes for evaluation of the effects of SC stimulation on spasticity or pain due to chronic SC injury or chronic radiculopathy. The epidural electrodes were placed over the dorsal aspect of the cord, approximately on the midline, from T9 to L1 vertebral levels. H reflexes were elicited with stimuli of 0.5 mS duration delivered to the tibial nerve at the popliteal fossa, at a rate defined by the patient's EKG. Myotatic reflexes (T-waves) were elicited by an electrodynamic hammer applied to the Achilles tendon at the same rate. Reflex responses were conditioned by the application of strong vibration applied to the triceps surae muscle. 16 or 32 responses were averaged for each recording. The response to electrical stimulation consists of a negative wave in the range of 10 to 100 microvolts, with an onset latency in the range 10 to 12 mS, a peak latency of approximately 15 to 16 mS, and a duration of approximately 6 mS, followed by a slower positive wave of lower amplitude. The response to mechanical stimulation was lower in amplitude and more dispersed. These responses reflect pre- and post-synaptic events generated in the root entry zone by the electrical or mechanical stimulus. By recording SC evoked potentials during electrically and mechanically elicited monosynaptic reflexes, we examined the contribution of cutaneous and proprioceptive afferent fibers in the generation of the evoked response. These SC evoked potentials were compared to SC potentials elicited during transitory hyporeflexia or areflexia induced by vibration of the Achilles tendon and to SC potentials observed during hyperreflexia due to postvibratory potentiation. Only minor changes of amplitude and waveform of the evoked spinal cord potentials were observed. In contrast, significant changes in the H- and T-reflexes were found. This suggests that generators of the SC potentials reflect more than proprioceptive input and changes to such input due to presynaptic inhibition induced by vibration.

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153.3 *NEUROMUSCULAR OSCILLATIONS ABOUT THE ANKLE JOINT IN THE CHRONIC SPINALIZED CAT.*
N.S. BRADLEY and J.L. SMITH. Neuromotor Control Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024.

Rapid, rhythmical oscillations of the neuromuscular system are associated with a number of neurological diseases and lesions. In the cat, these oscillations, generally referred to as clonus or tremor, may reach frequencies up to 20 Hz. Kittens spinalized at 2 wks or 3 mos of age generally develop flexor tremors at 8-12 Hz; the extensor muscles are seldom involved (Smith et al. Exp. Neurol. 76:1982). Our preliminary observations on cats spinalized as young adults suggested that extensor muscles were more frequently involved. This study was conducted to characterize neuromuscular oscillations about the ankle joint in 4 cats spinalized at the T-12 level as adults.

The animals were tested 1-2 times weekly during the first 3 mos of recovery. EMG from 2 ankle extensors, soleus (SOL) and lateral gastrocnemius (LG), and 1 ankle flexor, tibialis anterior (TA), were recorded during reflex testing, including tendon taps, manual stretch, paw pinch and minute vibration of the Achilles at 60 Hz. Automatic movements, airstepping (ASTP) and the paw shake response (PSR), were also examined for epochs of oscillations. Video records of the cat's limb movement were synchronized with the EMG. A total of 163 epochs of EMG oscillations, associated with rhythmical movements at the ankle, were examined.

Frequency ranges from 8 to 18 Hz were found in all animals, with the most common periods between 10-12 Hz. In the majority of records, the extensor muscles alone exhibited oscillations with no reciprocal activity in the TA. Of the 2 extensors, the SOL was more often involved than the LG. Higher mean frquencies were associated with stretch of the extensors (15 Hz) and vibration of the Achilles with the ankle held in 90° of flexion (15 Hz).

Oscillations involving reciprocal activity of the TA and SOL/LG were rare except when the animal was held suspended. Reciprocal oscillations occurred after ASTP or PSR and ranged 10-18 Hz, with a mean of 13 Hz. Normally, TA activity was initiated 50% into the extensor cycle. The average burst durations for the SOL (32 ms) and LG (26 ms) were similar to that for the TA (35 ms). These data show that the neural input associated with reciprocal oscillations were symmetrical for ankle flexors and extensors.

Although the rapid neuromuscular oscillations may be seen more typically in flexors or extensors, depending on the cat's age at spinalization, the EMG rhythms in all age groups agree with the predicted frequencies for the ankle muscles of the cat (Nichols, et al. Can. J. Physiol. Pharmacol. 56:1978). Since the rhythms never exceeded 20 Hz and were abolished when muscle tension increased, we hypothesize that the oscillations are produced by a combination of mechanical and reflex factors, rather than a central oscillator. Supported by NIH grant NS 16333.

153.4 ANKLE JOINT STIFFNESS IN A FUNCTIONALLY DEAFFERENTED HUMAN SUBJECT
P.L. Weiss, R. Forget† R.E. Kearney, I.W. Hunter and Y. Lamarre Biomedical Engineering Unit, Faculty of Medicine, McGill University and †Departement de Physiologie, Universite de Montreal, Montreal, Canada, H3G 1Y6

The relative contributions of the passive (muscle and joint visco-elasticity) and active (reflex and voluntary muscle activation) components to dynamic joint stiffness have been investigated in a subject who can vary the level of voluntary muscle contraction from full relaxation to a moderate level of activation but who is functionally deafferented. This subject has symptoms (i.e. lack of touch, vibration, pressure and kinesthetic senses) which indicate impairment of the larger diameter (groups I and II) peripheral sensory myelinated fibers below the level of C2. The results of electrophysiological tests (H-reflex and somatosensory evoked potentials), nerve biopsies and correlation functions calculated between changes in joint angle and agonist EMGs confirm the above clinical findings.

The paradigm used previously (Hunter & Kearney, J. Biomechanics, 1982), in which normal subjects were subjected to repeated stochastic ankle angular displacements during contractions of the ankle joint dorsi- or plantarflexors, was slightly modified to facilitate the investigation of dynamic stiffness at low frequencies.

The results of this investigation indicate that the behaviour of the ankle joint of this subject at frequencies above 5 Hz is very similar to that of normal subjects. As in previous work, ankle joint stiffness in the intact human can be modelled by a second-order transfer function having inertial, viscous and elastic terms. The inertial term remains constant but the viscous and elastic terms increase linearly with mean torque level. In contrast, the behaviour of the ankle joint at those frequencies at which one would expect the contribution of the reflex component to be relatively important (less than 1 to 2 Hz), was quite different than that shown by normal subjects. Whereas the squared coherence (a measure which provides an estimate of the proportion of the output variance accounted for at a given frequency by a linear dynamic relation between position and torque) for normal subjects was greater than 0.9 at low frequencies, it was less than 0.5 for this deafferented subject. Low coherence values will occur if (1) the relation between the input and output is non-linear, (2) there are additional, not accounted for inputs to the system being identified and (3) the output power is reduced. Our results indicate that neither (2) nor (3) are responsible for the low coherence values. Rather, it is likely that, in the absence of the possibility for reflex modification of ankle joint behaviour, the relation between changes in ankle joint angle and torque is non-linear.

153.5 EXCITABILITY CYCLE OF A LOW-THRESHOLD HUMAN FLEXION REFLEX, K.L. Robinson* and A.J. McComas (SPON: E.S. Werstiuk). Clinical Neurosciences, McMaster University Medical Center, Hamilton, Ontario. CANADA L8N 3Z5.

We have recently discovered a low-threshold cutaneous reflex in the human arm and have now analyzed the time-course of motoneurone excitability which follows the reflex discharge. The reflex is recorded from the posterior deltoid (PD) muscle with surface electrodes and can be elicited by single stimuli applied to digital nerve fibres or to cutaneous mechanoreceptors. Although the size of the reflex varies between trials and between subjects, a response can be recorded electromyographically in almost all normal subjects (19/20 volunteers). Using a conditioning testing paradigm, it can be shown that, after the reflex discharge, there is facilitation for 15 ms and this is succeeded by an inhibitory period lasting 180 ms. Similar results, following stimulation, can be obtained by rectifying and averaging EMG activity during steady voluntary contraction of PD. Two observations indicate that the major part of the inhibition cannot be due to recurrent inhibition of PD motoneurones; first, inhibition is present in PD after subthreshold conditioning stimulation and second, an inhibitory phase can be detected in the anterior deltoid without a preceding excitatory period. In previously untested individuals the inhibitory period is interrupted by a second excitatory phase at 100 ms but, interestingly, the latter is lost after repeated experimentation. The topographical organization of the excitatory components of the reflex indicate that the latter is part of a flexor withdrawal mechanism.

153.6 ISCHEMIC PRESSURE BLOCK: EFFECTS ON SECONDARY FACILITATION IN THE H-REFLEX RECOVERY FUNCTION.† R.T. Pivik and F.W. Bylsma*. Lab. Psychophysiology, Department of Psychiatry and School of Psychology, University of Ottawa, Ottawa, Ontario, K1H 8L6.

Spinal monosynaptic reflexes in man are facilitated if preceded 100-300 msec by a conditioning reflex response. The activation of long-loop reflexes, cutaneous afferents and afferent feedback following muscle contraction have been suggested as bases for this secondary facilitation. In the present investigation an ischemic pressure block was created to evaluate the effect of reducing or eliminating afferent feedback on secondary facilitation.

Twelve normal young adult volunteers (7 male, 5 female) participated in the investigation. Motor and reflex (H1, H2) responses recorded from the gastrocnemius-soleus muscles were elicited by pericutaneous stimulation of the tibial nerve in the popliteal fossa. Pairs of equal intensity .5 msec stimuli above threshold for direct responses were delivered at inter-stimulus intervals of 100, 200, and 300 msec (5 pairs/interval). Reflex measurements were taken immediately before the pressure block was put in place, at approximately 10 minute intervals during the block and immediately after the block was removed. The block was effected for 30 minutes by a sphygmamometer cuff attached to the leg between the stimulating and recording electrodes and inflated to 300 mm HG. Reflex amplitude was not appreciably diminished until the final minutes of the ischemic period (t20-t30). By t30 the ischemic effects began to spread above the cuff to the point of tibial nerve stimulation as indicated by marked decreases in amplitude of both H1 and H2. However, although both reflex responses were affected, the reduction in H2 was slightly enhanced relative to that of H1 (5.5% average greater decrement across intervals). This decrease accounts for approximately 20% of the reflex amplitude increase associated with secondary facilitation. However, the contribution of afferent feedback from muscle contraction to secondary facilitation may be more or less than the observed 20% since the ischemic block may have activated other influences, such as disinhibition of alpha motoneurons by inactivation of cutaneous afferents. The latter possibility is supported by the observation that amplitude decrements of 50% for H1 and H2 were associated with only a slight (6.1%) reduction in secondary facilitation.

†Supported by the Ontario Mental Health Foundation

153.7 REFLEX ACTION IN AN AGONIST-ANTAGONIST MUSCLE SYSTEM IN THE DECEREBRATE CAT. T.R. Nichols. Dept. of Kinesiology, Univ. of Washington, Seattle, WA 98195

Force of contraction and stiffness were measured in agonist and antagonist muscles with intact reflex connections during stimulation of various peripheral nerves. A flexor (tibialis anterior or extensor digitorum longus) and an extensor (soleus or gastrocnemius) were connected via individual myographs around the pulley of a printed motor in opposite directions. Cats were decerebrated at the premammillary level. Muscle temperature was regulated at 36°C. Ramp length changes lasting 100 ms and remaining at plateau for 300 ms were applied with alternating direction by the motor.

Peripheral nerve stimulation led to changes in muscle force. Stimulation of the contralateral tibial nerve (lower leg) at 50pps activated extensors at high intensities (>2XT) but frequently caused activation of flexors or transient coactivation at lower intensities. Ipsilateral tibial nerve stimulation usually led to flexor excitation and extensor inhibition at the higher intensities, but frequently led to extensor excitation at lower intensities. Ipsilateral sural nerve stimulation usually led to excitation of extensors at low intensities (<1.4 X T) and inhibition of extensors at higher intensities. Intermediate forces could be obtained by stimulating more than one nerve simultaneously.

Force responses to length change increased in a quasilinear way with ramp amplitude (0.25 - 8 mm) . Stiffness ($\Delta F/\Delta L$) was dependent upon initial muscle force, but relatively independent of the method of excitation. Occasionally transient decreases in stiffness were observed at the onset of stimulation. (cf. Hoffer and Andreassen, 1981. J. Neurophysiol. 45:267-285). The stiffness of extensors was usually greater than that of flexors. However, flexor stiffness was observed to equal or surpass extensor stiffness during flexor spasms. This effect could not be explained by changes in the initial force.

It is concluded that various peripheral reflexes affect motoneuronal excitability primarily, while stiffness is regulated by supraspinal influences.

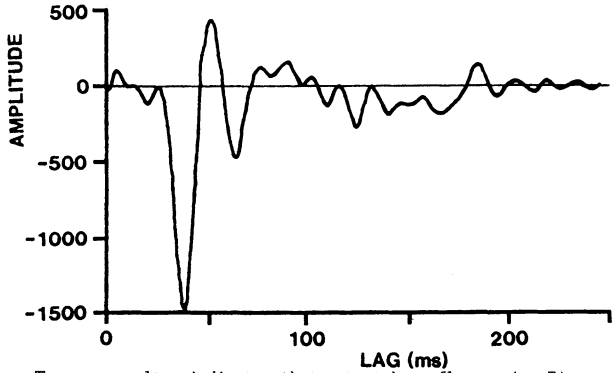
(Supported by the N.I.H. #NS17025 and by the Graduate School Research Fund of the University of Washington)

153.8 SYSTEM IDENTIFICATION OF HUMAN STRETCH REFLEXES: TIBIALIS ANTERIOR. R.E. Kearney, I.W. Hunter and P.L. Weiss. Biomedical Engineering Unit, Faculty of Medicine, McGill University, Montreal, Canada, H3G 1Y6.

System identification methods have shown that the stretch reflex in the human ankle extensor (triceps surae, TS) may be characterized as feedback of a velocity signal through a uni-directional sensitive nonlinearity via a single short latency pathway (Kearney & Hunter, Expt Brain Res, 1983). The present work has used the same methods to examine stretch reflexes in the ankle flexor (tibialis anterior, TA).

Five normal subjects maintained a tonic contraction of TA while subjected to repeated, stochastic perturbations of ankle position. Position, torque and smoothed rectified EMG's were recorded and ensemble averaged over 25 stimulus presentations.

Impulse response functions (IRF) relating TA EMG to ankle velocity accounted for 60-70% of the observed EMG variance and there was no evidence of any direction dependent nonlinearity. TA IRFs were all similar to that shown in the figure and are characterized by two distinct peaks of excitation separated by a period of inhibition. The IRF amplitude increased with increasing mean torque and decreased with displacement amplitude although the size of the effect on the two peaks was different.



These results indicate that stretch reflexes in TA are subserved by two separate mechanisms: a short latency pathway probably mediating spindle primary afferents and a second, longer latency pathway perhaps mediating spindle secondary afferents.

Supported by a grant from the Canadian MRC.

153.9 TESTING FOR REFLEX PARTITIONING IN THE MOTOR NUCLEUS OF THE CAT LATERAL GASTROCNEMIUS MUSCLE. S. Vanden-Noven, T. M. Hamm and D. G. Stuart. Department of Physiology, University of Arizona, Tucson, AZ 85724.

Recent studies have shown that: 1) the cat lateral gastrocnemius (LG) muscle has four anatomically separate "compartments" innervated by primary nerve branches (English and Weeks, Neurosci. Abstr. 8: 959. 1982); 2) the distribution of motoneurons supplying these nerve branches is topographically organized (Weeks and English, Neurosci. Abstr. 8: 959, 1982): and 3) the muscle compartments may be activated somewhat independently during movement (Russell et al., Neurosci. Abstr. 8: 948, 1982). These findings have prompted the present study which is designed to test the hypotheses that: 1) homonymous monosynaptic Ia EPSPs are not distributed homogeneously throughout the motor nucleus of LG, and 2) this heterogeneity is attributable to "own-branch" EPSPs being larger than "other-branch" EPSPs (Botterman et al., Neurosci. Letters 24: 35-41, 1981).

Intracellular recordings from LG motoneurons were made in anesthetized low-spinal cats during periods of electrical stimulation of the nerve branches supplying the four compartments of the LG muscle. Measurements were made of each cell's composite homonymous own- and other-branch monosynaptic Ia EPSPs evoked by stimulation of the test-nerve branches. To test the first hypothesis, the fractional EPSPs produced by the stimulation of single nerve branches (fraction of sum of EPSPs produced by all nerve branches) were normalized by the corresponding fractional dorsal-root volleys (fraction of total dorsal-root volley) for each cell. This procedure was designed to factor out variations in EPSP amplitude due to cell type (i.e., S, FR, FF) and variable afferent content among the nerve branches.

An analysis of variance of these data for all nerve branch -- motoneuron combinations indicates the presence of a significant ($p<0.005$) non-homogeneous distribution of monosynaptic Ia EPSPs in the LG motor nucleus. However, the present motoneuron sample size (N=44) is insufficient at this time to permit conclusions to be drawn concerning our second hypothesis. Supported in part by USPHS grants NS 07888, HL 07249, and NS 17887.

153.10 STRETCH REFLEXES AFTER SPINAL LESIONS IN THE CAT. M.F. Brothers, D.A. McCrea, W.G. Tatton and R.R. Tasker, Playfair Neuroscience, University of Toronto, Toronto, Canada. (Spon: J. Sharpe).

In order to provide quantitative data on the changes in reflexes following spinal lesions, we have studied the stretch reflexes obtained from ankle flexor and extensor muscles in awake, unanesthetized normal cat and several lesioned preparations: the chronic spinal (mid-thoracic cord transection, duration 3-4 months), the chronic hemisected (mid-thoracic), and the acute anemic decerebrate cat. Stretch reflexes were elicited by a torque motor which caused repeated passive ankle rotation at a variety of velocities; the evoked electromyographic activity (EMG) from medial gastrocnemius (MG) and tibialis anterior was recorded, and later digitized. Trials were sorted according to the velocity of ankle rotation; rectified EMG as well as tension and position signals were computer-averaged. Reflex EMG was quantitated through expression of its size as a percentage of the muscle's maximal electrically evoked EMG output (m-max) (Lenz, G.A., Tatton, W.G., Tasker, R.R., J. Neuroscience, 1983 (in press)). For earliest latency reflex activity, chronic spinal MG showed increased reflex "gain" (slope of EMG response vs. torque velocity), and decreased reflex threshold and latency, compared to normals. This hyperreflexia was apparent by 10 days post-transection, and at 3 months had not changed significantly. Later-latency reflex activity in MG was also increased in all lesioned animals, but with response characteristics that varied significantly amongst the preparations. Decerebrate cats displayed tonic EMG activity when the muscle was maintained in a static stretch position; in contrast, in the chronic spinal cats, reflex activity persisted only as long as movement continued (dynamic stretch reflex), with little response to maintained stretch. Two of the chronic spinal cats were decerebrated and MG stretch reflexes were obtained immediately before and after denervation of the distal hindlimb, except the nerve to MG. The hyper-reflexia in MG was still present indicating that activity in muscle afferents alone is sufficient to evoke this increased reflex response in MG.

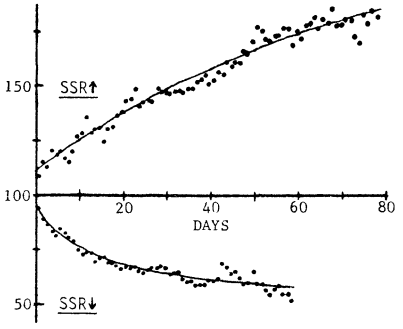
The effect of chronic spinal transection on tibialis anterior stretch reflex activity was opposite that of MG; both early and later latency responses were reduced in size; reflex gain was less, and latency greater. Thus the reflex changes occurring after chronic spinalization may be specific for a particular muscle group with hyper-reflexia in ankle extensors and hypo-reflexia in ankle flexors.
(Supported by the Multiple Sclerosis Society of Canada and the Medical Research Council).

153.11 ADAPTIVE PLASTICITY IN THE PRIMATE SPINAL STRETCH REFLEX: SLOW PROGRESSIVE CHANGE FOLLOWS IMMEDIATE SMALL CHANGE. J. R. Wolpaw. Center for Labs and Research, NYS Dept. of Health and Depts. of Neurology and Anatomy, Albany Med. Coll., Albany, N.Y. 12201.

Monkeys can gradually change the amplitude of the segmentally-mediated, largely monosynaptic, spinal stretch reflex (SSR), or MI, without change in background EMG or initial muscle length [Wolpaw et. al., Br. Res. 267 (1983) 196-200]. Increase (SSR↑ mode) or decrease (SSR↓ mode) develops over weeks and months, survives prolonged breaks in performance, and is relatively specific to the agonist, biceps, muscle (O'Keefe & Wolpaw, this vol.). Reversal and re-development of change follow the same gradual course. This study analyzes the course of SSR amplitude change for 2 months after onset of the SSR↑ or SSR↓ mode.

The figure below illustrates the main features of SSR change: 1) Monkeys respond within 1 day, probably within 6 hrs, with a 10% rise (SSR↑) or 6% fall (SSR↓) in SSR amplitude. 2) After this abrupt small change, SSR amplitude changes slowly and steadily throughout the duration of data collection. In the initial weeks, SSR↓ rate exceeds SSR↑ rate. 3) An equation of the form $y=(a/[b+(1/x)])+c$ closely fits both SSR↑ and SSR↓ courses, which approach asymptotes of 197% and 49% respectively (Fig).

The first day's abrupt change presumably indicates rapid onset of activity in descending spinal cord pathways which alters operation of the segmental arc of the SSR. The subsequent slow change, which continues for at least 2 months and survives prolonged breaks, is consistent with the hypothesis that continued presence of this descending activity produces gradually increasing persistent segmental alteration. Such segmental alteration would constitute a technically accessible substrate of memory.



Daily SSR amplitude after onset of SSR↑ or SSR↓ mode, as % of pre-onset SSR amplitude. Data are from 17 mode exposures (10 SSR↑, 7 SSR↓) in 9 monkeys. Lines are computer fits of the equation shown above.

153.12 ADAPTIVE PLASTICITY IN THE PRIMATE SPINAL STRETCH REFLEX: BEHAVIOR OF SYNERGIST AND ANTAGONIST MUSCLES. J. A. O'Keefe* and J. R. Wolpaw (SPON: K. D. Barron). Center for Labs and Research, NYS Dept. of Health, and Depts. of Neurology and Anatomy, Albany Medical College, Albany, NY 12201.

Monkeys can slowly increase or decrease the amplitude (amp) of the segmentally mediated, largely monosynaptic, biceps spinal stretch reflex (SSR), without change in initial muscle length or background EMG activity [Wolpaw et. al., Br. Res. 267 (1983) 196-200]. We investigated the concurrent behavior of synergist (brachialis and brachioradialis) and antagonist (triceps) muscles.

Five monkeys were implanted with chronic intramuscular EMG electrodes. Two pairs, a primary pair (biceps I) and a secondary pair (biceps II), were placed in the biceps, and single pairs were implanted in the brachialis, brachioradialis, and triceps. Each monkey then learned to maintain a 90° (±1.5) elbow angle against steady extension torque for a period which varied randomly from 1.2-1.8 sec and to keep the average absolute value of biceps I EMG for the final 0.2 sec of this period within a preset range. If it accomplished this two-part task, a brief additional extension torque pulse extended the elbow 2°-3° and elicited the biceps SSR. SSR amp was measured as average absolute value of EMG 14-24 msec after pulse onset, minus background EMG. Under the Control mode, reward occurred 200 msec after the stimulus. Under the SSR↑ or SSR↓ mode, reward occurred only if biceps SSR amp was greater (SSR↑), or less (SSR↓), than a criterion value. EMG and elbow angle were monitored continuously. All animals worked first under the Control mode for 10-30 days, and then under either the SSR↑ or SSR↓ mode for 20-100 days. Under the SSR↑ or SSR↓ mode (reward contingent only on biceps I SSR amp, never on synergist SSR amp) biceps I SSR amp increased to ca. 150% of control (SSR↑) or decreased to ca. 50% of control (SSR↓).

Biceps II data closely paralleled biceps I, indicating that comparable SSR change occurred throughout the biceps muscle.

Synergist background EMG remained stable despite marked change in biceps SSR amp. Triceps remained inactive throughout. Thus, biceps SSR amp change cannot be attributed to changes in synergist or antagonist background activity.

When biceps SSR amp changed under the impetus of the SSR↑ or SSR↓ mode, brachialis and brachioradialis SSR amps changed 72% and 33% as much as biceps SSR amp changed. Thus, greatest change occurred in the agonist, biceps. This relative specificity effectively eliminates a number of extra-neuronal mechanisms as potential causes of SSR amp change; and thus supports the possible value of the SSR as a system for studying the neuronal and synaptic bases of memory in a primate.

153.13 GENERAL CHARACTERISTICS OF SPINAL INTERNEURONS RESPONDING TO LONGITUDINAL TENDON VIBRATION. W.T. Rainey, K.G. Buahin, W.Z. Rymer. Dept. of Physiology, Northwestern Univ. Medical Center, Chicago, IL. 60611

Longitudinal tendon vibration is a strong, selective stimulus for primary spindle endings in passive muscle, and a powerful although less selective stimulus to these receptors in contracting muscle. We have used longitudinal tendon vibration, together with muscle stretch, electrically induced muscle twitch and reflexively mediated variations in tension of triceps surae muscles to assess muscle afferent projections to interneurons located in laminae V, VI and VII in the lumbosacral cord of the unanesthetized, decerebrated cat preparation. Interneurons were identified on the basis of latency of activation (typically less than 3 ms), synaptic jitter and absence of antidromic activation from muscle nerve or (in many instances) from rostral cord (T12).

Forty interneurons were activated by vibration, although not usually at the vibration frequency. Responses ranged from marked rate augmentation, to more modest responses, and down to just perceptible increases. Phase-locked discharge at a subharmonic of the vibration frequency was most common, but several interneurons showed more irregular, "unlocked" rate increases. Vibration sensitiye neurons responded to ramp stretch, varying considerably in their degrees of response to dynamic components of these stimuli. Superimposed ramp stretch and vibration often stabilized the rate of neuron discharge at a new firing rate, and eliminated ramp-related rate increases, suggesting that secondary ending actions were not prominent. Variations in isometric force during vibration or crossed extensor activation were not reliably associated with corresponding variations in discharge rate, suggesting that tendon organ convergence was also not prominent.

Most vibration-sensitive neurons responded to the cessation of vibration with a sharp reduction or even cessation of discharge. A small percentage showed considerable post-vibration discharge, which decayed in broad concordance with declining muscle tension or even adapted more slowly, over several seconds.

In summary, in the decerebrate preparation many interneurons in laminae V,VI and VII receive powerful Ia input, and appear to show little or no evidence of IB mediated activation. While we do not yet know whether the axonal projections of these neurons are excitatory or inhibitory, the location of many of these cells is dorsal to that of many identified Ia inhibitory interneurons. Those interneurons with prolonged after-discharge may function as neural integrators, contributing to the prolongation of force output after vibration offset, or during stretch reflex activation.

(Supported by NIH. NS 14959 - NINCDS.)

153.14 INPUT-OUTPUT PROPERTIES OF THE CLASP-KNIFE REFLEX IN THE CAT. W.Z.Rymer,C.L.Cleland(SPON:J.J.Pysh), Neuroscience Program and Dept. of Physiology, Northwestern University, Chicago, Il, 60611

The clasp-knife reflex, first described in spastic human patients, also occurs in decerebrated cats that have had their dorsolateral spinal funiculi sectioned. The reflex is characterized by powerful autogenetic inhibition which occurs when an extensor muscle is stretched beyond a certain length.

We studied the input-output properties of clasp-knife inhibition in 16 cats. Reflex effects of increases in muscle length and force were measured by recording the force and emg in the homonymous (soleus(SOL)), synergist (m.gastrocnemius(MG)) and several other extensor and flexor muscles of the hindlimb.

Autogenetic reflex inhibition could be due to increases in muscle length and/or force during stretch. By measuring the reflex inhibition in a synergist(MG) instead of the homonymous muscle(SOL), it was possible to dissociate the effects of force and length. To dissociate the reflex effects of force from length, we tetanically stimulated the SOL muscle nerve to produce increases in muscle force without increases in muscle length. Motor output in the MG was reflexely inhibited during the SOL force tetanus. Control experiments verified that the inhibition was not due to electrical stimulation of muscle afferents, unloading of spindle afferents, or mechanical coupling between the SOL muscle and sensory receptprs in the MG. The magnitude of inhibition of the MG produced by active, isometric force in the SOL was less than that due to SOL stretches that produced comparable, but passive, increases in muscle force. If the reflex actions of active and passive force are similar, then increases in both length and force in the stretched muscle contribute reflex inhibition of the synergist.

The input-output properties of clasp-knife inhibition indicate that it is more accurately thought of as a flexion withdrawal reflex rather than a specialized, regulatory or localized proprioceptive reflex. The spatial pattern of excitation and inhibition induced by stretching the SOL muscle, excitation of flexor and inhibition of extensor muscles, corresponds to that for flexion withdrawal reflexes. Stretch of a flexor muscle, the tibialis anterior, produces autogenetic excitation rather than inhibition, and the overall spatial reflex pattern is identical to that obtained for stretch of an extensor muscle. Extensor muscles are inhibited simultaneously with inhibition of all other extensor muscles and excitation of flexor muscles. Finally, the duration of reflex inhibition greatly outlasts the duration of the free nerve ending input that probably produces it. All these properties are similar to those of flexion withdrawal reflexes induced by other stimuli.

Supported by NIH 14959 and NIMH 5F31MH08593.

153.15 FUNCTIONAL CONSEQUENCES OF BETA-ACTION. Steve Grill*, W. Zev Rymer, Neuroscience Program and Department of Physiology, Northwestern University Medical School, Chicago, IL, 60611.

Skeletofusimotor (beta) fibers are motor fibers which branch to innervate both intrafusal (IF) and extrafusal muscle fibers (EF). Although they are abundent in both cat and monkey muscles, functional investigations have yet to be done. There are several features of beta MN's which distinguish them from gamma MN's.

Recordings from gamma neurons in monkey jaw muscles and cat hindlimb muscles have shown them to be recruited and their rates largely saturated below extrafusal threshold. Beta fibers may therefore be responsible for fusimotor effects occurring above extrafusal threshold. We have characterized the responses of 33 spindle afferents from triceps surae muscles in decerebrated cats to reflexively mediated increases in isometric tension. The rates of 58% of these increased in parallel with force and emg. After characterizing the afferents we anesthetized the cat and searched through the ventral roots for large diameter fibers which, when electrically stimulated, enhanced the afferent response to stretch (i.e. beta fibers). We found beta fibers innervating 71% of the spindle afferents. Afferents whose rates increased in parallel with motor output were more likely to show beta innervation ($P < .001$, W-test) suggesting that beta-innervation may be responsible for at least part of the fusimotor activity above extrafusal threshold.

To further examine this hypothesis, we used procaine to block gamma fibers on the peripheral nerve and then reexamined previously characterized spindle afferents. Although there was apparent fusimotor activity during gamma blockade for about half of the afferents studied, the effects were vastly diminished compared with before the block. It is possible that beta action is only significant when there is on-going gamma activity.

Another probable difference between gamma and beta MN's is that beta MN's (like alpha's) probably receive powerful excitatory feedback from spindle afferents whereas gamma's do not. Increased beta discharge will enhance spindle discharge which in turn will potentially increase further beta MN discharge, constituting a positive feedback loop. If the loop gain is significant, a component of the spindle length sensitivity would be due to the neural signals going around the loop. Interrupting the loop should remove this component and reduce the length sensitivity of spindle afferents. The length sensitivities of 9 out of the 12 afferents tested were decreased after interrupting the loop by cutting the dorsal roots. The positive feedback loop through beta MN's appears to be significant. According to control theory, this may help to reduce the sensitivity of the system to variations in muscle and load properties.

Supported by NIH NS17489

153.16 NONLINEAR VISCO-ELASTIC PROPERTIES OF THE HUMAN WRIST. L.E.Miller*,J.C.Houk,S.L.Marcus*,.C.C.A.M.Gielen*. Northwestern Univ. Dept. of Physiology and Rehab. Inst. of Chicago, Chicago, IL 60611. Supported by the Coleman, Hearst, J.M., Joyce, Regenstein, Searle Foundations, and Mr. and Mrs. L. Lavin.

Visco-elastic properties of muscle mechanics and stretch reflex actions play an important role in posture and movement. In the experiments reported here, the visco-elasticity of the human wrist motor servo was studied with the instruction, "Do not intervene voluntarily." Since subjects often have difficulty not intervening in response to perturbations, a new procedure was used to identify unintended interventions. These interventions may vary in time and amplitude and are superimposed on the more stereotypic forces due to the muscle mechanics and stretch reflex. Based on this characteristic, a trial comparison method was developed to select the most stereotypic responses from a set of non-intervention trials. The selected responses are thought to represent the mechanical properties and reflex actions of the motor servo uncontaminated by triggered reactions.

Elastic properties were studied by applying step force changes that stretched or released the muscles of the wrist. The response was basically spring-like but included a short-range (< 0.5 cm) enhancement of stiffness that gave rise to a prominent hysteresis. As a result, end position after a force change was dependent on the path to final position as well as final force. The high stiffness was appreciably diminished when a second force step was applied 300 ms following the cessation of movement caused by a prior force step. Some minimum latency may be required in order to reset the high stiffness when the limb reaches a new position.

Viscous properties were studied by applying position ramps of different velocities to the initially active limb. Responses of EMG and force could be described by a product relationship of terms related to position and velocity similar to those described for muscle spindles (Houk,J.C., J.Neurophysiol.:46,143-166,1981). EMG and force responses showed a linear increase with wrist displacement and a fractional-power dependence on velocity. Average values of the exponents were 0.30 (SD=0.09) for EMG and 0.17 (SD=0.05) for force. After ramp plateau had been reached, force and EMG began to decay slowly. After 5 seconds force traces still had not reached a constant level. This fact made it difficult to distinguish elastic from viscous properties and may be related to the phenomenon of hysteresis.

Nonlinear feedback from muscle spindles and short range elasticity of muscles may be responsible for the observed nonlinear visco-elastic properties of the wrist. These properties may be advantageous in the damping of postural responses and movements but may be disadvantageous for the accuracy of aimed movements.

153.17 LENGTH, VELOCITY AND HISTORY DEPENDENCE OF THE HUMAN STRETCH REFLEX. R.K. Powers* and W.Z. Rymer. (SPON: B.W. Peterson). Dept. Physiol., Northwestern University, Chicago, IL 60611

Previous work on the stretch reflex (SR) of the decerebrate cat suggests that during the dynamic phase of a ramp and hold stretch, the firing rate trajectories of primary and secondary spindle afferents, as well as the development of reflex EMG and force, are dependent upon the product of muscle length and a low fractional power of velocity (Houk et al., J. Neurophys., 46:143, 1981; Proc. Int. Congr. Physiol. Sci., 13:981, 1977). Another feature of spindle response that may be evident in SR behavior is the independence of spindle firing rate upon the past history of length change - after the initial burst, spindle firing rate depends only upon instantaneous length and velocity. We are attempting to determine to what extent these features are shared by the SR of human elbow flexor muscles.

We have studied the torque produced about the human elbow joint in response to 0.2 to 1.0 radian displacements at different constant angular velocities, and during transitions from one velocity to another. Elbow extension was produced by a torque motor controlled by a velocity servo. A computer controlled the presentation of stretch stimuli and the collection of position, velocity, torque and surface EMG data. Subjects were required to maintain a constant background flexion torque to initiate a trial, and were instructed not to intervene as the elbow was extended. Absence of intervention was verified by low intertrial variability for a given stimulus class (Crago et al., J. Neurophys., 39:925,1976).

SR torque usually rose linearly during extension, and both the slope of the torque-angle trajectory and the incremental torque developed after a given amount of extension exhibited a low fractional power dependence upon angular velocity. In contrast to muscle spindle behavior, torque-angle trajectories took from 200-400 msec to approach steady-state trajectories following an upward transition in velocity, and from 2-4 sec following a downward transition. Plots of the linear envelope of biceps and brachioradialis EMG (low-pass filtered, 7 Hz cuttoff) vs. joint angle often showed a curvilinear relation between reflex activity and angle, reflecting a rapid increase in EMG at stretch onset followed by little or no further increase with further stretch. The dependence of reflex EMG upon history of length change was less obvious than that of reflex torque, particularly for downward transitions where EMG often approached control trajectories within about 500 msec. These results suggest that at least part of the transitional behavior of reflex torque depends upon the mechanical properties of active muscle.

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153.18 ANATOMY AND PHYSIOLOGY OF SEXUAL REFLEXES IN MALE RATS: SPINAL ORGANIZATION OF DORSAL PENILE NERVE PRIMARY AFFERENTS. Ramón Núñez,* Gayle H. Gross* and Benjamin D. Sachs. The University of Connecticut, U-20, Storrs, CT 06268.

The dorsal penile nerve (DPN), a sensory branch of the pudendal nerve, carries sensory information mediating penile reflexes that have been functionally related to copulatory behavior. In this study we report the spinal segmental position of the dorsal root ganglion (DRG) cells that carry the genital afference, as well as their spinal cord projection patterns, conduction velocity, and synaptic organization.

In adult male rats pretested for strong penile reflexes, horseradish peroxidase crystals were applied to the DPN for 2 hr. Animals were allowed to survive for 72 hr before tissue preparation.

The cells of origin of the DPN were located exclusively in DRG L_6, within which they appeared to be randomly distributed. A mean of 642 ± 17 labeled DRG cells were observed, with a bimodal size distribution. Within the spinal cord these DRG cells send profuse collaterals to the substantia gelatinosa. In their dorsoventral trajectory the fibers course via the medial collateral pathway, exiting to form terminals on medial dorsal horn interneurons in laminae III to VI. At the anterior commissure they join fibers from the contralateral side to form a dense plexus, as well as terminals on lamina X interneurons. Thus, a striking feature of the central projection of the DPN afferents is their extensive distribution among laminae and spinal segments (T_{12} to S_3). Furthermore, we report the novel observation of termination of primary afferent fibers directly onto motoneurons of lamina IX in spinal segments L_2 to L_5, including the nuclei projecting to the striated penile muscles.

When recording from the DPN in response to dorsal root (DR) stimulation, only DR L_6 produced activity. This response had two components with conduction velocities of 32 m/sec and 7.1 m/sec respectively. When the reflex activity produced by electrical stimulation of the DPN was recorded from the ventral roots, the maximal polysynaptic response was obtained from L_2 and decreased in amplitude caudally. No monosynaptic response was obtained by DPN stimulation. However, stimulation of the posterior biceps muscle nerve produced a monosynaptic reflex that was facilitated by DPN stimulation.

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NEUROETHOLOGY: NONAVIAN AUDITORY SYSTEM AND VOCALIZATION

154.1 CRICKET (TELEOGRYLLUS OCEANICUS) PHONOTACTIC DIRECTION IS DETERMINED BY BOTH FREQUENCY AND TEMPORAL PATTERN. G.S. Pollack, F. Huber* and T. Weber*. Dept. of Biol., McGill Univ., Montreal, Quebec and Max Planck Inst. für Verhaltensphysiol, Seewiesen, FRG

Crickets locomote in a directed fashion in response to sound stimuli; they perform phonotaxis. We studied phonotaxis in T. oceanicus, with both a flight paradigm, in which steering attempts were signalled by postural changes, and in a walking paradigm, in which walking direction on a spherical treadmill was measured. Crickets steered toward, or walked in the direction of, a speaker broadcasting a model of the species calling song with a natural carrier frequency (5 kHz), and steered or walked away from a song model with 33 kHz carrier. Thus the frequency-dependent switch from positive to negative phonotaxis, previously reported for flight behaviour by Moiseff et al (PNAS 75: 4052; 1978) also occurs during walking.

Recently, Thorson et al (J. Comp. Physiol. 146: 361; 1982) discussed how changes in carrier frequency could, due to acoustic properties of the ears, result in alterations of the binaural difference in sound intensity which is thought to underly the cricket's determination of sound direction; directionality might thus be inaccurate at elevated carrier frequencies. Could errors in directionality explain positive and negative phonotaxis? To answer this question we studied crickets which were unilaterally deafened by amputation of one ear. Since these crickets received all acoustic information from the one remaining ear, their responses should not be affected by changes in binaural intensity difference. In the flight paradigm, one-eared crickets steered toward the intact side when a 5 kHz song model was broadcast, and away from the intact side when a 33 kHz model way played. In the walking paradigm, crickets turned continuously (circled) toward the intact side for the 5 kHz model, and away from the intact side for the 33 kHz model. Thus, low and high frequencies elicit opposite responses even when frequency effects on binaural intensity differences are precluded.

Low and high frequency responses had different temporal pattern requirements. A non-song pattern failed to elicit responses with 5 kHz carrier frequency, but with 33 kHz carrier it elicited negative phonotaxis in intact crickets, and orientation away from the intact side in one-eared crickets, in both the flight and walking assays. At an intermediate frequency, 15 kHz, response type depended on temporal pattern. The song pattern resulted in positive phonotaxis (intact crickets) or orientation toward the intact side (one-eared), and the non-song pattern in negative phonotaxis or orientation away from the intact side.

Frequency-dependent directionality errors alone cannot account for our findings. It seems likely that positive and negative phonotaxis are the products of different neural substrates.

154.2 SELECTIVITY OF MIDBRAIN NEURONS TO AMPLITUDE MODULATED WHITE NOISE: DIFFERENTIAL TUNING IN TWO SPECIES OF TOADS. G. J. Rose and R. R. Capranica. Section of Neurobiology and Behavior, Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

The mating calls of Bufo a. americanus and Bufo w. fowleri are very similar in terms of their frequency content but differ markedly in their temporal structure. Specifically, the call of Fowler's toad is amplitude modulated (AM) at a rate approximately three times that of the American toad, and is of shorter duration. Single unit recordings from the torus semicircularis in the midbrain revealed a population of neurons in each species that are tuned to particular rates of amplitude modulated white noise; these AM tuned units comprised 34% of all of the cells recorded. The modulation rate at which a unit responded maximally was termed its "best rate of AM". The distribution of best rates of AM was shifted to higher modulation rates in Fowler's toad, relative to the American toad. Further, neurons tuned to modulation rates greater than 75 Hz were only found in Fowler's toad. The results suggest that the tuning of AM-sensitive neurons in the anuran midbrain is species-specific.

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154.3 A SEX DIFFERENCE IN BASILAR PAPILLA TUNING IN THE HYLA CRUCIFER AUDITORY SYSTEM AND ITS BEHAVIORAL SIGNIFICANCE. Walter Wilczynski, Harold Zakon, and Eliot A. Brenowitz. Section of Neurobiol. & Behav., Cornell University, Ithaca, NY 14853.

The tuning of single eighth nerve auditory fibers in the spring peeper (a small treefrog) was investigated electrophysiologically in males and females collected from breeding choruses around Ithaca, NY. Two groups of fibers are present. One consists of low and mid frequency units with best excitatory frequencies (BEFs) ranging from 103-1200 Hz. These fibers presumably arise from the amphibian papilla (AP). There is no strong difference between males and females in AP tuning. However, a sex difference is apparent in the tuning of the second group of fibers, which are tuned to higher frequencies and presumably arise from the basilar papilla (BP). In both sexes, the BEFs of BP units in any individual are tightly clustered, but there is variation among individuals. In males, BP tuning averaged 3580 Hz (range: 3350-4000 Hz). In females average BP tuning is lower, 2939 Hz, and the variation in tuning among individuals is greater (range: 2100-3700 Hz). Male and female thresholds are comparable. Within females BP tuning is correlated with size such that as snout-vent length increases, BP tuning shifts to a lower frequency (r^2=0.58, p<0.025). (See also Shofner and Feng, 1981, J. Exp. Biol., 93: 181.) The sex difference in BP tuning may be due to size differences between males and females: females are generally larger than males. The smallest female tested was approximately the size of an average male, and had a BP tuned to 3600 Hz. Although the sex difference in BP tuning may be a passive result of male-female size differences, it has important behavioral consequences. In many anurans the male advertisement (mating) call has been implicated in both female attraction and intermale agonistic interactions. H. crucifer's call is a simple, tonal "peep". Its mean dominant frequency in males from the Ithaca area is 2895 Hz (range: 2588-3212 Hz). Thus males call where the females are tuned, which maximizes the distance over which females can be attracted. In doing this males become "detuned" to their own calls, although they can still detect the call frequencies with the low frequency slopes of their BP tuning curves. The high detection threshold this yields is used by the males to distribute themselves within the chorus. The average intensity of a male's call when it reaches his nearest neighbor was measured to be 79.1 dB SPL, which is just a few dB above the threshold for detecting the call frequencies. Thus males space themselves far enough apart so that they can barely hear their neighbors.

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154.4 TEMPORARY THRESHOLD SHIFT IN FROG AUDITORY NERVE FIBERS. R. Zelick* and P.M. Narins. Dept. of Biology, UCLA Los Angeles, CA 90024.

The treefrog Eleutherodactylus coqui communicates acoustically in a very loud sonic environment. Similar circumstances occur for many frog species in which calling males may commonly encounter continuous background levels of 80 to 90 dB SPL from the vocalizations of neighboring individuals. This prompted us to investigate whether these animals have adaptations which render them relatively insensitive to the temporary threshold shift (TTS) a mammal would experience in the same environment.

Under computer control, thresholds to tone bursts (50 msec duration) were determined for single fibers in the eighth cranial nerve of immobilized and topically anesthetized male E. coqui before and after a three minute presentation of a monaural continuous tone stimulus (CTS). For each neuron examined, the CTS was set to the best excitatory frequency (BEF) and to a level between 10 and 50 dB above the neuron's best threshold (115 dB SPL max). Immediately following the CTS, threshold determinations were made either every 200 msec or every second for a period of three minutes at the BEF and typically one and two octaves above and below the BEF.

Thresholds tracked before the CTS was presented did not change over time. After CTS exposure, both low frequency (amphibian papilla) and high frequency (basilar papilla) fibers exhibited thresholds elevated by as much as 40 dB. At any BEF there was a wide range of threshold shifts (>20 dB) observed for a given CTS level above threshold. TTS was greater at the BEF than at frequencies above and below the BEF. The amount of recovery (sensitivity regained) after three minutes was variable, even for neurons with similar BEFs, although all neurons recovered to some extent. Recovery functions were typified by a rapid initial phase (<30 sec) and a more gradual second phase. A stepwise linear regression procedure was used to determine the sources of variability for the dependent variables 'initial shift' and 'recovery'. Pre-CTS best threshold was the independent variable most highly correlated (p<.0001) with both dependent variables. CTS level above best threshold and BEF were also significantly correlated with the dependent variables.

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154.5 INTERFERENCE WITH TARGET RANGE DISCRIMINATION BY ECHOLOCATING BATS, CORRELATED WITH THE STRUCTURE OF ARTIFICIAL PULSES. R. Roverud * and A. D. Grinnell, Depts. Biology and Physiology, UCLA, Los Angeles, CA, 90024. (SPON: R. Barrett).

Bats of the species Noctilio albiventris were trained to discriminate differences in target distance by means of echolocation. During the discrimination trials, the bats repeatedly emitted pairs of pulses, the first a constant frequency (CF) signal approximately 12 msec long and 75 kHz in frequency, followed after 20-30 msec by a CF/FM pulse composed of a 75 kHz CF component of approximately 10 msec duration terminating in a 2 msec frequency modulated (FM) component sweeping downward to about 40 kHz.

Loud artificial pulses, simulating the bats' natural CF/FM echolocation sounds and presented to the bats during the behavioral trials, interfered dramatically with distance discrimination. Systematic modifications in the structure of the artificial pulses resulted in orderly changes in the degree of interference with the discrimination. Interference occurred when repetition rates exceeded 5/sec. Artificial pulses simulating the bats' natural CF sound alone or the FM component of the CF/FM pulse alone did not interfere with discrimination. 12 msec white noise pulses presented at 10 pulses/sec also had no effect, although constant white noise masked the behavior. Artificial CF/FM pulses with a reduced FM bandwidth were effective in disrupting performances when the FM sweep exceeded 10 kHz.

Interference occurred when the duration of the CF component of the CF/FM signal was between 2 and 30 msec, with maximal effect at durations of 8-20 msec. Presenting a brief (0.6 msec) CF signal 2-30 msec before the isolated FM component was equivalent, in effect, to CF/FM pulses having CF components of the same duration. Therefore it is the time interval between the onset of the CF component and the FM sweep that determines the extent of interference with the bat's interpretation of echoes of its own sounds.

The "jamming"effectiveness of the artificial pulses depended upon the absolute frequencies presented to the bat. Interference occurred when the CF frequency was between 77 kHz and 52 kHz, with a downward FM sweep of 25 kHz from the CF frequency. The degree of interference caused by the pulse also depended on the relative frequency of the CF and FM components. The pulses interfered with the ability of the bat to discriminate only when the CF frequency was very close to that of the beginning of the FM sweep.

These findings imply the importance for range discrimination of a population of neurons that are selectively activated by a combination of CF onset and FM components occurring at 10-20 msec separation, within a fairly broad frequency range, but with the requirement that the frequency of the CF and the onset of the FM sweep be nearly identical.

154.6 A QUANTITATIVE STUDY OF VOCALIZATIONS ELICITED BY HYPOTHALAMIC STIMULATION IN THE CAT. I. Altafullah,* C. Shipley* & J. Buchwald (SPON: E. Eldred). Brain Res. Inst., Ment. Ret. Res. Ctr., Dept. Physiology, UCLA Med. Ctr., Los Angeles, CA 90024.

Vocalizations evoked by electrical stimulation of the hypothalamus were studied in 6 adult unanaesthetized cats. Voiced ("Meows") and/or unvoiced ("Hisses") responses were evoked, but only the voiced calls will be discussed. The objectives of this study were: 1) to analyze quantitatively the calls evoked by electrical stimulation of the hypothalamus 2) to compare the evoked calls with spontaneous isolation calls of the animal and 3) to study the effects of parametric stimulus changes upon evoked calls.

Spontaneous calls were recorded with the animal comfortably restrained and in isolation. Stimulation was subsequently carried out with bipolar concentric electrodes delivering stimulus trains of 5 msec biphasic square waves. Stimulus parameters of interest were, frequency (30-150 cps), intensity (0.1-0.4 mA) and train duration (0.5-3 sec). At the end of the experiment the animals were sacrificed and histological confirmation of electrode sites obtained. Calls were analysed to determine latency from stimulus onset, duration and peak intensity. Acoustic analysis was performed after digitizing the call (sampling rate: 20 KHz.). The analysis program used linear prediction techniques and the fast Fourier transform. A number of acoustic parameters, including the fundamental frequency and harmonic structure of the call, were determined, which allowed quantitative comparisons between the spontaneous calls and calls evoked under various stimulus conditions.

A total of 297 points were stimulated along 61 electrode tracks in the hypothalamus. Vocalizations were elicited from 58 points and 470 evoked calls were recorded. Especially dense collections of responsive sites were seen in the pre-optic region, the lateral hypothalamus, the perifornical region and the area around the ventromedial nucleus of the hypothalamus. The threshold for response was, in most instances, between 0.1-0.2 mA, but varied depending on the animal, the site and the parameters of stimulation. Similarly, the latency from stimulus onset also varied, and ranged from 0.5 to 3 sec. Increasing the intensity of stimulation had no significant effect on the latency, intensity or duration of the evoked vocalizations. In contrast, increasing the stimulus frequency significantly increased the intensity and decreased the latency of the calls. Preliminary results from acoustic analysis suggest that voiced calls evoked by hypothalamic stimulation are very similar to spontaneous calls emitted during isolation. A detailed quantitative comparison of the evoked and spontaneous calls will be presented.

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155.1 PHYSIOLOGY AND MORPHOLOGY OF ELECTRIC ORGAN DISCHARGE COMMAND NUCLEI IN MORMYRID FISH. K. Grant*, S. Clausse*, C. Bell. Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190, Gif sur Yvette, France.

The spinal motorneurons which innervate the mormyrid electric organ are driven by a descending volley from the medullary relay nucleus (MRN). A recent anatomical study by Bell et al (J Comp Neurol, 1983) indicates that the MRN is in turn innervated by a small nucleus, nucleus C, located immediately ventral to the MRN. The goal of the present study was to characterize the neurons in these two command related nuclei and test the hypothesis, suggested by the previous work, that nucleus C is the EOD command nucleus. MRN neurons and nucleus C neurons were examined with intracellular recording, and with intracellular injection of horseradish peroxidase. Morphology was studied at both light and electronmicroscopic (EM) levels.

MRN neurons give a two spike volley before each EOD which is followed by an after-hyperpolarization (15-40 msec). Morphologically, each MRN cell body gives rise to 6 or 7 primary dendrites which ramify widely within the nucleus, contacting each of the other MRN somas (~20). EM examination shows gap junctions at these dendrosomatic contact points, and shows similar appositions between labeled and unlabeled dendrites. The above physiological results as well as electrical coupling and morphological gap junctions between MRN cells were described previously by Bennett et al (J Neurophysiol, 1967).

Nucleus C neurons also show a two spike volley before the EOD, with the first spike of the volley occurring 100-200 µsec before the first spike in MRN cells. Apparent synaptic noise and small (1-10 mv) all-or-none spike-like events (possibly axon spikes) are also seen. A pre-potential and one of the small spikes commonly precede the two full spikes of the pre-EOD volley. The synaptic noise and small spikes are blocked by a long-lasting (50-80 msec) hyperpolarization which follows the pre-EOD volley. The somas of nucleus C cells give rise to 2 or 3 main dendrites which branch slightly and extend far beyond the confines of their nucleus. The presumed axon arises laterally from a long initial segment and turns caudodorsally, giving off collaterals which appear to terminate immediately ventral to the MRN. The timing of the pre-EOD volley, the subthreshold potentials which precede the volley, the synaptic noise, and the morphology of nucleus C neurons support the hypothesis that these cells form an integrating center where the EOD command originates.

155.2 SINGLE ELECTROCYTES PRODUCE A SEXUALLY DIMORPHIC SIGNAL IN ELECTRIC FISH. M.M. Hagedorn* and C.E. Carr. Scripps Institution of Oceanography, La Jolla, CA., 92093.

Electric fish produce electric signals by means of an electric organ made up of modified nerve or muscle tissue in their tails. The electric organ discharge (EOD) of several species is sexually dimorphic during the breeding season, and the electric signals can be used in social communication. During the breeding season, male Hypopomus occidentalis, a pulse-type electric fish, produce a sexually dimorphic signal. The electric organ of Hypopomus is driven by spinal electromotor neurons which innervate the caudal face of large, modified muscle cells, called electrocytes; both faces of the electrocyte are excitable. When the fish fires its electric organ, the caudal, innervated face fires first producing the positive-going phase of the diphasic EOD. This depolarizes the electrocyte and causes the rostral face to fire producing the second, negative-going phase of the EOD. The second phase of a breeding, male's EOD has a smaller amplitude and a longer duration than the first phase, while nonbreeding and mature females have a symmetric EOD (see figure).

Adult males and females injected with 5αdihydrotestosterone (DHT) (5μg/g.b.w.) produce an asymmetric EOD, characteristic of a breeding male, while animals injected with saline maintain a symmteric EOD. After 1 week of treatment, the EODs of the animals were digitized on a PDP-11 computer and analyzed by a transient capture fast fourier program. DHT-treated animals had a low peak power frequency while saline-treated animals had a high peak power frequency. Steroid treated animals also showed an enlargement of the electrocytes in their tails.

Intracellular recordings were made from single electrocytes in animals treated with DHT and saline. Electrocytes were depolarized by an intracellular stimulating electrode and spikes were recorded across the caudal, innervated face as well as the rostral, uninnervated face. The electrocytes of saline-treated fish with symmetric EODs, produced similar spikes from both faces. The electrocytes of DHT-treated fish produced a normal spike from the caudal, innervated face and a spike of longer duration and smaller amplitude from the rostral, uninnervated face. This suggests that the sexually dimorphic EOD found in breeding, male Hypopomus occidentalis is produced by the action of steroids upon single electrocytes.

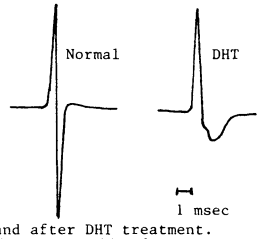

Figure: EODs of a female before and after DHT treatment.

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155.3 THE TORUS SEMICIRCULARIS OF GYMNOTOID ELECTRIC FISH: THE FUNCTIONAL ORGANIZATION OF A PARALLEL PROCESSOR. W.F.Heiligenberg and C.E.Carr. Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093.

The torus semicircularis of gymnotoid electric fish is a richly laminated, somatotopically organized midbrain structure (Carr et al, J.Comp.Neurol. 203: 649-670, 1981). The dorsal part of the torus, consisting of laminae #1 to #9, receives electrosensory input from the electrical lateral line lobe of the hindbrain (ELL) and projects topographically to the tectum, where visual and electrosensory images of the world are superimposed (Bastian, J., J.Comp.Physiol. 147:287-297, 1982).

The torus is of particular importance for the control of the Jamming Avoidance Response (JAR) which requires parallel processing of information about modulations in amplitude and phase of the electric signals in different parts of the body surface. Various cell types and their functional significance have been explored previously by extracellular recordings (Scheich, H., J.Comp.Physiol., 113: 181-255, 1977; Bastian,J. and Heiligenberg, W., J.Comp.Physiol. 136: 135-152, 1980; Partridge et al, J.Comp. Physiol., 145: 153-168, 1981). We are now in the process of anatomically identifying these cell types by intracellular recording and labelling.

Information about phase, i.e. the timing of zerocrossings of the sinusoidal electric signal, is received in lamina 6, and the difference in phase between signals arriving from different parts of the body surface is encoded by cells whose somata are located in lamina 8. Information about modulations in amplitude is received in laminae 5,7 and 8 and is then computed in reference to differential phase information. The outcome of this computation is encoded by cells in lamina 8 which project to the tectum.

Different steps in information processing are thus executed in different laminae of the torus, and, by virtue of their somatotopical organization, these laminae can readily compute spatial aspects of electric signals on the animal's body surface.

155.4 FREQUENCY TUNING OF ELECTRORECEPTORS IS LOWERED IN ANDROGEN TREATED MORMYRID FISHES. A.H. Bass. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853

In many species of electric fish the frequency tuning curves of electroreceptors track the peak frequency of the power spectrum (PPW) of the electric organ discharge (EOD). For mormyrids with a sex difference in the EOD, the male EOD is typically longer in duration and so has a lower PPW compared to females and juveniles. The male-type EOD can be induced in females and juveniles with gonadal steroids. Similarly, there is also a sex difference in the frequency characteristics of one class of electroreceptors (Knollenorgans) that are the putative communication sensors and are tuned lower in natural males for at least one species, Brienomyrus brachyistius triphasic. For this species, there is also a lowering of the best frequencies (as defined with frequency tuning curves) of Knollenorgans in androgen-treated females that coincides with the decrease in the PPW of the EOD. Androgens may exert their effects directly on Knollenorgans. Alternatively, changes in tuning may be induced by the power of the principal stimulus to the Knollenorgans which is the EOD itself. Such alternative hypotheses can be tested in mormyrids by silencing the electric organ and so removing the external EOD stimulus that may "entrain" the electroreceptor's best frequency . Silencing is accomplished by sectioning the spinal cord just anterior to the spinal electromotor nucleus located at electric organ levels, thereby interfering with a descending medullary signal that excites the electromotor nucleus and so the electric organ. Silencing experiments are being conducted with a mormyrid fish from Nigeria that also has a triphasic EOD waveform and shows a dramatic shift in the PPW of its EOD when treated with androgens. Preliminary results suggest that: (1) normal hormone treated fish show a decrease in frequency tuning that tracks the decrease in the PPW; (2) cholesterol treated fish show no change in tuning or PPW; (3) silenced fish kept in isolation and now deprived of the electrical stimulus show no drift in tuning; and (4) silenced fish treated with androgens show a partial shift in tuning. Gonadal steroids may alter the morphological and physiological features that define the passive and active electrical properties of the receptor cell membranes of Knollenorgans which, as other electroreceptors, are essentially modified hair cells. Studies of the development of frequency tuning in electroreceptors may provide a better understanding of the mechanisms underlying frequency tuning in other vertebrate hair cells such as auditory receptors.

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155.5 SEX STEROIDS INFLUENCE THE ELECTROSENSORY SYSTEM OF STERNOPYGUS: DIRECT EFFECT ON EOD-GENERATING CIRCUITRY, INDIRECT EFFECT ON RECEPTOR TUNING. H.H. Zakon and J.H. Meyer. Neurobiology Unit, Scripps Instit. of Oceanography, Univ. of Calif., San Diego, La Jolla, CA 92093.

Weakly electric fish detect their electric organ discharges (EOD)s via specialized receptor cells termed electroreceptors. Sternopygus dariensis produces a nearly sinusoidal EOD, the frequency of which displays an ontogenetic shift which is sexually dimorphic: juveniles discharge around 100 Hz and with age, the EOD frequencies of females increase (100-200 Hz), while those of males decrease (50-100 Hz). Systemically applied androgens decrease and estrogen increases EOD frequency. We have previously demonstrated that the androgen, 5α-dihydrotestosterone (DHT) can influence the frequency selectivity of the electroreceptors: receptors are always most sensitive to electric fields at the frequency of an individual's EOD and, after treatment with DHT, receptors can be "retuned" to a lower frequency which more closely approximates the new EOD frequency. However, since electroreceptors are also exposed to a new EOD frequency after DHT treatment, it is not clear whether the hormone itself or the electric field induces retuning of the receptors.

Baseline recordings were made of animal's EOD frequency and the tuning properties of their afferent electroreceptive fibers from the left lateral line nerve. For each fiber, best frequency (BF), Q_{10dB}, and threshold were measured. Then, the EOD was eliminated by a high spinal transection which disrupts descending input to the electric organ from a medullary pacemaker nucleus (PMN), but leaves the PMN intact. Fish were put in 2 groups, one given daily injection of DHT (5 µg/ g.b.w.), and the other given saline control injections. After 2 weeks of injections, receptor tuning characteristics of fibers in the right nerve and PMN discharge frequencies were determined. In the control group, PMN discharge frequencies and tuning characteristics of electroreceptive afferents were no different from baseline values. In the DHT group, PMN discharge frequencies were significantly lower. However, Q_{10dB} values and threshold did not differ after DHT treatment. BFs were also not significantly different in the DHT group, although 3/6 fish showed a slight decline in BF after the DHT treatment. Thus, 1) in the absence of an ongoing EOD, electroreceptors can maintain their tuning 2) DHT exerts a direct effect on EOD-generating circuitry within the CNS and can do so without concurrent reafference from electroreceptors 3) the EOD appears to be necessary for reliable and marked retuning, although DHT alone may be capable of inducing small decreases in receptor BF in some animals. (Aided by NIH grants to T.H. Bullock, W. Heiligenberg, JHM, HHZ and NSF grants to THB and WH.)

155.6 ANATOMICAL EVIDENCE FOR SYNCHRONIZATION OF FAST TOADFISH SWIMBLADDER MUSCLE, AN HRP STUDY. M.L. Fine and P.J. Mosca*. Dept. of Biology, Virginia Commonwealth Univ., Richmond, Va. 23284.

Toadfish sonic muscle is a classic example of a fast vertebrate muscle, capable of contracting several hundred times a second without tetanizing. Muscle contraction rate generates the fundamental frequency of the fish's courtship and agonistic vocalizations. In 1961 Skoglund demonstrated an 0.3 - 0.5 msec difference in onset of an action potential between the cranial and caudal ends of the muscle over a length of 40-50 mm, i.e. the muscle contracts in almost perfect synchrony. This time difference is less than would be predicted from the conduction velocity of the sonic nerve.

We looked for anatomical evidence of this synchronization by utilizing retrograde transport of horseradish peroxidase (HRP) injected into the sonic muscle. Since the muscle fibers are arranged vertically in a horizontal band along the bladder, and the sonic nerve courses horizontally (perpendicular to the fiber direction), the muscle is ideally suited for investigation of somatotopic mapping of motoneuron projections on muscle fibers. Individual 4 µL injections of 4% HRP (Sigma VI) were placed in either the cranial, mid, or caudal regions of sonic muscles. After survival times of 7-9 days, fish were processed for HRP following the procedure of Mesulam using tetramethylbenzidine as a chromagen. Serial frozen sections were cut horizontally through the SMN at 50 µm. The number and percentage of labeled motoneurons was recorded in each of 5 equally marked zones in the ipsilateral SMN. With minor exceptions, the distribution of labeled neurons in each of these zones was similar regardless of injection site. Small pin injections, one of which labeled only 2% of the ipsilateral neurons, confirmed this pattern. Accordingly, we conclude that a neuron in any part of the SMN has the potential to innervate fibers in any part of the sonic muscle. This is a simple but clear solution to the problem of muscle synchronization. The arrangement of toadfish sonic muscles is sufficiently different from mammalian muscles that caution must be used in suggesting that similar mechanisms control synchronous contraction of mammalian muscle.

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155.7 SPECTRAL SENSITIVITY IN HAPLOCHROMIS BURTONI (CICHLIDAE). T. E. Allen and R. D. Fernald. Inst. of Neuroscience, Univ. of Or., Eugene, Or. 97403

Previous investigations have shown that the African cichlid Haplochromis burtoni lives in a complex social system in which many of the encounters among individual fish are mediated by vision. Social status and motivational state are reflected in body color patterns which appear to be recognized by conspecifics (Fernald, 1977; Fernald and Hirata, 1977). The visual pigments, too, suggest that the perception of color is important to this species; while the rods contain the visual pigment $P500_1$, cones exist which contain $P455_1$, $P523_1$, and $P562_1$ (Fernald and Liebman, 1980). In order to determine the extent of color vision, it is necessary first to measure threshold as a function of wavelength. Since these are active and adaptable animals, I chose to use an operant conditioning paradigm with food reward in order to determine spectral sensitivity. A two choice chamber was constructed which could be inserted and removed from the home tanks of individual fish with minimal disturbance to them. I then designed and built an automated apparatus to select sides for stimulus presentation, to operate timers, shutters, and feeders appropriately, and to record the data. After initial training to high levels of performance at high stimulus radiances, a method of descending staircase sessions was employed. When the performance fell below criterion level (75% correct) the following session was conducted with an increased radiance. In this manner a stable threshold was found. The function derived from testing fish under dark adapted conditions conforms reasonably closely to the nomogram of a $P500_1$ pigment (confirming microspectrophotometric findings), but shows unexpectedly high sensitivity at short wavelengths (Allen and Fernald, 1981). Depending on assumptions about the precise angle of view of the stimulus, threshold at 500 nm is one quantum incident per second for every 5 to 50 rods. The spectral sensitivity function obtained under light adapted conditions is much broader and does not decline nearly so steeply outside the mid-spectral region; levels near to maximum are obtained from 450 nm to 600 nm. Several pigment classes are functional under these conditions. The form of the function suggests that an opponent processing mechanism similar to that found in all known trichromats is operating here (Allen and Fernald, 1982). Under light adaptation, threshold is found to be approximately 1,000 times higher than is the case for dark adaptation.

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155.PO THE NUCLEUS ELECTROSENSORIUS: A PROCESSING STATION INVOLVED IN THE CONTROL OF THE JAMMING AVOIDANCE RESPONSE. J. Bastian and J. Yuthas Dept. of Zoology, Univ. of Oklahoma, Norman, Ok 73069.

Weakly electric fish, Eigenmannia, respond to the electric organ discharges (EOD) of conspecifics having similar EOD frequencies by raising or lowering their frequency thereby reducing the deleterious effects of the summed EOD fields. Animals raise or lower their EOD frequencies in response to the sign of the difference frequency ΔF, where ΔF is defined as the frequency of the interferring fish, F2 minus that of the reference fish, F1, (ΔF= F2-F1). Positive ΔF results in fish 1 lowering its frequency and vice versa. Previous studies of this jamming avoidance response (JAR) have shown the torus semicircularis to be a major processing station for the control of this behavior (see Heiligenberg, Naturwissenshaften 67, 499-507 [1980] for review). Recent anatomical studies have identified a nucleus in the rostral mesencephalon, the pre-pacemaker nucleus (PPN), which projects directly to the medullary pacemaker nucleus (PM), and electrical stimulation of this nucleus verifies that it is capable of altering PM frequency. Short term increases or decreases in PM frequency occur depending on the time of stimulation relative to the phase of the EOD cycle. It remains to be determined by what pathways the outputs of the torus affect the PM frequency, presumably via the PPN.

We performed micro-stimulation experiments to try and identify brain areas which were capable of altering the PM frequency. We found that stimulation of a diencephalic site evoked increases in PM frequency having a time course similar to the EOD accelerations seen in the JAR. HRP deposition showed that the stimulation site correspondes to the recently described N. electrosensorius (Carr et al. J. Comp. Neurol. 203, 649-670, [1981]). Single unit recordings from this nucleus show two major cell types. One type is completely silent during $-\Delta F$ but becomes active during $+\Delta F$ stimuli. These cells show maximal activity with ΔFs ranging from 2-6 Hz. The second cell type is most active during $-\Delta F$ and activity is partially or completely suppressed during $+\Delta F$ stimulation. The activity of these cells continues to increase with increasing $-\Delta F$. These cells are probably not the final controllers of the pacemaker since short term changes in their firing frequency are not strongly correlated with changes in the PM frequency. Supported by NIH grant NS12237 to J. B.

156.1 COCKROACH ESCAPE BEHAVIOR: EFFECTS OF LESIONING INDIVIDUAL GIANT INTERNEURONS BY INTRACELLULAR INJECTION OF PRONASE. Christopher Comer. Dept. of Biological Sciences, Univ. Illinois, Chicago 60680

The ventral nerve cord of the cockroach, Periplaneta americana, contains 7 bilaterally paired 'giant interneurons' (GIs) which encode the direction of wind stimuli and which are believed to orient the animal's wind-triggered escape response. I was interested in directly testing the role of the GIs in the normal escape response and specifically in determining how spatial sensory information in these individually identified cells is translated into directed movement. To do this, I have begun analyzing the behavioral effects of lesions of individual GIs.

Cockroaches were immobilized ventral side up and the nerve cord was exposed through a small flap cut in the abdominal cuticle. GIs were impaled in the connective with glass micropipettes filled with 50mM KCl and .5% Pronase (Calbiochem). After a cell was tentatively identified from the pattern of its wind evoked activity, Pronase was pressure injected into the cell and the flap of cuticle was replaced and sealed with dental wax. Animals displayed normal activity and locomotion within 1hr. of this procedure.

Histological studies revealed that the axon of a GI completely degenerates within 48hrs. of Pronase injection. Standardized behavioral tests were conducted from 2-7 days after injection.

In 4 cases GI-1 was destroyed unilaterally. These animals turned incorrectly toward frontal winds on the side ipsilateral to the lesion ($p < .05$). 3 animals with unilateral deletions of GI-2 showed no abnormality in the orientation of escape turns. 4 animals with combined unilateral deletions of GIs 1 and 2 displayed incorrect turns toward ipsilateral wind stimuli, and this effect was more pronounced than in the case of lesioning GI-1 alone. Controls with pronase injected extracellularly or into non-giant interneurons showed no significant change in the orientation of escape turns.

These results provide direct evidence which implicates cockroach GIs in the control of directional escape behavior. Systematic study of the behavioral effects of various GI lesions should yield a description of the processes by which sensory information in this ensemble of cells is integrated to produce directional motor output.

156.2 CHANGES IN THE TEMPORAL PATTERNING OF ESCAPE SWIMMING IN CRAYFISH AS A CONSEQUENCE OF REPEATED STIMULATION. S. du Lac* and J. J. Wine. Dept. of Psychology, Stanford University, Stanford, CA 94305.

Crayfish escape swimming is an episodic rhythmic movement offering certain advantages for neural analysis. It was recently shown that the probability of swimming declines markedly with repeated stimulation (Reichert, H. and Wine, J.J., Nature, 296:86, 1982).). We wanted to know if the patterning of swimming was independent of response probability, or if it also changed as a result of stimulation. We recorded EMGs from phasic flexor and extensor muscles in 15 crayfish 5.5-9.0 cm in length, which were free to swim within the confines of a 5 gal aquarium (20 x 40 cm, 20 cm deep). Each animal was stimulated with a light tap on the adomen at one min intervals at least 20 times per day. Stimulus intensities were adjusted to hold mean responding close to 50%.

The first two swims of a series provided a measure of response patterning in unhabituated animals. The mean duration of such swims was 1151 msec (range 250-2850 msec), mean number of non-giant flexions was 11.4 (range 3-24), and mean frequency (based on inter-flexion intervals) was 8.7 Hz (range 4.2-14.7 Hz). We confirmed findings that extension leads flexion, and that the extension-flexion interval is neither phase nor latency constant (Schrameck, J.E., Science, 169:698, 1971).

With repeated stimulation, the proportion of responses that included swimming, as opposed to single, giant axon-mediated tailflips, declined from 86% on trials 1-5 to 36% on trials 16-20, thus confirming the earlier finding that swimming is more prone to habituation than is giant-mediated tailflipping (Reichert et al., ibid., 1982). In addition, the temporal properties of the swim changed over trials. The number of flexions per swim declined from 10.9 (± 5.4, S.D.) to 3.6 (± 1.0, S.D.), latency to onset of swimming increased from 140 to 180 msec, and mean frequency of swimming declined from 8.2 to 7.1 Hz (all measures were for trials 1-5 vs. trials 16-20).

A pronounced slowing of swimming frequency within swims was also noticed for about half of all swims. These results are inconsistent with the hypothesis that swimming is produced by a stable oscillator which is gated by a habituation-prone switch.

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156.3 THE BEHAVIOR OF APLYSIA CALIFORNICA: ETHOGRAM AND ETHOLOGICAL ANALYSIS OF MATING. Janet L. Leonard* and Ken Lukowiak (SPON: W.L. Veale). Dept. of Medical Physiology, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1.

As a complement to neuroethological studies, we have developed an ethogram for Aplysia californica, based on laboratory observations, and made detailed observations on the behavioral sequences involved in courtship, copulation and role-switching. Development of the ethogram was aided by the use of time-lapse videotape recordings. The ethogram is composed of 35 fixed action patterns (FAPs) (Schleidt, 1974; Lorenz, 1981), most of which are used in more than one context. Most FAPs represent subtle movements of specific regions of the body, primarily the head, and occur in more than one functional context. Many FAPs are superimposable.

The sexual behavior of A. californica is comparable in complexity and overall structure to that of Navanax inermis (Leonard and Lukowiak, 1982). A. californica tend to copulate in bouts, with each individual alternating between behaviorally identifiable "male" and "female" states. Courtship (= precopulatory behavior) is initiated when one individual (the "male") contacts a conspecific (the "female") with his head and begins exploring her body, using the FAP, Brushing. At some point after Brushing starts the male begins to show Rhinophores Laid Back. The female responds to Brushing, particularly when it is directed to her parapodia by first Flaring and then Drawing Down her parapodia. These FAPs expose the mantle and make the common genital aperture (CGA) accessible to the male. The female also stops Crawling. After Brushing the male uses the Frill Tucked posture to explore the body of the female, then begins Head-leaning. He eventually comes to rest with his head inside her anterior parapodia. He remains motionless in the Head-leaning posture during intromission. Intromission varies in durations from a few minutes to more than 3 hours. The female assumes a Tentacles Pointed posture at some point after Drawing Down and may show Tail Puffed. A striking feature of the sexual behavior of A. californica is that the male often remains motionless in the Head-leaning posture, in contact with the anterior parapodia of the female, after he has withdrawn his penis from the CGA. At some point, usually after the male has withdrawn, the female begins to shown male behavior either to her partner or to another individual.

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156.4 PHYSIOLOGICAL CONTROL OF PHEROMONE RELEASE BEHAVIOR IN FEMALE MOTHS (MANDUCA SEXTA AND UTETHEISA ORNATRIX). Haruhiko Itagaki and William E. Conner*. Department of Zoology, Duke University, Durham, NC 27706

The behavior associated with pheromone release in female moths is termed "calling", and consists primarily of the extrusion of the terminal abdominal segments to expose the pheromone glands located near the abdominal tip. The control of calling in female Manduca sexta(Lepidoptera:Sphingidae) and Utetheisa ornatrix (Lepidoptera:Arctiidae) were investigated using standard physiological methods.

Severing the ventral nerve cord (VNC) in the abdomen between the 1st and 2nd or 2nd and 3rd ganglia stopped calling in both species. Sham operated controls were unaffected. The removal of the two pairs of neurohemal organs associated with the brain, the corpora cardiaca and the corpora allata, did not affect calling behavior, contrary to the findings in Saturniid moths (Riddiford, L.M. and C.M. Williams, Biol. Bull., 140:1, 1971). Since there are other neuroendocrine organs in insects, non-calling Manduca were injected with 0.03cc of blood from calling females. Recipients of blood did not initiate calling earlier than normal. The gross electrical stimulation of the VNC in isolated abdomens of Utetheisa(@60Hz, 1 msec. duration, 5-40v, in 3 or 5 sec. bursts) was sufficient to induce the abdomens to extrude and retract the terminal segments in a rhythmic manner similar to normal intact females. The rhythmic extrusion and retraction could be elicited from abdomens with only the terminal ganglion present.

The control of pheromone release behavior in the moths Manduca sexta and Utetheisa ornatrix appears to be under strict neural control. There is no evidence for hormonal influences which initiate or modulate calling behavior. The source(s) of neural control, and the possible existence of a central pattern generator in the terminal abdominal ganglion is now under investigation.

156.5 SCORPION PECTINES: SENSORS OF SAND TEXTURE ? C. J. Harrington* and T. M. Root. Biology Dept., Middlebury College, Middlebury, VT 05753.

Pectines are a pair of unique, comb-like sensory appendages on the ventral surface of scorpions with unknown function. Each pectine consists of a long basal element upon which numerous "teeth" are articulated. On the basis of limited work, some workers have suggested that pectines may detect the texture of sandy substrates, which is important for spermatophore deposition during courtship, but direct evidence of this mechanoreceptive role has been lacking.

In this study, scorpions were given a choice of substrates comprised of one of four sand grain sizes, ranging from 0.5 to 2.5 mm. in diameter. Chi square analyses of their choice at five minute intervals over a thirty minute period revealed that scorpions consistently preferred the smaller grain sizes. When the pectines were coated with wax however, this preference was lost.

To determine if the pectines were sensitive to differences in sand texture, electrical recordings from them were made in restrained animals. The pectines were sensitive to mechanical deformation of the teeth, upon which are located a series of microscopic sensory pegs. Recordings were subsequently made from freely-walking scorpions with chronically-implanted platinum wire electrodes. When a tethered animal dragged its pectines on a sandy substrate, the pectines responded phasically, with complex spike bursts involving at least 9-13 separate cells. The time course and pattern of impulses from the pectine nerve suggests that they are capable of almost immediate and complex responses to mechanical deformation. This work therefore supports the theory of Carthy (1968) that the pectines respond to mechanical distortion of sensory fields on individual teeth, but how the complex information from each pectine is used to provide a basis for sand selection behavior is unclear.

156.6 DOMESTICATION DID NOT AFFECT WING-FLAPPING IN CHICKENS. R. R. Provine, C. S. Abrams* and B. J. Harrison*. Dept. of Psych., Univ. Md. Baltimore Co., Catonsville, MD 21228.

The development of wing-flapping rate, lateral flight, wing area, and the ratio of wing area to body weight were described in three chickens (Gallus gallus) to assess the effects of domestication. The chickens were the White Leghorn (a commercial egg producer), the Cornish X Rock (a commercial meat producer), and the Red Jungle Fowl (the probable ancestor of domestic chickens). All birds performed drop-evoked, bilaterally symmetrical wing-flapping on the day of hatching, at least one week before lateral flight was possible. Flapping rate of chickens doubled between hatching (4-6 Hz) and 13 days (9-12 Hz), after which it leveled off. The JF and WL developed lateral flight at 7-9 days. The CR first flew 1-2 weeks later but subsequently became flightless. The WL and JF had similar ratios of wing area to body weight; the ratios increased to a peak at 11-15 days and later declined. The ratio of the very heavy, essentially flightless, CR was approximately one-half that of the flighted WL and JF. The wing-flapping frequencies of the domestic WL and CR chickens approximated that of the JF, suggesting that domestication did not affect the motor pattern generator for flight. The artificial selection of the CR for high body weight drastically diminished its flight performance by producing an unfavorable ratio of wing area to body weight. The JF and the domestic WL both flew well and had similiar ratios. Domestication affected flight performance but not the neural circuitry producing the basic, bilaterally synchronized motor pattern of wing-flapping. Previous work indicated that the neural circuitry producing flight was altered during the evolutionary history of flightless ratites and penguins (Provine, R. R., Soc. Neurosci. Abstr., 8: 612, 1982) but not during short intervals of movement restriction during the lifetime of individual chickens (Provine, R. R., Behav. Neur. Biol., 27: 233, 1979; Dev. Psychobiol., 14: 279 and 481, 1981).

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156.7 PRIMATE MODELS IN BIOMEDICAL RESEARCH. F.R. Ervin, R.M. Palmour* and S.N. Young*. Dept. Psychiatry, McGill Univ. Montreal PQ CANADA H3A 1A1.

The world supply of well-described primates, e.g. M. mulatta, has precipitously declined. Simultaneously, the importance of appropriate primate models for biomedical research has increased. In particular, biological differences between man and non-primate experimental animals suggest that the medical applicability of basic or preclinical studies carried out on rodents and carnivores may be limited. One important problem is the individual variability of primates with respect to virtually any biological or behavioral parameter. Might not the innate heterogeneity found in a primate population actually be a useful resource in developing clinically appropriate models for study?

One approach to this goal would be to identify individual monkeys which show characteristics relevant to specific investigations. We have used this concept in initiating studies of an isolated Caribbean population of Cercopithecus aethiops, a non-endangered, readily available and generalized primate. Like other investigators (Angelakos et al., 1979), we have confirmed that 5-10% of this population has consistently elevated blood pressure and have formed breeding groups of high blood pressure animals and, separately, of very low blood pressure animals. Within a stable enclosure, two matrilines showing strong familial predisposition to elevated blood pressure have been identified. Studies of possible biochemical and/or genetic markers in these groups have only recently been initiated.

We have further been interested in models for multifactorial human diseases. An initial step is to characterize the population genetically with respect to loci which show electrophoretically detectable variation in man. 12 of 60 blood proteins thus far scored show polymorphism; rare variants are present in perhaps half that number of loci. Investigation of 50 additional loci is underway, including ones on each chromosome, based on the human gene map. Of particular interest are variants at loci which may have pharmacological or toxicological consequences--serum pseudocholinesterase, α-1-antitrypsin, etc.

Studies of CSF levels of catecholamines, indoleamines and their metabolites reveal that the African green monkey shares some important neurochemical similarities with man, while levels of the compounds in rat CSF are quite different. Mean rates of 5-HT, DA and NE metabolism in the brain are higher in female vervets than in males. Significant relationships between amino metabolites and amino acid precursors were found.

The Caribbean vervet population promises to be a useful model for the continued study of basic and clinical questions.

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157.8 NEUROANATOMICAL SUBSTRATES OF JUVENILE PLAY IN RATS. S.Siviy*, J. Panksepp and K. White*, Dept. of Psych., Bowling Green State University, Bowling Green, OH 43403.

Little is known about the brain mechanisms which control juvenile play. In these experiments, we sought to identify brain areas which mediate juvenile play-fighting behavior in weanling rats. Male and female Long-Evans rats were lesioned electrolytically (age 20-23 days) and play behavior, as indexed by frequency of pinning, was followed through puberty. A social deprivation paradigm was used with pups housed individually and observed for 5 minute periods daily (as in Panksepp & Beatty, Behav. Neural Biol., 1980, 30, 197). Brain areas studied were amygdala, septum, ventromedial hypothalamus (VMH), dorsomedial thalamus (DMT) and parafascicular area of thalamus (PFA). As in previous work (Beatty, et al., Physiol. Behav., 1982, 28, 649), septal lesions markedly increased play, while amygdala lesions decreased play. VMH lesions decreased play, probably because of many aggressive encounters with serious biting during which protagonists became timid and defensive. DMT lesions produced a moderate 15% decrease in pinning, but pin durations were increased markedly by 120%, suggesting possible disruption of play sequence termination factors. Lesions of the PFA decreased play markedly by 65%, and behaviorally the animals seemed less able to initiate pinning sequences. Morphine (1 mg/kg) increased pins in control and DMT lesioned animals, but not in PFA animals, suggesting areas important for opioid modulation of play (Panksepp, Neurosci. Abstr., 1979, 5, 172.)

The results suggest that septal circuits normally inhibit play, while amygdala, and a dorsal diencephalic circuit running through DMT and PFA elaborate excitatory influences, with the DMT involved in the termination of play bouts and the PFA participating more in play initiation. The VMH may normally inhibit aggressive overtones from arising during play.

156.9 THE IMPORTANCE OF OVIDUCTS FOR ONSET OF REPRODUCTIVE BEHAVIOR IN FEMALE FROGS. C. Diakow, C. Scharff*, and L. Aronow*. Biology Dept., Adelphi University, Garden City, NY 11530.

Onset of receptivity in female frogs is associated with ovulation. As ovulation proceeds, eggs are released into the body cavity, pass through the oviducts, and are stored in the uterus. By the time ovulation is complete, there are several thousand eggs in the uterus. We hypothesized that the onset of receptivity depends on some aspect of egg passage. We injected adult, female R. pipiens with one pituitary gland each to induce ovulation, and tested for receptivity. In testing, we recorded the number of vocalizations (release calls) in 30 sec. of manual clasping. Frogs were tested twice before pituitary injection, and every 4 hrs from 16 to 72 hrs after injection. A decline in the number of calls indicates receptivity. Data are presented below as the median and range of the median number of calls per female.

In Exp I, oviducts were removed from Group 1 (n=6); Group 2 was sham operated (n=9). The oviductectomized females did not become receptive.

	SHAM	OVIDUCTECTOMY
before pituitary inj.	37 (10-44)	36 (14-47)
52-72 hrs after inj.	0 (0-0)	28 (16-33)

In Exp II, Group 1 was ovariectomized (OVX: n=7) and could not ovulate. Group 2 was intact but the oviducts were ligated (OVID; n=3) so eggs could not pass through the oviduct. The uterus was cut open in Group 3 (UT; n=6) eggs could pass through the oviduct but could not accumulate in the uterus. Group 4 had sham operated controls (SHAM; n=7). Receptivity occurred in both groups in which eggs passed through the oviduct, but not in either group in which eggs could not do so.

	OVX	OVID	UT	SHAM
before pituitary inj.	30 (23-47)	37 (24-41	29 (22-40)	34 (17-41)
52-72 hrs after inj.	34 (18-42)	33 (22-36)	1 (0-1)	3 (0-30)

We conclude that onset of receptivity is influenced by passage of eggs through the oviduct.

156.10 MODEL OF A NEURAL NET SUBSERVING WORM-ANTIWORM DISCRIMINATION IN FROGS AND TOADS.[1] F. Cervantes-Perez*[2] and M. A. Arbib (SPON: A. Barto). COINS Dept. and the Center for Systems Neuroscience, University of Massachusetts, Amherst, MA 01003.

It is well known that Anuran Amphibians use mainly visual input to guide their interactions with the external world. In the process of adapting adequately to that world these animals present very stereotyped cognitive and motor behaviors.

Neuroethological studies (Ewert, 1980 and Ingle, 1982) have shown that there are innate mechanisms in frogs and toads that recognize key-stimuli in the environment to elicit the proper motor response. It has been shown that both the size of a moving stimulus and its geometry in relation of the direction of motion play an important role in the prey-catching behavior of these animals: small objects whose longitudinal axis moves parallel to the direction of motion ("worm-like") are considered as prey; whereas if an "antiworm-like" stimulus (larger objects moving with their longest axis orthogonal to the direction of motion) is presented the animal does not exhibit prey-catching orienting behavior or may assume a freezing posture or may exhibit avoidance behavior. This worm-antiworm discrimination has been proved to be invariant to the direction of motion and there is still debate about this process being also invariant to the velocity function of the stimulus. Ewert (1970) and Ingle (1975) have also shown that lesions of the dorsal pc/pl region within the thalamus (Pretectum) disrupts the animal's ability to discriminate different configurations of the stimulus. Furthermore, animals with pretectal lesions snap indiscriminately to any moving object, even to those resembling the probability of fitting the predator category. We present a neural net model based on anatomical, physiological and neuroethological grounds (Szekely and Lazar, 1976; Ewert, 1980; Grusser and Grusser-Cornhels, 1976), that raises specific hypotheses of how the interactions among several brain regions (Retina, Optic Tectum and Pretectum), formed by multiple groups of neurons structured in layers, use a parallel distributed processing of the visual input to control the animal's motor response. The model reproduces adequately the experimental observations, and allows us to analyze qualitatively the neural mechanisms that might be subserving worm-antiworm discrimination, with direction invariance of this phenomenon being a consequence of the tectal architecture, size selection and changes in response latency. The two latter processes depend on the animal's motivational state.

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[2]On leave from Centro de Investigaciones en Fisiologia Celular, Universidad Nacional Autonoma de Mexico.

156.11 AFFERENTS OF SOME DORSAL RETINORECIPIENT AREAS OF THE BRAIN OF BUFO BUFO. A. Weerasuriya* and J.-P. Ewert (Spon. C.H. Hockman). Neuroethology lab., GhK, Kassel, Federal Republic of Germany.

A predominant part of the behavioural repertoire of anurans is guided visually. In toads, as in other anurans, retinofugal fibres terminate in targets distributed bilaterally in the di- and mesencephalon. The sensory analysis underlying such visually guided behaviours as prey capture and avoidance of enemies and obstacles, requires interaction between retinorecipient dorsal thalamic areas and the tectum. The following results are based on consistent retrograde cell labelling seen after successful placement of iontophoretic deposits of HRP in the caudal, lateral and rostral tectum, posterior nucleus, caudal thalamic neuropil, lateral anterior nucleus, and a rostral area encompassing the nucleus and neuropil of Bellonci. The rostral and lateral tectum receive input from ipsilateral medullary reticular formation. All 3 tectal regions receive bilateral input from nuclei isthmi. The nucleus profundus mesencephali, pretoral grey, pretectal grey and tegmental nuclei provide ipsilateral afferents to all 3 tectal regions with the lateral tectum receiving a contralateral input from the latter two areas. The lateral tectum also receives input from the posterior tubercle, ventral (bilateral) and lateral hypothalamus. Lateral, posterior (bilateral), and central nuclei project to all 3 tectal regions. The ventromedial nucleus projects to the caudal tectum, whereas the suprachiasmatic, preoptic and venrolateral nuclei project to the lateral tectum. Anterior entopeduncular nucleus and nucleus of Bellonci provide input to the rostral tectum. An area at the ventral edge of the lateral ventricle projects bilaterally to the lateral tectum. Posterior nucleus receives afferents from medullary reticular formation, tegmental anterodorsal nucleus, lamellar and principal nuclei of torus, tectum, pretoral and pretectal grey, ventral hypóthalamus, contralateral posterior nucleus, anterior, central, ventrolateral and -medial nuclei and nucleus of Bellonci. Injection sites confined to the caudal thalamic neuropil produced cell labelling in tectum (bilateral), posterior, central, lateral, ventromedial and -lateral, anterior, and posterior entopeduncular nuclei and nucleus of Bellonci. If injection sites extended to the lateral anterior nucleus too, additional labelling was seen in the tegmental anterodorsal nucleus, magnocellular nucleus of torus and ventral habenular nucleus. In both cases, anterograde fibre labelling could be followed up to the striatum via the lateral forebrain bundle. Injection sites encompassing the nucleus and neuropil of Bellonci labelled cells in tectum, ventral hypothalamus, and posterior (bilateral), central, ventromedial and -lateral, anterior (bilateral), and posterior entopeduncular nuclei and the contralateral nucleus of Bellonci.

157.1 RESPONSES TO ACOUSTIC STIMULI IN THE AVIAN TRACHEOSYRINGEAL NERVE. H. Williams* and F. Nottebohm. (SPON: M. Constantine-Paton). Rockefeller University, New York, N.Y. 10021.

Microstimulation of the principal avian forebrain song control nucleus, hyperstriatum ventrale pars caudalis (HVc), is known to produce evoked potentials in the tracheosyringealis branch of n. XII (nXIIts) which innervates the avian vocal organ (A.P. Arnold, Am. Sci., 68:165, 1980). HVc is also known to respond to acoustic stimulation (L.C. Katz & M.F. Gurney, Br. Res., 221:192, 1981). We have found that under some conditions acoustic stimuli will elicit activity in nXIIts as well. The nXIIts response has a long latency (30-100 ms) and follows the HVc response with a latency of ~15 ms; this is equal to the latency seen when recording from nXIIts after microstimulation of HVc. Thus, it seems possible that the pathway for this peripheral acoustic response involves HVc, a forebrain nucleus.

The response evoked in nXIIts by microstimulation of HVc is gated by respiratory activity, with more axons firing during expiration than during inspiration (K.R. Manogue & J.A. Paton, Br. Res., 247:383, 1982). The auditory-evoked nXIIts response is, however, not respiratorily gated; this may imply that some functional difference between these two responses exists.

Characterization of the stimuli most effective in evoking the nXIIts response is underway. Clicks, pure tones, and song playback have been shown to evoke the response in zebra finches and canaries.

A similar response, the laryngeal muscle acoustic reflex, has been described in the bat (P.H.-S. Jen & N. Suga, Science, 191: 952, 1976). But the response latency in the bat is 6 ms, which precludes mediation by a long pathway. Acoustic reflexes also occur in the decerebrate cat (C.G. Wright & C.D. Barnes, Br. Res. 36:307, 1972). However, the avian nXIIts acoustic response differs from these reflexes in its much longer latency (and hence its greater potential for involvement of central processing) and in the implication that it is mediated by telencephalic nuclei. Its function, as yet undetermined, may involve such higher order behaviors as song learning and production.

157.2 VOCAL LEARNING IN ZEBRA FINCHES: THE ROLE OF HYPOGLOSSAL AFFERENT FIBERS. A.P. Arnold and S.W. Bottjer. Dept. of Psychol., UCLA, CA 90024

Sensory afferent fibers originate in the vocal organ (syrinx) of zebra finches (Poephila guttata) and travel along the hypoglossal nerve (Bottjer & Arnold, 1982). A question of great theoretical interest centers on whether feedback along these fibers contributes to vocal learning in songbirds, as does auditory feedback. Neither auditory feedback nor feedback along hypoglossal afferent fibers is important for maintenance of adult song patterns (Konishi, 1965; Bottjer & Arnold, in prep). The present study was designed to investigate the role of hypoglossal afferent fibers in song learning by juvenile zebra finches.

Subjects were 27 male zebra finches ranging in age from 22 to 36 days. Experimental birds (n=14) were anesthetized and the anastomosis between the hypoglossal and vagal nerves was exposed. The descending branch of the vagus was teased back slightly on the left side and the XII afferents were sectioned. The same operation was performed on the right side, except that the descending vagus was not teased back (thus, descending X was sectioned along with hypoglossal afferents). Efferent hypoglossal fibers travel separately and were not disturbed. The remaining birds underwent one of two different control treatments: All control birds were subjected to the same procedure as experimental subjects, but no nerves were actually cut in some birds (n=6), whereas the right descending vagus was sectioned in others (n=7). The song patterns of all birds were recorded periodically until they were 90 days old. A sound spectrograph was used to analyze recordings made for each bird at approximately 75 days of age. A blind observer listened to the songs, examined the spectrographs, and assigned a score to each bird ranging from 1 (abnormal) to 5 (normal). Songs were scored based on stereotypy and complexity of the song pattern and degree of similarity of individual notes to those of normal adult males. There were no differences in the song performance of control subjects which were therefore collapsed into one group. The median scores were 4.00 ($\pm$1.44) and 2.25 ($\pm$1.76) for the control and experimental groups, respectively. This difference did not reach significance (U = 63.5, $p > .10$).

We believe these results to be highly suggestive of a significant role for feedback along hypoglossal afferent fibers in the development of learned song behavior. Although the effects obtained did not achieve significance, they may have been mitigated by regrowth of the sectioned XII afferent fibers. Our current objective is to trace the central termination site of these afferent axons and lesion this site at various stages of vocal development.

157.3 QUALITY OF SONG AND DEGREE OF MASCULINIZATION OF THE BRAIN IN FEMALE ZEBRA FINCHES. G. Pohl-Apel* (SPON: M. Gurney). Univ. of Chicago, Dept. Pharm. Phys., Chicago, Illinois 60637.

In the Zebra finch the sexes show a marked dimorphism. In females, certain brain nuclei are reduced in size and correlated with this, females do not sing. By treating female nestlings with estradiol the song system becomes male-like and if the adult bird is subsequently treated with testosterone, she may sing.
The aim of this study was to investigate the correlation between the degree of female brain masculinization, produced by varying the duration of estradiol-treatment, with the quality of song.

Sixteen female nestlings were implanted with silastic capsules filled with estradiol. Treatment started between day 2 and day 10 after hatching and ended between day 8 and day 18. The birds were raised by their Zebra finch parents. When adult (150 days) the birds were implanted with testosterone-propionate to induce song. Song was recorded 6 weeks later. Four weeks after the removal of the testosterone implant the birds were killed and their brains examined.

Song was observed in 11 out of 16 birds. Within 3 to 6 weeks birds gained their individual song quality which remained unchanged even after several more weeks of testosterone treatment. The song quality can be classified into 3 categories: I The song is very similar to adult male song (i.e. temporal patterning, number of elements, repetition of elements in the same manner). (n=3). II The song is similar to early juveniles song, i.e. this song is poor in structure and not crystallized (no element is repeated for a second time in exactly the same manner). (n=4). III In between these categories are females with an intermediate song type which is similar to late juveniles song in males. (n=3).
Anatomical data show that song quality correlates with degree of masculinization of the song system. Females singing the typical male song (I) have a mean volume of Nucleus robustus archistriatalis (RA) of 0.07 mm^3 (0.06 - 0.08; mean RA volume in males: 0.24; 0.22 - 0.27, n=5); females with early juveniles song (II) have a mean RA volume of 0.03 mm^3 (0.02 - 0.04). This volume is still larger than that of nonsinging females (mean: 0.010, 0.007 - 0.010, n=4). The volume of the females with an intermediate song type (III) is between that of category I and II (mean: 0.04, 0.03 - 0.05).

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157.4 DEVELOPMENTAL CHANGES IN EFFECTS OF LESIONS IN FOREBRAIN SONG-CONTROL NUCLEI OF PASSERINE BIRDS. S.W. Bottjer, E. Miesner* and A.P. Arnold. Dept. of Psychol., U.C.L.A., CA 90024.

Male zebra finches (Poephila guttata) learn the song typical of their species during a "critical" period of development (Immelmann, 1969). Song patterns become progressively more stereotyped during the period from 50 to 90 days, and do not change thereafter. Two telencephalic nuclei, HVc and RA, are known to form part of the major efferent pathway of the neural system controlling song; bilateral lesion of either HVc or RA severely disrupts vocal behavior in adult songbirds (Nottebohm, et al., 1976). Two additional nuclei in the anterior forebrain, MAN and Area X, are monosynaptically connected with HVc and/or RA, and are therefore defined as part of the song system, although little is known concerning their function. The purpose of the present study was to determine if MAN and/or Area X are involved in vocal development.

30 zebra finches ranging in age from 35 to >90 days received bilateral lesions aimed at either MAN or Area X. Birds 50 days of age and older were recorded while singing before undergoing surgery. Electrolytic lesions were produced using monopolar stainless steel insulated electrodes to pass anodal DC current of 90 to 100 ua for 60 sec. The song patterns of all birds were recorded post-operatively at two-week intervals until the birds were at least 90 days old, at which time they were sacrificed. Their brains were fixed, embedded, sectioned at 40 um, and stained with thionin. Sections were examined using a microprojector in order to verify the exact location of the lesion.

Birds which received complete bilateral lesions in either MAN or Area X when they were 35 to 50 days old produced severely abnormal vocalizations from 50 to 90 days of age. Their "songs" usually consisted of 1 or 2 highly abnormal syllables, often produced at very low volume. In contrast, birds which received "control" (i.e., off-target) lesions showed normal song development. Birds which received complete bilateral lesions in MAN or Area X after 60 days of age were affected little, if at all. Because MAN and X are quite close to each other, it is conceivable that lesions in MAN interfere with axons projecting into Area X from HVc, but preliminary neuroanatomical tracing studies argue against this possibility.

Lesions in MAN and X may exert their effects by interfering with normal neural development in HVc and RA, although preliminary examination of these brains do not support this idea. Alternatively, these results may indicate that functions important for vocal learning are being carried out in MAN and X during a restricted period of development. Thereafter, vocal development either becomes independent of these functions, or they are somehow "transferred" to another area of the brain.

157.5 IMPORTANCE OF THE VOCAL CONTROL NUCLEUS HYPERSTRIATUM VENTRALE, PARS CAUDALE IN REPRODUCTIVE BEHAVIOR OF THE FEMALE CANARY. J. M. Greenspon* and D. A. Ingram* (SPON: D. Durham). Dept. of Psychol., Hobart and William Smith Colleges, Geneva, N.Y. 14456.

Female canaries learn conspecific male songs, for the most part, during their first 30 d. post-hatching (Greenspon, J.M., East. Psychol. Assoc. Abstr., 54th Ann. Mtg., p. 75, 1983). In addition, adult female canaries are attentive to attributes of conspecific male song as evidenced by their exhibiting significantly more robust forms of reproductive behavior (e.g., nest-building and egg laying) when exposed to complex as opposed to simple songs (Kroodsma, D.E., Science, 192: 574, 1976).

The hyperstriatum ventrale, pars caudale (HVc) is thought to be an important brain area for auditory memory formation in canaries (Nottebohm, F., Stokes, T.M., and Leonard, C.M., JCN, 165: 457, 1976). Therefore either left (n=6), right (n=6) or bilateral (n=5) ablations of HVc were performed on female canaries in order to selectively create auditory memory deficits. Brain-damaged females were then exposed to complex songs while having their reproductive behaviors monitored. Left, right and bilaterally damaged females all significantly ($p<.05$) outperformed sham controls (n=5) on measures of total number of strings gathered and placed in nest, and in latency to lay eggs; all of which are sensitive measures of female canary reproductive behavior. In addition, nest-material placing behavior over the first 12 d. was most robust in the bilaterally-damaged group, with both left and right-damaged groups exhibiting slightly less and the shams even less.

Loss of auditory memory would result in an increased probability of hearing novel sounds. That bilateral, as opposed to unilateral or sham damage to HVc resulted in increased reproductive activity in females exposed to complex male songs indicates that novel aspects of song, in contrast to learned aspects, result in increased reproductive arousal. This might be due to females being habituated to learned sounds and maximally sensitive and thereby aroused by novel sounds.

157.6 NEURAL CORRELATES OF AVIAN SONG DUETTING. F.A. Brenowitz* and A.P. Arnold (SPON: D.B. Lindsley). Dept. of Psychol. and Brain Res. Inst., UCLA, Los Angeles, CA 90024.

Studies of the neural network that controls song in canaries (Serinus canarius) and zebra finches (Poephila guttata) have demonstrated striking sexual dimorphism. The volumes of the caudal nucleus of the hyperstriatum ventrale (HVc) and the robust nucleus of the archistriatum (RA) are significantly larger in males, who sing, than in females, who do not (Nottebohm and Arnold 1976). Cell body size and dendritic field spread of cells in RA are also larger in males (DeVoogd and Nottebohm 1981; Gurney 1981). We report here the results of a preliminary investigation of the neural correlates underlying song in the white-browed robin-chat (Cossypha heuglini), a species in which both sexes participate in complex vocal duets.

In January 1983 two female and three male chats were caught in Kenya, East Africa. Song was heard from all of these birds before capture. They were anesthetized and perfused with 10% formalin. Their brains were frozen-sectioned and stained with thionin. The volumes of HVc and RA were determined by standard procedures. The sizes of neuronal somas distributed throughout RA were measured in two males and two females by tracing somal perimeters through a camera lucida. Using estimates of neuronal density and volume of RA, total neuronal number in RA was calculated.

Both RA and HVc are considerably larger in males (RA: mean $\pm$ SD=0.828 $\pm$ 0.149 mm^3; HVc: 3.692 $\pm$ 1.190 mm^3) than in females (RA: 0.325 $\pm$ 0.091 mm^3; HVc: 1.262 $\pm$ 0.515 mm^3). The density of neurons within RA is greater in females (4.6-5.7 x 10^4 neurons/mm^3) than in males (2.6-4.0 x 10^4 neurons/mm^3). Given the greater volume of RA in males, we estimate that the RA of male chats contains more neurons (2.2-3.3 x 10^4) than that of females (1.6-2.0 x 10^4)(not corrected for cell splitting). There does not, however, appear to be any sexual difference within RA of maximum somal diameter (male mean $\pm$ SD = 16.97 $\pm$ 4.15 um, n=375; female 16.79 $\pm$ 3.44 um, n=491) or somal area (male 140.74 $\pm$ 48.84 um^2; female 138.66 $\pm$ 39.23 um^2). One male and female had nearly identical mean cell body diameters and areas, as did the other male-female pair.

It thus appears that the sex difference in the volumes of RA and HVc in chats is as large as that found in canaries, a species in which only males sing. However, cell body size within RA of the duetting chats is sexually monomorphic. These results suggest that cell size is more directly related to vocal performance than overall volume of brain regions or total number of neurons.

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157.7 BRAIN STIMULATION EVOKED VOCALIZATION IN THE DOMESTIC CHICK: A 2-DG ANALYSIS. P.W. Chatfield* and N.C. de Lanerolle, Psychology, Wesleyan Univ. & Neurosurgery, Yale Univ. Sch. of Med. CT.

Domestic chicks vocalize in several emotional contexts. Short calls are produced in 'pleasure' situations whereas peeps are given in 'stressful' situations. Electrical stimulation (ES) in diencephalic areas evoke peeps, whereas short peeps or trills are evoked from the midbrain nu. intercollicularis (ICO)/ area C region. ES at call sites in each of these regions was combined with the 2-deoxyglucose (2-DG) method to determine the entire spectrum of neural substrates involved in the production of these vocalizations. Midbrain ES resulted in increased metabolic activity in the ipsilateral caudal medulla where treacheosyringeal motoneurons and motoneurons innervating upper neck muscles are located; in midbrain area C which influences respiratory rate; in ICO; nu. tegmenti pedunculo-pontinus pars compacta (TPc) (dopamine neurons) and optic tectum. All these regions are associated with vocal pathways traced from the ICO/area C call sites by silver degeneration methods. In addition several ipsilateral lateral diencephalic areas, the ectostriatum, the lateral part of paleostriatum augmentatum (PA), paleostriatum primitivum (PP), nu. intrapeduncularis (INP) and archistriatum were active. ES at diencephalic peep sites resulted in conspicuous ipsilateral activity in all parts of the hypothalamus (with highest levels in the lateral hypothalamus) and a periventricular strip dorsal to it. ES in all of these active diencephalic areas evokes peeps (de Lanerolle, 1972). Hypothalamic areas were not active in midbrain stimulated birds, and tectal areas were not active with peep site stimulation. The telencephalon of peep site stimulated birds has strong uptake bilaterally in the ectostriatum, and on the ipsilateral side similar high uptake extended into the surrounding neostriatum dorsally and ventrally in the lateral part of the PA, PP and INP. The nu. taenia of the archistriatum was also more active on the ipsilateral side. This study demonstrates the brain areas in which visual information, important in the causation of chick vocalizations, can interface with motor control pathways for vocalization. A key site in this regard is the ectostriatum, which receives visual information via the tecto-rotundal pathway. This pathway has been reported, by others, to provide the basis for attention-directing and scan initiating functions, which functions were shown previously to be crucial in the causation of chick vocalizations (Brain Behav. Evol., 10: 377f & 10: 354f). Ectostriatal connections with the neostriatum intermediale may also provide access to memory functions important for emotional responsiveness. Further, a dopaminergic projection from the TPc to the PA may underlie the dopaminergic mechanisms involved in the causation of vocalization (J. Comp. Phys. Psychol., 92:416 & 94:346).

158.1 STABILIZATION OF HEBBIAN NEURAL NETS BY INHIBITORY LEARNING. Paul Easton* and Peter E. Gordon*. (SPON: Isabelle Alter). Brain Research Labs, NYU Medical Center, NY,NY, 10016 and Physics Department, U. of Mass. at Boston, Boston, Mass. 02125

In Hebbian neural models synaptic reinforcement occurs when the pre- and post- synaptic neurons are simultaneously active. This causes an instability toward unlimited growth of excitatory synapses. The system can be stabilized by recurrent inhibition via modifiable inhibitory sysapses. When this process is included, it is possible to dispense with the non-linear normalization or cutoff conditions which were necessary for stability in previous models. The present formulation is response-linear if synaptic changes are slow. It is self-consistent because the stabilizing effects will tend to keep most neural activity in the middle range, where neural response is approximately linear.

The linearized equations are tensor invariant under a class of rotations of the state space. Using this, the response to stimulation may be derived as a set of independent modes of activity distributed over the net, which may be identified with cell assemblies. A continuously infinite set of equivalent solutions exists.

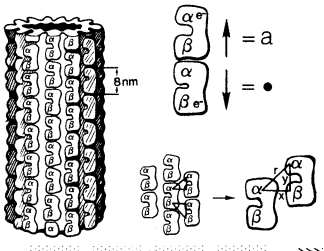
158.2 A LEARNING RULE GOVERNING WEAKENING OF NEURAL INTERCONNECTIONS COMPLEMENTS HEBB'S RULE AND ENABLES REPRESENTATION OF CONCEPT HIERARCHIES WITHIN STRUCTURALLY HOMOGENEOUS NEURAL NETWORKS. Rolf Martin. Biochemistry Lab., Chemistry Dept., Brooklyn Coll., Brooklyn, NY 11210.

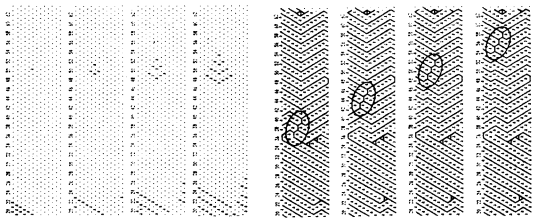
A photograph of a male and female lion and three cubs is frequently identified as "lions"; a photograph of a lion and tiger is very often labelled "lion and tiger"; a photo containing a lion, tiger, leopard and lynx is generally identified as "cats"; a photo of a lion, tiger, leopard, zebra, antelope and buffalo is frequently labelled "cats and hoofed mammals"; and a photograph of a wolf, horse, leopard, beaver, rabbit and porpoise is very often labelled "mammals". Note the shift to more inclusive labels as the diversity and number of concepts within each photograph increases. This shift can be simulated in simple, theoretical neural networks governed by Hebb's learning rule[1] if, and apparently only if, a second learning postulate is added which specifies that a connection between two neurons is weakened when the presynaptic neuron discharges but the postsynaptic neuron does not. Other capabilities required for appropriate use of concept hierarchies can also be simulated, including: listing the names of concepts within each category, listing the defining attributes of each category, separately listing the defining attributes of each concept within each category, correct categorization of novel data (which represent concepts not previously "seen" by the neural net), and automatic construction of alternative hierarchies when appropriate. The neural nets used in these simulations are basically the same as those used previously[2-3] for simulation of associative and sequential recall. Layering of neurons to provide a structural basis for representing levels within hierarchies is not required (cf. 4). The proposed rule governing the weakening of neural connections also appears to be necessary for relatively accurate representation of probability relations and for risk benefit assessment.

1) D.O. Hebb postulated that connections between neurons are strengthened when they discharge concurrently, so that they become more likely to trigger one another in the future (The Organization of Behavior, Wiley, N.Y., 1949).
2) K. Nakano (1972) I.E.E.E. Trans. SMC 2, 380.
3) K. Fukushima (1973) Kybernetik 12, 58.
4) J. Grinberg-Zylberbaum (1976) J. Theoret. Biol. 56, 95.

158.3 INTRANEURONAL PATTERN PROCESSING: CELLULAR AUTOMATA IN CYTOSKELETAL LATTICES. S.R. Hameroff, S.A. Smith, R.C. Watt. Dept. Anesthesiology, Univ Arizona Health Sci Ctr, Tucson, AZ 85724 and Computing Division, Los Alamos National Laboratories, Los Alamos, NM 85745

Rudimentary aspects of cognition may depend on intra-neuronal information processing which subserves inter-neuronal synaptic activities. Nonlinear electrodynamic effects such as Frohlich coherent dipole oscillations (Nature 228:1093, 1970; PNAS 72: 4211, 1975) in subunits of cytoskeletal lattices such as microtubules (MT) have been implicated in such information processing (J Theor Biol 98:549, 1982). Cellular automata (CA) (Sci Am 244:112, 1971) are dynamical systems which can generate and process patterns via lattice neighbor interactions in discrete time intervals. Based on MT structural geometry and Frohlich coherent oscillations of 10^{-10} to 10^{-11} sec, we calculated the electrostatic forces among MT subunit (α, β dimer) neighbor dipoles (Fig). The net forces are equal to $\sum_{i=1}^{n} \frac{y}{r^3}$ (n=7 neighbors, y="vertical" neighbor distance, r=direct distance) and, when solved for MT lattice dimensions, lead to "rules" determining dimer state at subsequent time intervals. Lower figures represent CA patterns of an initial single α state which grows (left), and (right, dots connected) a later pattern moving (8 nm/10^{-1} sec) and interacting through the stable lattice. These dynamic patterns could manifest protein binding sites or represent information computing and interacting in cognitive processes.





158.4 EXTRINSIC AND INTRINSIC INTERACTIONS AMONG SIMULTANEOUSLY RECORDED NEURONS IN THE NUCLEUS PARABRACHIALIS MEDIALIS DURING SLEEP-WAKING STATES. R. D. Frostig*, Z. Frostig*, G. C. Sieck and R. M. Harper (SPON: M. Nuwer). Dept. of Anatomy and the Brain Research Institute, UCLA, Los Angeles, CA 90024.

Respiratory-related neurons in the nucleus parabrachialis medialis, recorded from intact, drug-free, unrestrained cats, exhibit variations in their short-term discharge correlations (<2 msec) between different sleep-waking states (Harper and Sieck, Brain Res., 199:343-358, 1980). Variations in these correlations may arise from local intra-network functional changes or from external functional sources. In order to distinguish between these possibilities, a new analytical procedure for evaluating spike train dependencies was used. This procedure is based on filtering a subset of the presynaptic spike train which includes only spikes that are responsible for the time-locked primary effects in a cross-correlogram. Different subsets can be filtered for different parts of the primary effects (i.e., a subset for interaction effect, a subset for shared input effect, etc.) and may be compared over different sleep-waking states. All neurons were found to be strongly influenced by multiple sources of shared input that were most likely extrinsic. Both these extrinsic sources and local direct connections were excitatory. When calculated across sleep-waking states, some of the local connections showed a polarity reversal between pre- and post-synaptic neurons. However, no change in parameters such as rate or coefficient of variance was demonstrated. The shared input sources showed statistically significant ($p<0.05$) state-related changes in these parameters. Thus, we suggest that the state-related variation in short-term correlation discharge is due to variations in the shared input to the neuronal network, rather than to intra-network interactions.

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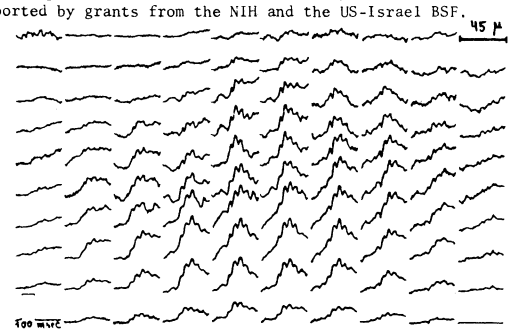
158.5 OPTICAL MONITORING OF NATURALLY EVOKED DYNAMIC PATTERNS OF NEURONAL ACTIVITY FROM THE INTACT FROG OPTIC TECTUM USING A PHOTODIODE ARRAY. A Grinvald, L. Anglister*, R. Hildesheim* and J.A.Freeman
Dept. of Neurobiology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Optical recording methods were developed in order to investigate information processing, functional organization and pharmacology of the intact vertebrate brain (see also Orbach et al., Neurosci.Abstr. 8, 936, 1982; *ibid* 9, 1983). The exposed frog's optic tectum was stained for 20 min by topical application of a Ringer solution containing 1.6mM RH-414 (a new voltage sensitive fluorescent probe). In order to record and visualize the spatio-temporal patterns of evoked activity we used a 100-elements photodiode array and a computerized optical monitor (J.Physiol. 333, 269, 1982). Complex dynamic patterns were detected in the tectum in response to visual patterns (stationary or moving, spots, bars and annuli) presented to the eye. Each optical signal contains 5-12 peaks which are correlated with the peak of the electrically recorded evoked potentials. The spatial resolution depends on the objective and varies from 10 to 400 μm. Laminar analysis can be achieved by differential optical recording of the activity at different depths. In many instances signals are sufficiently large so that signal averaging is not required, and many measurements can be made on a single preparation.

Simultaneous optical recording from hundred loci appears to offer some unique advantages in monitoring dynamic patterns of neuronal activity in the intact brain because it provides both electrophysiological and "anatomical" information in a single experiment. The figure shows the pattern response to a light bar stimulus presented to the contralateral eye.

Supported by grants from the NIH and the US-Israel BSF.



158.6 **IDENTIFICATION OF ACTIVE SINGLE NEURONS IN LOCUST NEURAL CIRCUITS USING ^{3}H-2-DEOXYGLUCOSE AUTORADIOGRAPHY.** **Aguan Wei* (SPON: G. Hoyle). Institute of Neuroscience, University of Oregon, Eugene, OR 97403.**

2-Deoxyglucose (2-DG) autoradiography (ARG) has been developed as a method to screen and mark neural metabolic activity which reflects electrical activity. A modified freeze-substitution proceedure (Sejnowski, T.J., et al., Nature, 287:449, 1980) minimizes label diffusion, allowing for single cell resolution. Applied to invertebrate nervous systems with large and typically located identified neurons, 2-DG ARG offers the possibility for the identification of entire behavioral circuits, including behaviorally identified interneurons. The present investigations were performed to test the applicability of this technique to the locust nervous system.

Metathoracic Fast Extensor Tibiae (FETi) motoneuron somata were used in feasibility tests in a semi-isolated preparation in which all nerves and connectives to the meso- and metathoracic ganglia were cut, except metathoracic nerve 5. In the presence of ^{3}H-2-DG (100μC/ml), FETi on one side was stimulated antidromically at 10 Hz, using bipolar electrodes implanted in the muscle, its contralateral homologue serving as an unstimulated control. After stimulation, for varying durations, the ganglia were dissected and washed in three changes of isotonic saline for 30 minutes to remove non-specific uptake. The tissue was then either processed for autoradiography, or individual FETi somata dissected and assayed directly for uptake by scintillation counting. Stimulated FETi somata showed significantly greater uptake of 2-DG over unstimulated controls. This stimulation-dependent uptake appeared to increase linearly as a function of the duration for at least the first 4 hours of stimulation.

The isolated metathoracic ganglion has been demonstrated to produce ventilatory rhythmicity endogenously (Lewis, G.W., et al., J. exp. Biol., 59:149, 1973). Autoradiographs of isolated, unstimulated ganglia reveal a set of 17 intensely-labelled, and 13 lightly-labelled cells in the fused abdominal region of the metathoracic ganglion. The positions and numbers of the heavily-labelled cells appear to be invariant between animals. Intracellular recording and dye injection confirm the presence of bursting neurons with activity in phase with ventilation, with soma sizes and positions matched with those seen labelled. These neurons are likely to be elements of the central pattern generator for ventilation.

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158.7 ULTRASTRUCTURE OF SYNAPSES BETWEEN IDENTIFIED NEURONS IN THE LEECH. L.P. Tolbert and R.L. Calabrese. Dept. Anatomy, Georgetown Univ. School of Medicine, Washington, DC 20007 and Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

The rhythmic constriction of the heart tubes in the leech Hirudo medicinalis is controlled by an identified set of motor neurons (HE cells) and interneurons (HN cells) (reviewed by Stent, Thompson, and Calabrese, Physiological Reviews 59:101). Electrophysiological recordings have established that the HE and HN cells interact with each other in a precise pattern. HE cells, found in ganglia 3-18, innervate the heart-tube muscle and are electrically coupled to their contralateral homologues (Peterson, Biophysical Journal, in press). They are tonically active; rhythmic inhibitory synaptic input from certain HN cells coordinates their activity to produce functional firing patterns. HN cells are found in ganglia 1-7. While some HN cells synapse directly onto HE cells, others synapse only onto other HN cells. The synaptic connections among HN cells are inhibitory and differ from ganglion to ganglion.

In the present study, the synaptic framework mediating the interactions among the HE cells and HN cells is being examined in an effort both to further our understanding of the circuitry responsible for the heartbeat and to elucidate basic cellular mechanisms of integration. The electrophysiological results described above indicate particular relationships between identified neurons. Using electron microscopy of physiologically identified, HRP-injected cells, we are examining the zones of interactions and types of contacts between specific cells.

Each HE cells sends numerous fine processes to the ganglionic midline, where they interdigitate with the fine processes of their contralateral homologue. In the electron microscope, the HE cells are seen to contact each other frequently. Areas of direct juxtaposition of HE-cell processes are extensive, measuring as much as 6 μm in length. In much of the area of juxtaposition, the apposed membranes are rigidly parallel, as is typical of gap junctions. The membranes in these areas are spearated by an intercellular gap of 6nm, like that between S cells (Muller and Carbonetto, J. comp. Neurol. 185:485). Throughout much of the neuropil, the HE processes are wrapped by glial sheets. HN cells also contact the processes of HE cells. The HN cells in the third ganglion are known to inhibit the HE cells in that ganglion. In the electron microscope, we see synaptic contacts made by HN processes onto multiple thread-like extensions of HE-cell processes; these contacts may account for the inhibitory influence. In addition, HN and HE processes have been seen to be postsynaptic at the same synapse made by a third, unknown, neuron. We are currently investigating HN-to-HN-cell contacts.

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158.8 ASCENDING INTERNEURONS IN THE VENTILATORY SYSTEM OF THE DRAGONFLY LARVA. A. Komatsu. Dept. of Physiology, Tokyo Women's Med. Coll., Shinjuku-ku, Tokyo 162, Japan.

In the dragonfly larva, Anax parthenope julius Brauer, the main oscillator of the respiratory motor rhythm is located in the terminal abdominal ganglion, gA8 (Komatsu, 1982). To bring about synchronous contraction in all abdominal segments, the existence of interneurons is necessory which convey the respiratory signal from the main oscillator located in gA8 to the segmental respiratory motor systems.

To search for interneurons 4th to 7th abdominal ganglia were penetrated by glass microelectrodes. Activity of the respiratory interneurons was recorded in the deep midline region (depth 90-110 μm from the dorsal surface of the ganglion). These interneurons produced rhythmic bursts of action potentials in phase with expiration. These action potentials are considered to be axonal spikes, because they showed no prepotentials. Their structure was determined by intracellular staining with the fluorescent dye Lucifer Yellow. These neurons had their cell body in gA8 from which an axon ascended to the anterior ganglia via the ipsilateral connective. They also sent out secondary processes bilaterally in all abdominal ganglia in which their structure was determined. I designated them the ascending expiratory interneurons (AE neurons).

Intracellular recordings from the segmental respiratory motoneurons have revealed a pattern of alternating excitatory and inhibitory synaptic potentials (Komatsu, 1980). To test the possibility that the AE neurons provide some of the synaptic input to the segmental motoneurons, depolarizing current pulses were injected via the recording electrode into an AE neuron whereas activity of the respiratory motoneurons was monitored extracellularly from the peripheral nerves. The amplitude but not the timing of respiratory motor output could be altered by injecting current into an AE neuron. Some expiratory motoneurons were excited and an inspiratory motoneuron was inhibited by the evoked discharges of the AE neuron. A number of tests failed to demonstrate that the AE neurons have an innate rhythmicity, or that they are elements involved in the rhythm generating system.

Electrical stimulation of a sensory nerve of gA8 during inspiration resets the respiratory motor rhythm (Mill and Hughes, 1966). When stimulus was applied during inspiration, an expiratory burst of impulses was evoked in an AE neuron and the successive burst rhythm was reset. When stimulus was applied during expiration, the activity of the AE neuron did not show a noticiable change.

These results suggest that the AE neurons form part of the drive to the segmental respiratory motor systems and that they are under the control of the rhythm generating system.

158.9 FREQUENCY MODULATING INTERNEURONS IN THE VENTILATORY SYSTEM OF THE CRAB, Carcinus maenas. R. A. DiCaprio* and C. R. Fourtner. Dept. of Biological Sciences, SUNY Buffalo, Buffalo, NY 14260

Gill ventilation in decapod crustacea is produced by rhythmic movements of the scaphognathite (SG). The motor pattern consists of alternating bursts of action potentials in the motor neurons innervating the levator and depressor muscles of the SG. The neuronal central pattern generator, originally thought to be a single endogenously oscillating non-spiking neuron in each hemiganglion (Mendelson, M., Science 171: 1170, 1971), has recently been demonstrated to consist of a number of non-spiking interneurons (Simmers, A. J. and B. M. H. Bush, Brain Res. 197: 247, 1980; DiCaprio, R. A. and C. R. Fourtner, Soc. Neursci. Abst. 7: 744, 1981). We report here on three physiological types of non-spiking interneurons which can modulate the frequency of the ventilatory rhythm.

The first type of modulatory interneuron has small amplitude (<1 mv.) membrane potential oscillations in phase with the ventilatory motor pattern. The hyperpolarizing phase of the oscillation occurs at the onset of the depressor activity. In a quiescent preparation depolarizing current applied to this interneuron will initiate the SG rhythm. In rhythmically active preparations depolarizing current increases the frequency of the rhythm, while hyperpolarizing current slows and eventually stops the motor pattern. This neuron appears physiologically identical to an interneuron described by Simmers and Bush.

The second type of modulatory interneuron also has a small membrane oscillation with the same period as the motor output but its hyperpolarizing phase occurs near the end of the levator burst slightly preceeding the hyperpolarization of the first type. Applied currents produce effects on the rhythm which are opposite to those produced by the first type; depolarization of this neuron decreases the rate of ventilation while hyperpolarization increases the ventilatory rate. The frequency modulation effect is also observed in the contralateral SG rhythm. Injection of the dye Lucifer Yellow into this neuron reveals a ventrolateral soma, a large diameter neurite which crosses the midline, and an asymetrical arborization in the ventilatory neuropile of each hemiganglion.

The third type of frequency modulating interneuron shows no oscillations during the ventilatory rhythm, but does produce alterations in the rhythm which are identical to those produced by the second type when depolarizing and hyperpolarizing currents are applied. In addition, there is some evidence that depolarizing currents stronger than those necessary to stop the rhythm may trigger bouts of reversed ventilation. The cell body of this neuron is located on the ganglionic midline and a symmetrical branching pattern overlaps both ventilatory neuropiles. (Supported by grant 1F32NS06277 to R. A. D.)

158.10 HOW OFTEN MUST A LOCUST CATCH ITS BREATH?: EXPERIMENTAL IMPLICATIONS OF A MODEL OF RHYTHMIC PATTERN GENERATORS WITH RANDOM NEURAL INPUTS. Simon F. Giszter*(SPON. M. Posner),Lab. of Dr. G. Hoyle, Inst. of Neuroscience, Dept. of Biology, University of Oregon, Eugene, Oregon.

How separate oscillatory portions of cyclic central pattern generators are coupled to one another and to the environment to produce viable behaviours is an important problem for understanding rhythmic motor control systems.How much random interference an oscillator system can cope with and the arrangements which are consequently best for achieving coordination have not been examined systematically from a motor control perspective. However they must be important evolutionary 'design' factors in most rhythmic behaviours such as walking, flight, ventilation and swimming. In locust ventilation several apparently redundant phase-resetting mechanisms occur and the question arises as to their functional importance.

To examine these problems a mathematical model was constructed of relaxation oscillators with pulsed cycle to cycle phase resetting. The model was examined analytically and the results confirmed and extended using computer simulations. The effects of altering the variance of added gaussian noise, types and forms of phase transition curves, and the number of oscillators interactions, were examined.

The model showed several interesting features. Using the terminology of Winfree(1980): 1) Even master/slave arrangements with type 0 oneway phase transition curves (which always reset to a fixed phase) cannot produce complete phaselocking of two oscillators corrupted by gaussian noise. Control by a single master oscillator could not reproduce the observed ventilatory behaviour in the locust. 2) Two type 1 phase transition curves allowing feedback can produce larger, more frequent periods of phase locking than one type 0 interaction. Thus apparent coupling redundancy in the locust abdomen may be an adaptation to random variations in cycle length from various sources.

These results suggest sets of biological oscillators ought to cope with random events by use of inter oscillator feedback or 'memories' of disturbances. One way to generate these adaptive properties is to reciprocally couple several simple oscillators using simple phase transition curves as described. The organization of the locust abdomen seems to be such an adaptation. Using physiological parameters and connections the frequency of loss of coordination between oscillators in the model is similar to that measured between segments in the locust.

[This work was supported by an SRC overseas award, and a Morgenroth Scholarship to S.F.Giszter and NSF Research Grant BNS 82-41884 to Dr. G. Hoyle].

158.11 MAINTENANCE OF TRITONIA SWIMMING BY RECIPROCAL EXCITATION. P.A. Getting and M.S. Dekin. Dept. of Physiol. and Biophys., Univ. of Iowa, Iowa City, IA 52242.

Escape swimming of the mollusc, Tritonia, consists of 2-20 cycles of alternating dorsal and ventral flexions and is initiated by brief, noxious stimuli to the epithelium. Swim activity can be observed in two distinct neuronal populations; a network of 12 premotor, pattern-generator interneurons (PGI) and a large pool of motor efferent cells termed flexion neurons (FN). In all these cells, the neural correlate of swimming is a large, ramp depolarization (RAMP) which is largest just after the initiating stimulus and then dissipates slowly throughout the swim. Superimposed upon the RAMP are cyclic bursts that correspond to the dorsal or ventral flexion phases of a swim. The source of the RAMP, however, remains unknown. Two possibilities are the RAMP could be generated by long-lasting effects of sensory pathways extrinsic to the swim network or the RAMP could be generated within the swim network by re-excitation. These possibilities were tested for each cell type by 1) preventing cyclic activity within the swim network and 2) by artificially driving cyclic activity. The amplitude and time course of the membrane currents underlying the RAMP were measured by voltage clamp.

The RAMP in FNs was dependent upon cyclic activity of the premotor PGI and appeared to arise from the summation of long-lasting excitatory components of the monosynaptic connections from the PGI to the FN. Likewise, the RAMP in two of the three subgroups of PGI (C2 and ventral swim interneurons) was heavily dependent upon cyclic bursts within the swim network. In contrast, the RAMP in the dorsal swim interneurons (DSI) (a third subgroup of PGI) was only partially dependent upon cyclic swim activity and appeared to be generated by both long-lasting excitation from sensory sources and by excitatory connections within the swim network.

The DSI are coupled reciprocally via monosynaptic excitatory synapses and via a polysynaptic pathway mediated by an inhibitory interneuron. In a quiescent preparation the polysynaptic inhibition is stronger than the monosynaptic excitation so that activity in one DSI leads to inhibition of the other DSI and swimming is thereby prevented. The inhibitory interneuron, however, is inhibited by C2. During a swim when C2 is firing, the inhibitory interneuron would by inactive and the DSI will mutually excite each other contributing to the maintenance of the ramp depolarization initially set-up by the sensory stimulus. Swimming, therefore, is released and maintained in part by disinhibition as well as an initial, transient excitation.

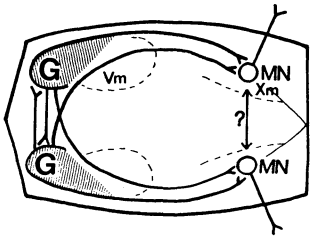
158.12 A TRIGEMINAL COMPONENT OF THE CENTRAL PATTERN GENERATOR FOR RESPIRATION IN THE ADULT LAMPREY. Carl M. Rovainen, Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110.

Unidentified generator interneurons drive branchial motoneurons (MNs) in the isolated brain stem of the adult lamprey to produce spontaneous fictive breathing activity. In the larval lamprey the pattern generators (Gs) are present in the trigeminal region of the brain stem and drive both the velar MNs in the V motor nuclei and the branchial MNs in the IX-X motor nuclei (J. Comp. Physiol. 104: 175). After metamorphosis, the trigeminal muscles are no longer used for respiration. The question is whether the rostral pattern generators are retained in the adult lamprey.

In both larval and adult lampreys, hemisection of the brain stem behind the V motor nucleus eliminates periodic bursts in IX-X MNs on the same side. This indicates that an ipsilateral descending pathway is necessary for periodic excitation of branchial MNs. The rostral medulla but not the midbrain is required for fictive breathing in adult lampreys.

One site for bilateral coordination of generators in the adult lamprey is the midline of the rostral medulla. Extracellular recordings at this site show periodic spike bursts which precede those in the IX-X nerves. Electrical stimulation at the same site excites IX-X MNs, elicits bursts in the nerves, and can reset and entrain fictive breathing. Cuts along the midline which spare the rostral medulla allow synchronous bursts in MNs on both sides. Alternatively, cutting the rostral midline still allows coordination through a caudal commissural system between the IX-X motor nuclei. Complete transection of the brain stem behind the V motor nuclei silences the IX-X MNs, but periodic bursts can still occur at the rostral midline. Therefore, at least some of the components of the pattern generators for respiration in the adult lamprey are in the rostral medulla.

The proposed locations of the pattern generators (G) and their connections are diagrammed to the right.



158.13 RESPIRATORY PUMPING IN APLYSIA IS MEDIATED BY TWO COUPLED CLUSTERS OF INTERNEURONS. J. Koester. Center for Neurobiology & Behavior, Columbia Univ., College of Physicians & Surgeons, and N.Y. State Psychiatric Institute, N. Y., N. Y. 10032.

Respiratory pumping is a brief (3-10 sec), stereotyped all-or-none behavior that consists of synchronous gill, siphon, mantle shelf and parapodial contractions, heart inhibition and vasodilation. It occurs spontaneously at intervals ranging from several seconds to several minutes, and its frequency can be modulated by a variety of stimuli, including noxious and tactile stimuli, light, hypoxia, feeding, and copulation (Eberly et al., 1981, Behav. Neural Biol.). The motoneurons that mediate this behavior have been identified previously by various investigators. Byrne (1983, J. Neurophysiol.) has previously identified a cluster of interneurons, called L25 cells, on the left side that mediate respiratory pumping. I describe here a second cluster of interneurons, called R25 cells, that also mediate this behavior.

The R25 cells are located in the right hemiganglion, symmetrical to the L25 cluster. They have several features in common with those previously described for the L25 cells: (1) There are as many as 7 R25 cells per ganglion; (2) The R25 cells burst during spontaneous respiratory pumping episodes; (3) Firing a single R25 cell can trigger a regenerative burst in the entire L25-R25 network; (4) Hyperpolarizing a single R25 cell can decrease the rate of spontaneous respiratory pumping; (5) The R25 cells make mutually excitatory connections to each other and to the L25 cells, mediated at least in part by electrical coupling; (6) The R25 cells are excited by tactile stimuli to the gill or siphon; (7) When respiratory pumping occurs in a steady, rhythmic fashion, the L25 and R25 cells exhibit slow, depolarizing pacemaker-like ramps between bursts.

Newly described features of the L25 and R25 cells are: (1) Individual R25 cells and L25 cells that trigger a burst when depolarized also connect directly to motoneurons in the abdominal ganglion; some R25 cells also connect directly to neurons in the branchial ganglion; (2) The L25 and R25 cells are non-homogeneous with regard to their synaptic projections and to their synaptic input; (3) The L25 cluster can burst independently of the R25 cluster, resulting in weak, low intensity episodes of respiratory pumping; (4) Some L25 cells produce slow IPSPs in other L25 cells and in R25 cells; (5) The L25-R25 cell network can be made to switch to the rhythmic bursting mode by exciting the R20 cells, a newly described pair of electrically coupled cells located near the R25 cluster.

These results, together with Byrne's data, indicate that: (1) Initiation and maintenance of the burst in the L25-R25 network is mediated by mutually excitatory connections that lead to reverberation within the network; (2) Burst termination is in part produced by slow inhibitory synaptic potentials generated by some of the L25 cells; (3) Some L25 and R25 cells that contribute to burst initiation within the interneuron network also project directly to motoneurons that drive the behavior. (NS19328)

158.14 GANGLIONIC BLOOD FLOW INFLUENCES THE ACTIVITY OF NEURONS REGULATING THE HEART AND VASCULATURE OF APLYSIA. S.M. Furgal* and P.H. Brownell. Dept. of Zoology, Oregon State Univ., Corvallis, Oregon 97331.

The abdominal ganglion of the mollusk Aplysia californica contains several identifiable motor neurons, neurosecretory cells and an interneuron that are known to regulate cardiac and vascular functioning. In previous studies, we observed that the somata of these neurons generally lie adjacent to major branches of the artery serving the ganglion, and that brief interruptions of arterial blood flow tended to excite neurons that excite the heart. Since the abdominal ganglion receives blood shortly after it leaves the heart, we reasoned that a simple mechanism for mediating feedback regulation of cardiac output might involve direct, compensatory actions of pressure, oxygen tension or other blood parameters on central neurons controlling circulation.

To further investigate this possibility we manipulated ganglionic blood flow and oxygen tension in a dissected preparation while simultaneously monitoring cardiac activity (pressure transducer) and intracellular electrical activity of cardiovascular-control neurons. Our results show that all neurons that act to increase cardiac output or peripheral vascular resistance were excited by decreases in blood flow to the ganglion. Thus, the heart excitor interneuron (L10), excitor motor neurons (RB cells), the vasoconstrictor motor neurons (LB_{VC}) and neurosecretory cells (R9 - R12) were reversibly excited by decreased flow while many other ganglionic neurons were unaffected or inhibited. The response of L10 was especially strong, usually transforming this cell from tonic to bursting mode of discharge. Similarly, a decrease in pO_2 (<20 mmHg) of blood perfusing the ganglion excited these neurons although this stimulus was notably less effective than decreased blood flow. Together these reflexive influences of blood pressure and oxygen tension on central neurons should act to stabilize and regulate cardiac output as a function of circulatory and metabolic demand in intact animals.

Supported by Am. Heart Assoc. grant 80-962 and NIH grant NS18681.

CARDIOVASCULAR REGULATION: HYPERTENSION AND STRESS

159.1 MODULATION OF BARORECEPTOR REFLEX SENSITIVITY BY NALOXONE AND CLONIDINE. Julianna E. Szilagyi. Baylor College of Medicine, Houston, Texas 77030.

Central control of the cardiovascular system is now known to be influenced by the opiate system (eg, central pressor actions of angiotensin II, development of hypertension in SHR's, various forms of shock). Additionally, the actions of the antihypertensive agent clonidine have been linked to the release of opiates. Naloxone, an opiate antagonist, can block the reduction in blood pressure due to clonidine administration in SHR's. In fact, morphine and clonidine produce similar cardiovascular effects via actions on the sympathetic and parasympathetic nervous systems. This study was designed to determine whether these effects involve an action of naloxone and clonidine on baroreflex sensitivity.

Eight dogs (18-22 kg) were anesthetized with pentobarbital (30mg/kg) and a femoral artery and vein cannulated. Blood pressure was raised or lowered pharmacologically with phenylephrine (20µg/kg) and sodium nitroprusside (100µg/kg) and heart rate was determined at several levels of pressure during the response. These variables were recorded before and after administration of low dose naloxone (0.04mg/kg) and clonidine (0.01mg/kg). Baroreceptor sensitivity was determined from the slope of the regression line relating mean arterial pressure and heart period (reciprocal of heart rate,msec). In one group of animals (n=4), when compared to control slopes, naloxone reduced baroreflex sensitivity to both phenylephrine and nitroprusside. The slopes of the regression lines were reduced by -52% and -20% respectively. Peak changes were not different. In contrast, introduction of clonidine in four separate dogs markedly increased sensitivity to both drugs. In this case the slopes of the regression lines were increased by 295% (phenylephrine) and 221% (nitroprusside). When low dose naloxone was given to these same animals baroreceptor reflex sensitivity was partially normalized. Compared to clonidine responses naloxone lowered the slopes of the baroreflex sensitivity curves to phenylephrine by -45% and to nitroprusside by -15%. Again, when compared, peak responses were not statistically different.

These data indicate that 1) blockade of endogenous opiates can diminish baroreflex sensitivity and, 2) the actions of clonidine on baroreceptor reflex sensitivity also involve opioid modulation. (Supported by NIH-NIRA and AHA-Texas Affiliate).

159.2 INCREASED CENTRAL ALPHA$_2$-ADRENOCEPTOR RESPONSIVITY IN GENETICALLY HYPERTENSIVE RATS. J. Marwaha, Pharmacology & Physiology, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Recent studies (1, 2) indicate that there exist some commonalities in central circuits involved in control of blood pressure and nociception. Clonidine, a clinically useful antihypertensive drug, has been shown to be effective in blocking the effects of noxious stimuli in rodents (3). In the present study, we examined the antinociceptive effects of clonidine in related strains of genetically hypertensive (SHR) and normotensive (WKY) rats.

Age-matched SHR (Okamoto-Aoki) and WKY (Wistar-Kyoto) rats were used. These 30-week-old male rats were purchased from Laboratory Supply Company, Inc., Indianapolis, IN.

Nociceptive thresholds were tested by the tail flick method. Tail flick was tested by placing the tail of the animal in a thermostatically-controlled water bath, set at 55°C. Cut-off time in absence of a response was 10 sec. The results were expressed as the mean latency ± S.E.M. There were no differences in baseline latency values between SHR and WKY rats. After administration of clonidine, (1 mg/kg, i.p.) tail flick latencies in spontaneously hypertensive rats (SHR) were significantly greater than those in normotensive (WKY) controls. The antinociceptive effect of clonidine was significantly antagonized by yohimbine, an alpha$_2$-adrenoceptor antagonist (2 mg/kg, i.p.-administered 15 min. before clonidine). Oxymetazoline (up to 6 mg/kg, i.p.), a poorly permeable alpha$_2$-adrenoceptor agonist, did not produce antinociceptive effects in either SHR or WKY rats.

Our results suggest that SHR rats differ from WKY animals in the following respects: (A) SHR rats have decreased responsiveness to pain as assessed by administration of clonidine; (B) SHR rats have increased responsiveness (increased receptors!) to alpha$_2$-adrenoceptor agents as assessed by yohimbine antagonism; (C) In SHR rats the more responsive alpha$_2$-adrenoceptors are probably centrally located as assessed by oxymetazoline.

Supported in part by USPHS Grants SO7RR 5371, AG 03737, AA 05871, and an Indiana State University Grant #6703.

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159.3 LONG-TERM ENHANCEMENT OF INTRASPINAL SYMPATHETIC TRANSMISSION FOLLOWING BRIEF PERIODS OF INTENSE ACTIVATION. Alan F. Tramposch*, Ralph L. Myers*, and Donald N. Franz. Dept. of Pharmacology, University of Utah, Salt Lake City, Utah 84132.

Sympathetic discharges recorded from upper thoracic preganglionic rami were evoked by stimulation of descending excitatory pathways in the cervical dorsolateral funiculus of unanesthetized, spinal cats at 0.1 Hz. At this stimulus rate, the sizes of evoked discharges, analyzed on-line by signal averaging, remained stable for more than 6 hr. Brief periods of repetitive stimulation produced long-term potentiation (LTP) of subsequent discharges evoked at 0.1 Hz. The size and the duration of LTP were dependent on frequency (10-75 Hz) and duration (5-20 sec) of tetanic stimulation. Following tetanic stimulation at 50 Hz for 10 sec, subsequent discharges reached maximal sizes of 120-600% of control values within 90 sec and thereafter slowly declined to control or higher values by about 20 min. Depression of intraspinal transmission by clonidine (20 ug/kg), 5-HTP (40 mg/kg), or morphine sulfate (1 mg/kg), which act by depressing the excitability of sympathetic preganglionic neurons (SPGNs), did not depress the absolute size or duration of LTP. In contrast, chlorpromazine HCl (4.5 mg/kg) which appears to depress intraspinal transmission by blocking the excitatory influence of descending norepinephrine pathways, almost completely prevented the development of LTP. These results suggest that enhanced release of norepinephrine is largely responsible for LTP.

Repeated tetanization of intraspinal pathways (50 Hz for 10 sec), at four, 20 min intervals produced incremental increases in steady state transmission to SPGNs in 47 of 66 experiments (average, 180%; range, 110-335% of pretetanic control values). The increases in transmission were sustained for up to 3 hr and were directly correlated with the average maximal size of LTP following the four periods of tetanization ($r=0.775$; $P < 0.001$). These results suggest that long-term increases in intraspinal sympathetic transmission produced by intermittent, intense activation of descending excitatory pathways to SPGNs may contribute to the development of primary hypertension.

(Supported by NIH grants HL-24085 and GM-07579 and the Utah Heart Association.)

159.4 CENTRAL EFFECTS OF ANGIOTENSIN II OPPOSE SYMPATHOINHIBITION INITIATED BY BARORECEPTORS. R.D. Stein*, R.B. Stephenson*, and L.C. Weaver (SPON: J.R. Hoffert). Dept. of Physiol., Mich. St. Univ., E. Lansing MI 48824.

Central administration of angiotensin II (AII) causes increased arterial pressure which may be mediated, in part, by effects upon central sympathetic neurons or by interference with central or peripheral components of the baroreceptor reflex. The purposes of this investigation were to determine 1) if central excitatory effects of AII on splanchnic sympathetic activity are evident in the presence of concomitant baroreceptor activation and 2) if peripheral actions of AII decrease the sensitivity of the carotid sinus baroreceptors. Discharge rates of carotid sinus afferent and splanchnic sympathetic efferent nerves were monitored in 12 chloralose-anesthetized cats. Neural responses during increases in arterial pressure caused by intracarotid or intravenous administration of AII were compared to responses to equivalent increases in arterial pressure induced by intravenously injected dextran or phenylephrine.

Intracarotid injections of AII caused a mean increase in arterial blood pressure of 16 mm Hg and variable individual splanchnic sympathetic responses consisting of increased, decreased, or unchanged activity. Mean splanchnic nerve activity within the group remained unchanged. In contrast, sympathetic activity was consistently inhibited (-24%) during equipressor infusions (18 mm Hg) of dextran or phenylephrine. AII had no apparent effect on the transduction characteristics of the arterial baroreceptors, as carotid sinus afferent activity was increased 35% by the intracarotid AII injections and 31% by the intravenous dextran or phenylephrine infusions.

Splanchnic and carotid sinus nerve responses to intravenously injected AII were not significantly different from responses to dextran or phenylephrine infusions. Intravenous AII caused a pressor response of 19 mm Hg, splanchnic nerve inhibition (-21%), and carotid sinus nerve excitation (+34%). Equipressor doses of dextran or phenylephrine induced the expected decreases in sympathetic discharge (-23%) and increases in baroreceptor activity (+32%). These data demonstrate that AII can act centrally to excite sympathetic outflow or to interfere with the sympathoinhibitory baroreceptor reflex. Through such actions, AII may contribute to the genesis and maintenance of the renal hypertensive state.

Supported by grant HL21436.

159.5 DECREASED SODIUM EXCRETION DURING BEHAVIORAL STRESS IN DOGS: ROLE OF CENTRAL BETA-ADRENOCEPTORS AND RENAL NERVES. J. P. Koepke*, K. C. Light*, A. Grignolo*, and P. A. Obrist* (SPON: C. L. Lee). Dept. of Psychiatry, University of North Carolina School of Medicine, Chapel Hill, NC 27514.

Exposure to behavioral stressors has recently been shown to reduce renal sodium excretion in saline-infused dogs and rats (1-3) and in water-loaded young men with borderline hypertension or hypertensive parents who showed evidence of high sympathetic activity during stress (4). To clarify possible neural mediating processes, further studies in dogs were performed comparing excretory responses to signalled shock-avoidance before and after i.v. infusion (1 mg/kg) of three non-selective beta-adrenergic antagonists or surgical renal denervation.

In 7 dogs, decreases in excretion of sodium and water shown during avoidance without propranolol (-47% and -45% from baselines of 345 μEq/min and 2.1 ml/min) were abolished with propranolol (-2% and -5% from 527 μEq/min and 3.0 ml/min). In 5 other dogs showing similar stress-induced decreases in sodium and water excretion without beta-antagonists (-48% and -40%), significant decreases in excretion remained after infusion of timolol (-38% and -36%) or oxprenolol (-25% and -19%). Timolol and oxprenolol concentrate highly in the kidney and timolol is 5-10 times as potent in beta-antagonism as propranolol, indicating that blockade of renal beta-receptors does not prevent stress-induced changes in sodium excretion. Instead, since propranolol has been shown to cross the blood-brain barrier more readily than timolol or oxprenolol (5,6), its effectiveness appears to be due to receptors within the central nervous system.

Renal excretory responses to avoidance were also compared in 10 dogs before and after surgical renal denervation or sham-denervation. Decreases in sodium and water excretion shown before sham-denervation (-41% and -42%) persisted and were even slightly greater after (-55% and -57%). In contrast, average decreases seen before renal denervation (-41% and -44%) were largely absent after (-14% and -11%); in fact, denervation totally abolished these changes in 4 dogs but had no effect in one. Thus, in most dogs, the decreased sodium excretion induced by this stressor appears to be mediated directly by the renal nerves.

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159.6 MEDIAN PREOPTIC LESIONS INTERFERE WITH REFLEX COMPENSATION DURING HEMORRHAGE. D. K. Hartle, F. Cheung* and J. W. Manning. Dept. of Physiology, Emory University, Atlanta, GA 30322.

The median preoptic nucleus (MePON) has been recently implicated in central nervous control of the circulation. It appears to be an integrative site for both neural and endocrine mechanisms that regulate sympathetic vasomotor activity. Destruction of the MePON eliminates the central pressor actions of angiotensin II, produces chronic deficits in vasopressin regulation and impairs baroreflex function. Destruction of the MePON has been demonstrated to both prevent and reverse many different forms of experimental hypertension. Two vasoconstrictor pathways descend from the MePON that are differentially required for the development of renin-dependent and non-renin-dependent forms of renal hypertension. One pathway is activated by angiotensin II and the other pathway is apparently activated by relief of inhibition by baroreceptor-mediated input. Animals with MePON destruction do not have these central mechanisms for increasing sympathetic nervous activity and consequently do not develop hypertension.

This experiment was designed to test whether these central control mechanisms were required for homeostatic compensation during blood volume depletion. A group of five conscious unrestrained rats with previous radiofrequency destruction of the ventral MePON were compared with a group of six sham-operated rats. The animals were hemorrhaged by 1.6% of their total body weight over a 5 min period. The MePON lesion group became hypotensive with this bleed (average 59% decrease in mean arterial pressure). The sham-operated control group had no significant change in blood pressure from control. The difference between the responses of the two groups was statistically significant, $p<.01$.

Bleeding was continued at the reduced rate of 1ml/7 min until irreversible shock caused death in each animal. MePON lesion animals were significantly more susceptible to irreversible shock. Death occurred in this group with 2.8% body weight blood loss compared with 4.4% in the sham group. These data suggest a role for neural circuitry involving the MePON in compensatory augmentation of sympathetic vasoconstriction and vasopressin release required for blood volume mobilization to maintain blood pressure during hemorrhagic crisis. Supported by GA Heart Association.

159.7 THE EFFECT OF LESIONING THE AV3V REGION UPON THE CENTRALLY MEDIATED PRESSOR RESPONSE TO ANGIOTENSIN II IN THE DOG. O. Marson*, C.L. Chernicky, K.L. Barnes and C.M. Ferrario, Research Division, Cleveland Clinic Foundation, Cleveland, Ohio 44106.

The administration of angiotensin II (Ang II) into either a brain ventricle or the cerebral circulation of several species causes a prominent cardiovascular hypertensive response. Although in the dog the area postrema is a receptive site for the pressor effects of Ang II given via the vertebral arteries, it is not yet known what region or structure accounts for the activation of sympathetic vasoconstrictor outflow when the hormone is given into the cerebrospinal fluid (CSF).

The changes in mean arterial pressure (MAP) and heart rate (HR) due to infusions of Ang II into either a third cerebral ventricle (IVT) or the common carotid artery (ICA) were measured before and following electrolytic lesion (4 mA DC, 20 sec) of the periventricular tissue surrounding the anterior portion of the ventral third cerebral ventricle (AV3V) in six dogs anesthetized with chloralose .66 mg/kg, IV) after they were medicated with morphine (2 mg/kg, IM). In another three dogs (sham control group) an electrolytic lesion was placed at sites either rostral or caudal to the AV3V region. In both sham and AV3V lesioned groups, the pressor activity of the peptide was assessed at the following doses: 1, 10, 20 and 50 ng/kg/min for the ICA route; 50, 100 and 200 ng/kg/min for the IVT route. All infusions lasted two minutes.

In all dogs smaller quantities of Ang II were needed to produce a pressor response via the ICA equivalent to that obtained when the peptide was infused IVT. In addition, the increases in pressure were always associated with tachycardia following IVT administration of Ang II; in contrast, the pressor response due to ICA Ang II consistently caused bradycardia. Lesion of the AV3V region produced a significant blunting of the pressor effects of Ang II administered via a third ventricle at all doses tested (i.e.: 14 ± 2 and 22 ± 4 mmHg before versus 7 ± 3 and 14 ± 3 mmHg after AV3V lesion at the doses of 50 and 200 ng/kg/min respectively). In contrast AV3V lesioned dogs did not show any change in the pressor activity of Ang II given via the ICA route.

These findings demonstrate that in the dog the AV3V region contributes to the pressor activity of Ang II delivered via the cerebrospinal fluid. However, it appears not to be active as a site of action for blood-borne angiotensin II. (Supported in part by NHLBI grants HL-6835 and HL-24100).

159.8 CARDIOVASCULAR AND APPETITIVE EFFECTS OF ESTRADIOL. T.A. McCaffrey* and John A. Czaja. Department of Psychological Sciences, Purdue University, West Lafayette, IN. 47907.

The influence of estrogens in cardiovascular and appetitive modulation is well documented but poorly understood. Yet, simultaneous measures of these related parameters are rarely taken. The present study documents the ability of estradiol-17beta to lower blood pressure (BP) and pressor responses (PR) to norepinephrine (NE) in conscious, unrestrained guinea pigs (GP). Female GP were ovariectomized 19 days prior to implantation of carotid artery and jugular vein catheters (PE50 and PE10, respectively). Measurement of direct arterial blood pressure and response to 30 ul of intravenous NE (52 ug/ml) was performed on 7 GP just previous to subcutaneous injection of 30 ug estradiol-17beta and on 7 GP receiving only the corn oil vehicle. Measurements of resting and NE-modified systolic, diastolic, and pulse pressure, and HR were quantified by a microcomputer system at hour 0 and 1, 3, 6, 12, 24, 36, 48, 60, and 72 hours post injection. Food intake, water intake and body weight were measured daily throughout the experiment. The data was expressed as a change from zero-hour and compared between groups using a Student's t-test. Results indicate that estradiol depressed resting pressures up to 12% and PR up to 20% in a period from 12 to 48 hours post-injection. Only minor changes in pulse pressure, heartrate or heartrate depression induced by NE were observed. An analysis of the average NE infusion profile indicated that estrogen produced a pressure dependent decrease in the response to NE with no change in the half-life of the response. When expressed as a change from the two days previous to the injection and compared between groups, estradiol exhibited statistically significant behavioral effects. Food intake was depressed for three days following the estradiol, body weight was depressed for two days, and water intake for one day following the estradiol. A significant correlation was observed between the 24 hour change in systolic pressure and the concurrent change in water intake ($r(5) = .80$, $p < .05$). The correlation could not be easily attributed to a generalized estrogen sensitivity since the depression in systolic pressure did not correlate well with the 24 hour change in food intake ($r = .42$, NS), body weight ($r = .20$, NS), or systolic pressor response ($r = -.02$, NS). In a related study, total 24 hour food and water deprivation could not be shown to decrease BP or pressor response to NE. Thus, the correlation between the vascular and drinking changes following estradiol treatment implies that the reduced blood pressure and vascular tone may be indicative of systemic changes in cardiovascular stimuli for thirst.

159.9 BURST DURATION AS AN INDEX OF RENAL SYMPATHETIC NERVE ACTIVITY IN DOCA HYPERTENSIVE YUCATAN MINIATURE SWINE. R. R. Notvest*, E.J. Zambraski* and C.D. Ciccone* (SPON: F. E. Horvath). Physiology, Rutgers Univ., New Bruns., NJ 08901.

Absolute values for sympathetic nerve activity are difficult to obtain because of the limitations of the present methods used to quantify multiunit nerve activity (voltage integration, frequency counts). This difficulty in measuring absolute activity is especially apparent when comparing nerve activity among different animals (e.g. normotensive/hypertensive). We recently have explored a novel approach for quantifying renal nerve activity (RNA) which relies on the measurement of RNA burst duration (BD). As previously reported, renal nerves discharge in pulse-synchronous bursts. We recently observed that during a phenylephrine-evoked pressor response, RNA BD decreased as mean arterial pressure (MAP) increased. There was a direct positive correlation between the time interval of individual BDs and the integrated voltage per burst ($r=0.76$; $P< 0.01$). This finding suggests that the BD parameter is a valid index of total RNA. We have utilized analysis of BD to compare RNA between 9 control (CON) and 7 DOCA hypertensive anesthetized swine. MAP, which was significantly elevated in conscious DOCA animals, was reduced by pentobarbital anesthesia. Under these experimental conditions the mean BD for the CON and DOCA groups were not significantly different. The mean BD per minute (normalized for heart rate) was elevated in the DOCA group although the difference was not significant.

	MAP-Cons. (mm HG)	MAP-Anes. (mm HG)	HR (bts/min)	BD (msec)	BD/min (sec)
CON	132 + 5	135 + 6	157 + 15	196 + 22	30 + 4
DOCA	170 + 7	140 + 7	174 + 14	201 + 25	35 + 5

The lack of a significant difference between the BD parameters of CON and DOCA groups may have been due to the effects of the anesthesia on nerve activity and the reduced MAP in DOCA animals. Further studies in the conscious state will be required to determine whether BD, as an index of total RNA, is altered in DOCA hypertension.

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159.10 EFFECTS OF ACUTE BARODENERVATION IN NORMOTENSIVE & DOCA-SALT RATS. N. Gravel* and J. de Champlain. Dept of Physiology, Université de Montréal, Montréal, Québec, Canada.

Chronic barodenervation (BDX) of the sino-aortic nerves induces significant increases of the systolic arterial pressure and of circulating catecholamines (CA) in rats (Alexander, N., Am. J. Physiol. 238:H-521, 1980). However, chronic observations of these animals reveals a lability of the blood pressure rather than a consistant increase (Norman, R., Hypertension, 3:119, 1981). Since arterial baroreceptors are involved in rapid adjustments of blood pressure, it is of interest to evaluate the acute cardiovascular response to BDX. Furthermore, the possibility of an association between baroreceptor dysfunction and hypertension warrants further investigation in hypertensive animals. This study was carried out to examine the cardiovascular response of normotensive (NT) and DOCA-salt hypertensive (HT) rats submitted to acute carotid and aortic BDX. Plasma norepinephrine (NE) and epinephrine (E) concentration were used as indices of sympathetic activity. NT and HT animals were either barodenervated (BDX) or sham-operated (SHAM) under fentanyl anaesthesia. Mean arterial pressure (MAP) was monitored continuously in the awaken animal and recorded every 5 min during 1 hour while blood samples (0.5ml) were withdrawn at 20, 40 and 60 min for measurements of plasma NE and E (ng/ml). CA concentrations were measured by radio-enzymatic assay using thin layer chromatography for separation of amines.

MAP is significantly higher in NT-BDX than in NT-SHAM animals until 20 min (127 ± 3 vs 80 ± 9 mmHg) and decreases thereafter to comparable levels 40 min later. However, plasma NE and E concentrations are consistently greater in NT-BDX (1.4 ± 0.1 and 3.6 ± 0.5 ng/ml) than in NT-SHAM rats (0.6 ± 0.1 and 0.5 ± 0.03 ng/ml). Furthermore, CA levels increase steadily until 60 min in NT-BDX rats whereas these values remain relatively constant in NT-SHAM. In HT animals, MAP tends to be greater in HT-BDX (158 ± 14 mmHg) than in HT-SHAM (152 ± 7 mmHg) until 20 min and decreases thereafter below basal values. Plasma NE and E levels are greater in HT-BDX (1.8 ± 0.2 and 4.0 ± 0.9 ng/ml) than in HT-SHAM (0.97 ± 0.1 ng/ml and 1.0 ± 0.2 ng/ml). However, in this group of rats, CA concentrations are greatest at 20 min and decrease until 60 min in HT-BDX. These findings demonstrate a close association between arterial pressure and sympathetic response at 20 min following acute BDX. The decrease in MAP observed both in NT-BDX and HT-BDX from 20 to 60 min would be associated to a decrease of sympathetic activity only in HTp-BDX since CA levels remain elevated in NT-BDX. The lesser sympathetic effect of BDX in HT animals suggest the possibility of a dysfunction at the level of baroreceptor in DOCA-salt treated rats. Supported by Medical Research Council of Canada and Québec Heart Foundation.

159.11 HYPOTHALAMIC AVP RELEASE IN THE DEVELOPMENT OF DOCA/NaCl HYPERTENSION. Y. F. Chen, B. Stamoutsos*, M. D. Lindheimer* and S. Oparil*. Cardiovascular Research and Training Center, Univ. of Alabama in Birmingham, Birmingham, AL 35294. Dept. of Medicine, Univ. of Chicago, Chicago, IL 60637.

The role of arginine-vasopressin (AVP) in the development of deoxycorticosterone-NaCl (DOCA/NaCl) hypertension was studied by measuring plasma AVP levels and basal release of AVP from superfused hypothalami of uninephrectomized male Sprague-Dawley rats subcutaneously implanted with DOCA (100mg/kg) and drinking 1% saline and three control uninephrectomized groups (H_2O, saline, and DOCA alone). Three days, 1 wk, and 3 wks after DOCA implantation, animals were sacrificed by decapitation and blood was collected for determination of plasma AVP. To measure AVP release, whole hypothalamic tissue fragments were dissected out and superfused in vitro. AVP collected in the superfusate and plasma AVP were quantified by radioimmunoassay.

Plasma osmolarity and body weight increases were similar among the 4 groups. Systolic blood pressure (BP) in mmHg in DOCA/NaCl rats was significantly ($p<0.01$) elevated above the H_2O control group [185.4±6.3 (14) vs 129.4±4.4 (16)] at 3 wks. BP in the group treated with DOCA alone was also elevated to 158.8±3.1 (13) at 3 wks. Results [Mean ± SE (n)] of plasma AVP and basal release of AVP from superfused hypothalami are:

	H_2O	NaCl	DOCA/H_2O	DOCA/NaCl
I. Plasma AVP (pg/ml)				
3 days	2.9±0.9 (9)	2.5±0.8 (9)	15.6±3.7(10)*	8.8±1.1 (9)*
1 wk	1.4±0.3 (7)	2.8±0.6 (9)	15.9±3.3 (9)*	7.6±1.3 (8)*
3 wk	1.2±0.4(19)	1.2±0.2(20)	10.2±1.8(18)*	4.8±0.6(23)#
II. Spontaneous release rate of AVP from hypothalami (pg/min/Hypo)				
3 days	6.4±1.0 (8)	5.1±0.5(10)	8.2±1.3(10)	6.3±0.9(10)
1 wk	7.2±0.7 (8)	5.6±0.7 (8)	10.1±1.8(10)	7.0±1.0(10)
3 wk	2.5±0.5(20)	3.3±0.6(18)	12.0±1.3(18)*	7.0±0.6(17)*

*$p<0.01$, #$p<0.05$ compared with H_2O control group

Our results demonstrate that both the spontaneous release of AVP from hypothalami and plasma AVP are increased in DOCA/H_2O and DOCA/NaCl hypertensive rats. The observation that both hypothalamic AVP release and plasma AVP levels are increased in DOCA/H_2O animals suggests that DOCA alone may stimulate the release of AVP from the central nervous system. Further study is needed to determine the mechanism of this effect and the role of increased AVP release in the pathogenesis of DOCA/NaCl hypertension.

159.12 ATTENUATION OF BLOOD PRESSURE IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) BY RAUWOLSCINE. S.J. Goldman, T.R. Hansen, G.A. Oltmans and S.A. Berenbaum. (SPON: R. WEISZ). Depts. of Pharm. Physio. and Psychol., Chicago Medical School, N. Chicago, IL 60064.

The spontaneously hypertensive rat (SHR) exhibits markedly increased systolic and diastolic blood pressure in comparison to normotensive WKY controls. The SHR is also behaviorally hyperreactive compared to the WKY, and exhibits a significant increase in locomotor activity in a novel environment. Although the mechanisms responsible for the hypertension and hyperreactivity are not known, a CNS component has been implicated in both instances. In this respect, significant increases in central α-1 receptors have been reported in the SHR (Pullen, et al. 1982) in areas believed to be involved in the regulation of blood pressure. Since receptor increases frequently are associated with decreased neurotransmitter activity, in the current study the α-2 antagonist rauwolscine was administered to SHR and WKY controls in an attempt to increase central adrenergic activity. The effects on blood pressure and locomotor activity were then assessed.

Fifteen minutes after injection, rauwolscine treatment (16mg/kg, i.p.) significantly reduced the tail artery systolic pressure of unanesthetized SHR (predrug=175.5±4.6mm Hg; postdrug=108.3±8.8; $p<.01$) but did not significantly change that of WKY controls (predrug=111.7±4.1; postdrug=104.0±3.5). A partial recovery of pressure occurred in the SHR during the next 15 minutes (to 139.6±7.4) and blood pressure remained at this level for the next 30 minutes. The blood pressure in the SHR was significantly below baseline at least 60 min following rauwolscine treatment. In contrast, no significant change in blood pressure was found in the normotensive WKY controls at any of these times.

In contrast to the effects on blood pressure, rauwolscine did not affect the activity levels of either SHR or WKY rats in a novel environment. Baseline activity levels of the SHR in the novel environment were significantly higher than those of WKY controls (2193±75 counts/30 min vs 1635±118, respectively). Following rauwolscine treatment, the activity levels of drug-treated SHRs were not significantly different from those of saline-treated SHRs, and both of these groups were significantly more active than drug- or saline-treated WKY controls.

The results suggest a selective hypotensive effect of rauwolscine in the SHR, and further indicate that this effect is separable from an effect on activity. (Supported by BRSG grant 5366).

159.13 ALTERATIONS IN MEDIAL LEMNISCAL FIELD POTENTIAL RESPONSES IN SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE WISTAR KYOTO RATS. James T. Garsik*, Walter C. Low, and David Whitehorn. Department of Physiology and Biophysics, University of Vermont, Burlington, VT 05405.

The spontaneously hypertensive rat (SHR) is a commonly used model for human essential hypertension. Increased sympathetic activity and reactivity in the SHR compared to the normotensive Wistar Kyoto rat (WKY) has been shown to contribute to the elevated blood pressure of this strain. Recent evidence suggests that this feature of the SHR is part of a generalized hyperexcitability affecting all excitable tissue. To test this hypothesis we have directly determined the responsiveness of a sensory pathway in the SHR and WKY with electrophysiological studies of transmission through the dorsal column nuclei (cuneate nuclei). Input-output (I-O) relationships were constructed which describe the medial lemniscal responses (output from the dorsal column nuclei) as a function of electrical stimulations applied to the animal's forepaw (input to the dorsal columns). In these experiments four groups of animals were used: SHR (uSHR), SHR maintained normotensive with hydralazine from weaning (tSHR), WKY (uWKY), and WKY given the same hydralazine treatment at tSHR (tWKY).

The uSHR displayed a more rapidly rising I-O relationship than did the uWKY. This difference persisted between the tSHR and tWKY indicating that this is an inherent alteration in the SHR unrelated to blood pressure. A comparison between tSHR and uSHR demonstrated that the tSHR had an I-O relationship shift upward signifying that elevated blood pressure inhibits transmission through the dorsal column nuclei (tWHY and uWKY did not differ).

Two subsequent approaches demonstrated that acute and chronic elevations in blood pressure affect dorsal column nuclei transmission. In the acute experiments medial lemniscal evoked potentials were compared during saline or phenylephrine (PE) infusion to pre-treatment responses in individual WKY. PE infused animals had a reduction in the medial lemniscal response which was not seen in the saline treated rats. The chronic experiments consisted of a comparison of the I-O relationships of sham operated and Goldblatt renal clip hypertensive Sprague Dawley rats. The I-O function of the hypertensive rat group was shifted downward from that of the sham operated animals.

Our results are consistent with a theory of generalized neuronal hyperexcitability in the SHR and confirm that transmission through the cuneate nuclei is inhibited by hypertension.

159.14 LIMBIC HYPOTHALAMIC ELECTRICAL ACTIVITY IN SPONTANEOUS HYPERTENSIVE RATS R.J. Morgan, C.C. Turbes, G.T. Schneider* and T.N. Solie* Dept. of Biophysics and Physiology, Colorado State Univ., Ft. Collins, CO 80521.

Anatomical studies have shed some light on the neural mechanisms in the brain stem, hypothalamus and limbic regions that serve to integrate autonomic and neuroendocrine responses to pertubations in blood pressure. Few studies have addressed the related changes in electrical activity of these regions. Chronic electrodes are implanted in the amygdala(AMY), the preoptic area(POA), arcuate nucleus of the hypothalamus(ARC) and the hippocampus(HIP) of normal (NCR) and spontaneous hypertensive rats (SHR). The central nervous system (CNS) signals are recorded simultaneously for the first ten minutes of each half-hour for eight hours sequentially for four days. The analog recordings are analyzed using signal processing procedures including cross-correlation, coherence spectra and frequency time delay plots. This study will be concerned with female rats both NCR and SHR. There are differences in the cross-correlation and coherence spectra findings in the SHR rats from the NCR rats. There is a reversal of signal flow in the brain regions studied of the SHR rats from the NCR rats noted in the frequency time delay plots. The signal flow in the NCR rats correlate with the four days of the reproductive cycle. In the SHR rats there is a lower correlation of the signal flow patterns that relate to the reproductive cycle. In certain brain areas of the SHR rats, the signals are reversed at certain periods of the reproductive cycle as compared with the NCR rats.

159.15 HISTOCHEMICAL ANALYSIS OF VASOPRESSIN-NEUROPHYSIN AND NOREPINEPHRINE IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEI OF THE SPONTANEOUSLY HYPERTENSIVE RAT.
John R. Sladek, Jr. and Celia D. Sladek, Depts. of Anatomy and Neurology, University of Rochester Sch. of Med., Rochester, NY 14642.

Alterations in vasopressin (VP) and norepinephrine (NE) content of the supraoptic (SON) and paraventricular nuclei (PVN) have been reported in spontaneously hypertensive rats (SHRs) compared to normotensive Wystar-Kyoto (WKY) controls. The present study was initiated to evaluate whether these changes in content reflect alterations in the specific distribution of these substances within the PVN and SON. A combined histofluorescence and immunohistochemical technique was applied to the brains of 15-16 week old WKY and SHR rats, 3 of each strain. At this age blood pressure is chronically elevated in the SHRs (198 ± 2 mm Hg vs 142 ± 2 mm Hg in WKY, p .001). Alternate sections were examined either for NE fluorescence or vasopressin-associated neurophysin (VP-NP) with comparator bridge microscopy. A marked increase was seen in the density of NE varicosities in the SON. While the ventral and caudal regions contain a maximal density of varicosities in the SHR, this density was augmented by increased densities in dorsal and rostral regions also. These same animals also showed an increase in VP-NP staining in the SON; a larger proportion of neurons in the dorsal and rostral regions of the nucleus were stained. Increased VP-NP staining also was seen in the PVN; the majority of neurons appeared maximally stained in the SHR whereas in the WKY a larger proportion of the neurons were lightly stained. Unlike the SON, the PVN showed a reduced density of NE varicosities over portions of the nucleus and a reduction in the overall size of the PVN. The reduced densities appeared in the form of a pocket within the dense pattern of NE innervation. In the SON, the increased distribution of NE varicosities in the rostral portion of the nucleus mirrors the increased representation of VP-NP cells in this area. This represents either an increase in the number of VP neurons or an increase in the staining intensity which thereby increases the number of cells visualized. In PVN, the observation of NE deficient regions in the SHR corresponds to the reports of decreased norepinephrine content in the PVN of SHRs (Saavedra et al., Circ. Res. 42:529, 1978). The increased density of VP-NP immunoreactivity in SHR requires further analysis. It may reflect an increase in VP biosynthesis and turnover, because it has been reported previously that the VP content of the PVN determined by radioimmunoassay is decreased (Morris and Keeler, Br. Res. 249:173, 1982). Thus, the VP prohormone complex may be transported away from the nucleus before processing occurs thereby preventing expression of the VP immunoreactivity. Another possibility is that there is a decrease in the number of VP producing cells in the PVN of SHRs resulting in activation of the remaining cells. These possibilities will be addressed in subsequent studies. Supported by NIH grants HL-18172 and NS-15816.

159.16 VASOPRESSIN AND RENIN RESPONSE TO PLASMA VOLUME LOSS IN THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR). M.L. Blair, C.D. Sladek and Y.-H. Chen. Dept. Physiol., Neurol. and Anatomy, University of Rochester Medical School, Rochester, NY 14642

Both vasopressin and renin-angiotensin may contribute to the development of hypertension in the SHR. We have evaluated the possibility that stimulation of vasopressin and renin release elicited by blood volume loss might be exaggerated in the SHR in comparison with the normotensive Wistar-Kyoto rat (WKY). Rats were studied during the early stages of hypertension development (6 weeks old) and after hypertension was fully established (18 weeks old). Rats were sacrificed 3 hr. after IP injection of 2 ml/100 gm body weight of 0.9% saline (control group), 20% polyethylene glycol (PEG) or 30% PEG. Trunk blood was collected for radioimmunoassay of serum arginine vasopressin concentration (AVP) and serum renin activity (SRA). The magnitude of plasma volume loss was evaluated from the microhematocrit values (HCT). At 6 weeks of age systolic blood pressure was 129 ± 2 mm Hg in SHR and 87 ± 2 mm Hg in WKY ($P<.001$). At six weeks of age neither AVP nor HCT was different in the SHR and WKY control groups (SHR: 1.7 ± 0.6 pg/ml, n = 6 samples pooled from 10 rats; hct = 42 ± 1, n = 10; WKY: 2.7 ± 0.4 pg/ml, n = 6 samples pooled from 11 rats; hct = 40 ± 1, n = 11). For rats which showed hemoconcentration to HCT $\geq$ 46 after 20 or 30% PEG injection, both SHR and WKY had AVP values which were significantly greater ($p<.005$) than those in the control group. However, AVP values in SHR (26.0 ± 10.1 pg/ml, n = 4 samples from 10 rats) were significantly greater ($p<.025$) than in WKY (6.0 ± 2.0 pg/ml, n = 9 samples from 15 rats).

SRA in control 6 week old rats was not different for SHR (1.2 ± 0.2 ng AI/ml·hr, n = 6) and WKY (1.8 ± 0.3 ng AI/ml·hr, n = 6). In a separate group of 5 week old rats total kidney renin content and kidney renin concentration were also the same in SHR (31.1 ± 1.8 mg AI/kidney·hr; 64 ± 6 ng AI/ mg·hr, n = 5) and WKY (31.3 ± 2.1 mg AI/kidney·hr, 74 ± 5 ng AI/mg·hr, n = 5). SRA increased ($p<.001$) in both groups following PEG injection and the response was not different for SHR (15.7 ± 1.6 ng AI/ml ·hr; n = 5) and WKY (16.5 ± 1.5 ng AI/ml·hr; n = 7) for rats with hemoconcentration to a HCT $\geq$ 46.

At 18 weeks, systolic BP was 194 ± 3 mm Hg in SHR and 144 ± 2 mm Hg in WKY ($p<.001$). AVP in control, 20% PEG and 30% PEG groups was 3.2 ± 0.2 (n = 7), 6.1 ± 2.8 (n = 7), and 13.0 ± 3.2 (n = 5; $p<.05$) pg/ml respectively for SHR and 3.2 ± 1.5 (n = 7), 4.2 ± 0.5 (n = 7) and 9.1 ± 1.9 (n = 7; $p<.05$) pg/ml for WKY. Neither control nor stimulated AVP values were significantly different for SHR versus WKY.

In summary, SHR show an exaggerated AVP response to plasma volume loss early in development of hypertension (6 weeks) but not after hypertension is fully developed (18 weeks). In contrast, renin release is not augmented in young SHR. Supported by NIH Grant 1-R01-HL-28172 and RCDA to M.L.B. K01-HL-00966.

159.17 AGE, SEX AND STRAIN DIFFERENCES IN ACTIVITY AND HABITUATION PATTERNS IN SHR AND WKY RATS. E.D. Hendley, D. Wessel*, D.G. Atwater*, J. Gellis*, D. Whitehorn and W.C. Low. Dept. Physiol. Biophys., Univ. Vermont, Burlington, VT 05405.

Wistar-Kyoto derived spontaneously hypertensive (SHR) and normotensive (WKY) rats, bred at the Univ. of VT, were examined with respect to their spontaneous activity in a novel environment, as well as their habituation to the activity test cage during repeated exposures over a 4 h period. The test cage consisted of a plastic cage equipped with 4 sets of light beams and photodetectors. Spontaneous activity (locomotor as well as stationary behaviors) was taken as the number of beam breaks in a 15 min period in naive rats. Habituation was determined by re-testing the rat in 3 additional 15 min trials, at 1 h intervals between trials. A total of 197 rats of both sexes were tested at ages 4 to 56 weeks. The effects of sex, age and strain (3-way ANOVA), or of sex and age within each strain (2-way ANOVA), on activity in the first trial were determined using the SPSS computer program. These results showed a strong main effect of age on activity, as well as interactions of age with sex and strain, therefore further analyses were done using rats divided into 3 age groups: 0-18, 19-36 and greater than 37 weeks. These analyses confirmed our earlier findings, and those of others, that SHR are more active than WKY rats, at all ages and in both sexes. Within each strain, females were more active than males. Across ages, WKY rats were more active in the middle age group than at either the young or older ages, whereas SHR showed 2 peaks in activity scores over a range of 52 weeks. One peak was from 6-15 weeks of age, and the other from 35-52 weeks. Accordingly, strain differences in activity are best detected in young (e.g. 12 weeks) or older (e.g. 40 weeks) rats in these strains. Patterns of habituation to the test cage were determined using ANOVA with repeated measures (trials 2,3,4), expressed as % of trial 1 (computer program BMDP). These analyses revealed marked strain differences in pattern of habituation. WKY exhibited a marked drop in activity levels across trials, falling to about 20% of trial 1 by the fourth trial, and no differences were observed with age or sex in this strain. In contrast, SHR habituated poorly, particularly in the older rats where trial 4 activity scores were 95% of trial 1. These findings further characterize the behavioral differences between SHR and WKY rats, and emphasize that although activity levels fluctuate with age in these strains, the patterns of habituation tend to remain fixed over at least one year of time. Supported by MH36064, PHS5429-19-18, HL24110, F32-HL06339 and VT Heart Assoc. Grants-in-Aid to EDH and DW.

159.18 CARDIOVASCULAR AND SYMPATHOADRENAL RESPONSES TO SWIM AND SHOCK STRESS IN TRAINED AND UNTRAINED RATS. R. H. Cox, J. W. Hubbard, J. E. Lawler, V. P. Mitchell* and B. J. Sanders.* Dept. of Psychology, University of Tennessee, Knoxville, TN. 37916.

Swim training in rodents has been shown to result in no change or a reduction in chronic (resting) blood pressure (BP)--an adaptation quite different than the increase produced by repeated exposure to tail shock. This study sought to determine if a quantitative difference exists in the acute physiological response produced by these stressors. A more intense response might be associated with a pathological adaptation. A second question of the study involved the specificity of the adaptations produced by swim training. Would the attenuation of autonomic and hormonal responses to exercise, characteristic of swim trained animals, be manifested in a different stress situation? Cardiovascular and hormonal responses to a novel stress, viz, predictable tail shock (.2-.4mA), were measured in swim trained and control male Long-Evans rats. Swim training, which was 5-7 weeks in duration, 5 days/week for 1 hr/day, utilized two percent of bwt attached to the tails as added resistance. Non trained (NT) rats received similar handling but no exercise. The heart rate (HR), systolic (S) and diastolic (D) BP and plasma levels of norepinephrine (NE), epinephrine (E) and corticosterone were measured after 20 min of each stress in rats implanted with aortic catheters. Overall swim and shock elicited similar SBP/DBP levels in T & NT rats, but T rats showed a lower HR to swim which was evident after the first 4 min.

		Rest	Swim	Shock
T (N=11)	SBP/DBP	140/95	167/110	162/106
	HR	338±11	406±11	433±11
NT (N=8)	SBP/DBP	142/94	177/116	159/104
	HR	348±10	440±13	423±10

After 20 min of swim T rats showed lower blood levels of E (332 vs 739 pg/ml), corticosterone (62 vs 32 ug/dl) and lactate (.9 vs 2.0 m moles/l) compared to NT rats. However, T & NT rats did not differ during shock on any of the measured parameters. The results support the concept of specificity of training and also suggest that the differences in adaptation produced by chronic exposure to swim and shock can not be attributed to differences in the severity of the stresses. Supported by NIH (HL-06036 & HL-19680).

159.19 ULTRASTRUCTURE OF MONOAMINERGIC SYNAPTIC VESICLES IN AN EXPERIMENTAL MODEL OF ARTERIAL HYPERTENSION. A. Pellegrino de Iraldi, C. Rojas Marcos Pereda and J.P. Corazza. Instituto de Biología Celular, Facultad de Medicina, Paraguay 2155, Buenos Aires 1121, Argentina.

Clinical and experimental evidence suggest that in arterial hypertension there is an alteration in the inactivation processes of noradrenaline (NA). Taking into account that the main mechanism of inactivation is the uptake of NA by nerve terminals and its storage in synaptic vesicles, we studied under the electron microscope the synaptic vesicles of different nerves, after using the experimental model of de Champlain et al (Supp I, Circulation Research, 24 and 25: 1, 1969). Adult Wistar male rats, uninephrectomized, injected s.c. with desoxycorticosterone (DOCA), (10 mg/ per week), drinking ad libitum a solution of 1% sodium chloride, were killed by decapitation after 6 weeks of treatment: (Group I). Untreated and uninephrectomized rats were used as controls. In another group treated and untreated rats wer injected with 5-hydroxydopamine (5-OH-DA), i.p., 50 mg kg, 30 min before killing. In every case the pineal gland, the vas deferens and the splenic artery were fixed in OsO_4 or glutaraldehyde OsO_4 and processed for electron microscopy. It was observed in the group I that the dense cores were increased in number and size in the pineal gland, greatly decreased in the vas deferens and not significantly changed in the splenic artery, related to controls. The storage of 5-OH-DA followed a similar pattern of variation in control and experimental animals. These findings seems to indicate that the storage of noradrenaline or substances structuraly related are altered in arterial hypertension being increased, decreased or not changed according to the studied territory. These results are in line with distribution pattern of endogenous and exogenous noradrenaline reported by de Champlain et al. in their experimental model. These authors described a decreased storage capacity in heart, gut, muscle, spleen and kidney and an increased or not changed storage capacity in the salivary glands. This work was supported by a Grant from SUBCYT, Argentina.

159.20 H_1-HISTAMINE RECEPTOR ANTAGONISTS INDUCE VASOSPASM ON INTACT AND ISOLATED CEREBRAL BLOOD VESSELS: RELATION TO SEDATIVE AND SOPORIFIC EFFECTS OF ANTIHISTAMINES. Bella T. Altura*, Burton M. Altura and Samuel Lassoff. Dept. of Physiology, SUNY Downstate Medical Center, Brooklyn, NY 11203

H_1-histamine receptor antihistamines (e.g., diphenhydramine-DI, pyrilamine-PY, pyribenzamine-PYR, promethazine-PR, chlorpheniramine-CHL) are known to possess sedative and soporific actions. No satisfactory explanation or mechanism has been presented to explain these side-effects. We undertook quantitative in-situ and in-vitro studies to determine the direct actions of H_1-receptor antihistamines on pial terminal arterioles (PTA) and muscular venules (PMV) in the young intact rat, using quantitative high-resolution television microscopy, and on isolated canine basilar (BA) and middle cerebral arteries (MCA). Perivascular application of the H_1-receptor antihistamines produced concentration-dependent constriction of PTA but not PMV; the relative order of sensitivity was: CHL $>$ PY $>$ PYR $\cong$ PR $>$ DI. Doses of H_1- receptor blockers from 10^{-6} to 10^{-1}mg reduced diameters from 5-35%. Addition of these H_1-receptor antihistamines to isolated canine BA and MCA suspended in Krebs-Ringer bicarbonate solution, in concentrations of from 10^{-7} to 10^{-4}M, produced dose-dependent degrees of cerebrovasospasm; maximum contractile tensions were 60-95% of maximum KCl-induced contractions. No known pharmacologic amine antagonist or opiate-receptor blocker attenuated, abrogated, or prevented the micro- or macro-vascular spasms induced by these antihistaminic drugs. In view of these new findings we conclude that: 1) H_1-receptor antihistamines seem to produce cerebrovasospasm by a direct action on cerebral vascular smooth muscle cells; and 2) the potent vasoconstriction of cerebral arterioles and arteries produced by H_1-histamine receptor blockers might produce cerebral hypoxia which in turn could produce sedative and/or soporific qualities. (Supported by Research Grant HL-29600 from the USPHS.)

CARDIOVASCULAR REGULATION: CENTRAL TRANSMITTERS I

160.1 IDENTIFICATION OF SUBSTANCE P AND CHOLECYSTOKININ IN RENAL AFFERENT PATHWAYS IN THE CAT. J.J.Oravitz*, D.C.Kuo, M.G.Backes* and W.C.deGroat, (SPON: O. Reinmuth) Department of Pharmacology, Medical School, University of Pittsburgh, Pittsburgh, PA 15261

Recent horseradish peroxidase tracing experiments in the cat identified the segmental distribution and central projections of afferent neurons innervating the kidney. In the present experiments we have used fluorescent dye tracing combined with immunohistochemistry to study the distribution of neuropeptides in the renal afferent pathways.

The central end of the transected left renal nerve in anesthetized cats was exposed for one hour to true blue, a fluorescent dye. Following a transport time of 1-2 weeks colchicine was applied locally or injected into the ipsilateral upper lumbar dorsal root ganglia. After 24-48 hours the animals were perfused with saline and 4% paraformaldehyde fixative. The thoracolumbar dorsal root ganglia and sympathetic chain ganglia along with the coeliac-superior mesenteric ganglion complex were removed, sectioned in a cryostat, examined for dye labelled cells and then processed by indirect immunofluorescence methods for substance P, cholecystokinin, somatostatin, and vasoactive intestinal polypeptide (VIP).

The segmental distribution of dye-labelled renal afferent and sympathetic postganglionic neurons matched the distribution of these neurons identified in previous experiments by HRP-tracing techniques. Labelled cells were present in ipsilateral sympathetic chain ganglia and dorsal root ganglia from T_{13} to L_4 and in the superior mesenteric ganglion. Dye labelled dorsal root ganglion cells exhibited substance P and cholecystokinin immunoreactivity, but did not contain somatostatin or VIP. Substance P, which in comparison to cholecystokinin was present in a larger number of the lower thoracic and lumbar dorsal root ganglion cells, was also identified in a larger proportion (approximately 30%) of dye labelled renal afferent neurons.

In summary, the present results suggest that both substance P and cholecystokinin may be neurotransmitters in afferent pathways from the kidney. Supported by the Western Pennsylvania Affiliate of the American Heart Association.

160.2 ROLE OF SPINAL OPIATE RECEPTORS ON THE AFFERENT COMPONENT OF THE SOMATOAUTONOMIC REFLEXES IN THE EXERCISING DOG. G. Pomeroy*, J. F. Ardell* and R. D. Wurster (SPON: T. Khan). Department of Physiology, Loyola University Medical Center, Maywood, IL 60153.

Muscle afferents mediate, in part, the blood pressure and heart rate changes associated with exercise. Spinal cord opiate receptors have been located in the dorsal horn of the spinal cord and have been implicated in modulating ascending afferent activity. We hypothesize that these opiate receptors modulate muscle afferent activity mediating the cardiovascular responses to muscle exercise.

To test this hypothesis, dogs were trained to run on a treadmill on their hind legs for five minutes each at 2, 4, and 6 Km/h (10% grade), successively. Simultaneously, heart rate and blood pressure responses to non-ischemic and ischemic exercise following lumbosacral intrathecal (IT) administration of either saline, morphine, or naloxone plus morphine were recorded. Under pentobarbital anesthesia to chronically instrument the dogs, skin tubes were made around the carotid arteries. This allowed for the recording of carotid arterial blood pressure by pericutaneous insertion of a needle connected to a pressure transducer and oscillograph. To produce unilateral muscle ischemia, a pneumatic occluder was placed around the iliac artery. The occlusion response was tested for 90 s at each exercise level. A silicon rubber catheter was placed in the lumbosacral IT space via a partial laminectomy at the T12 vertebral level.

Several days after recovery from instrumentation, IT administration of 0.5 ml of saline or the drugs dissolved in saline were made with at least three days between testing intervals. Exercise was initiated 10 minutes after IT injection. Blood pressure usually increased with the increment of treadmill speed. Blood pressure increased further with the 90 s occlusion and returned to preocclusion levels before the treadmill speed was incremented. In a dose-dependent manner, morphine (0.1 to 1 mg, IT) lowered the non-occlusion blood pressure as well as the increment due to muscle ischemia when compared to control IT saline injection responses. Naloxone (0.4 mg, IT) administration increased the blood pressure before and during ischemic exercise. The decreased responses following morphine (1 mg, IT) were blocked by prior administration of naloxone (0.4 mg, IT). The heart rate responses were much more variable although they, in general, showed the same trends.

Summary: Spinal cord opiate receptors modulate ascending afferent activity involved in cardiovascular responses to exercise suggesting a possible enkephalinergic modulation of these autonomic reflexes. (Supported by NIH Grant HL27612)

160.3 RESPONSE OF CENTRAL SYMPATHETIC NEURONS TO SUBSTANCE P AND ELEDOISIN RELATED PEPTIDE. Leslie P. Felpel and Ronald D. Huffman, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX 78284

The intermediolateral (IML) cell column receives input from numerous brainstem areas and contains a number of putative neurotransmitters, including monoamines and substance P (SP). Descending SP projections from the ventral medulla to the IML have been demonstrated in the rat (Helke et al., 1982). Because the ventral medulla may play a role in the regulation of sympathetic control of the vasculature, and SP has depolarizing action on many central neurons, we have investigated the effect of SP and eledoisin related peptide (ERP), a substance P analog, on central sympathetic neurons in the thoracic spinal cord. Adult female cats were anesthetized with an i.p. injection of a solution of alpha chloralose (40 mg/kg) and urethane (800 mg/kg) and all surgical procedures performed. Single IML units were recorded extracellularly at the seventh or eighth thoracic (T7-T8) level of the spinal cord with multiple barrel micropipettes and were identified by their orthodromic response to splanchnic nerve stimulation. Drug containing barrels were filled with solutions of monoamines as well as SP (1 mM in 0.1M NaCl) and ERP (1-5 mM in 0.1M NaCl). Localization of units was histologically verified by placing a dye mark at the recording site through the recording barrel (2M NaCl and Fast Green).

Of the twelve spontaneous or d,l-homocysteic acid-evoked IML neurons, SP and ERP excited three (25%), inhibited five (42%) and had no effect on the remaining four (33%) neurons. Excitation by both SP and ERP was slow in onset (11-74 seconds), and the duration (and sometimes peak) of the excitatory effect usually extended beyond the termination of the iontophoretic application of either drug. Such an effect is in agreement with neuropharmacological studies on other spinal (Henry et al., 1975) and central neurons (Krnjevic and Morris, 1974; Phillis and Limacher, 1974). Relatively high doses (80 nA or greater) of SP and ERP were generally required to evoke either excitation or inhibition, and the two substances were approximately equipotent. While our data support a possible role of SP in the regulation of blood pressure, they would suggest that SP acts more in the capacity of a modulator than as a rapid-acting and direct neurotransmitter at lower thoracic central sympathetic neurons. (Supported by the Biomedical Research Grant Program, Division of Research Resources, NIH #3-P01-NS 14546-0551.)

160.4 CATECHOLAMINES AND NEUROPEPTIDES IN THE VAGAL MOTOR NUCLEI: EVIDENCE FOR TOPOGRAPHICALLY ORGANIZED SUBPOPULATIONS OF CHEMICALLY SPECIFIED PREGANGLIONIC NEURONS. P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

A combined retrograde transport-immunofluorescence method was used to identify neuroactive substances that may be contained within vagal preganglionic neurons. Fifty-100 nl of a 5% suspension of a retrograde tracer, True blue, was injected beneath the sheath of the cervical vagi, bilaterally, in adult male rats. After 3-5 days the animals were sacrificed, and series of sections through the medulla were counterstained using antisera against the catecholamine-synthesizing enzymes tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) or any one of a number of neuropeptides. To enhance the staining of peptidergic perikarya, some animals were pretreated at 1, 2, or 3 days prior to sacrifice with 50 µg colchicine (icv).

In non-pretreated rats, small to moderate numbers of retrogradely-labeled neurons at the rostral and caudal poles of the dorsal motor nucleus (DMX) were found reliably to stain positively for the presence of TH or DBH, suggesting the existence of norepinephrine- (or epinephrine-) containing vagal motor neurons at these loci. Near the midextent of the DMX a prominent cluster of cells could be doubly labeled with anti-TH, but not with anti-DBH, indicating that dopaminergic preganglionic neurons occupy this region. In animals pretreated for 2-3 days with colchicine small numbers of True blue labeled neurons in the DMX could also be shown to contain any one of several neuropeptides including neurotensin, leu-enkephalin, somatostatin, and substance P; each such subpopulation displayed a distinctive topography. Retrogradely labeled neurons in and around the nucleus ambiguus (NA) could be doubly labeled (in pretreated rats) only with antisera against neurotensin or calcitonin gene related peptide (see Nature, 298:240, 1982). In each case, however, large subpopulations of neurons in the rostral half of NA were involved. Because of questions pertaining to the validity of localizations seen in colchicine pretreated animals, a unilateral ligation of the cervical vagus was performed in 6 otherwise normal animals, which were then perfused after 1, 3, or 5 days. At 5 days post-ligation, cells in the ipsilateral vagal motor nuclei could be stained for each of the neuropeptides (except substance P) mentioned above, and displayed a distribution similar to that seen after colchicine treatment.

These results indicate that vagal motor neurons contain neuroactive substances other than, or in addition to, acetylcholine, and suggest that specificity of function in these cell groups may be chemically, as well as topographically, encoded.

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160.5 AN IN VITRO INVESTIGATION OF SYNAPTIC TRANSMISSION IN THE MEDIAL NUCLEUS OF THE SOLITARY TRACT. D.L. Kunze and R. Miles* Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550.

On entering the medulla baroreceptor afferent fibres travel in the solitary tract and terminate to a large extent on neurones located medial to the tract. It has been suggested that significant neuronal processing including the depression of reflex effects at high stimulation frequencies (Seller and Illert, Pflug. Arch., 306: 1, 1969) may occur here. We have developed two in vitro techniques to investigate synaptic transmission in this area.

Longitudinal guinea pig medullary slices of thickness 400 µm were prepared so that the solitary tract could be stimulated while intracellular recordings were made from neurones medial to it at levels close to the obex. Cells were typically spontaneously active with overshooting action potentials followed by a prominent after hyperpolarization. On stimulating the tract an epsp was evoked in all neurones tested. Often several epsp's with different latencies were apparent while in a few cases an ipsp followed the initial epsp. The amplitude of the synaptic potential was progressively depressed as the frequency of stimulation was increased above 5 Hz.

In order to examine pure postsynaptic responses to putative neurotransmitters single neurones from the area medial to the solitary tract were enzymatically dispersed using the technique of Numann et al (Soc. Neurosci., 8: 413, 1982). The cells obtained after 2 hrs. exposure to 5% papain in physiological saline and mechanical dispersion possessed a morphology similar to that of Golgi stained neurones from this area. Recordings were made with 0.150 M KCl filled electrodes of resistance 2-10 MΩ using the suction pipette technique. In voltage clamp we tested neuronal responses to pressure ejection of 10^{-4} M L-glutamate which it has been suggested may be the transmitter released by afferent fibres. A conductance increase associated with a current which was inward at negative potentials and which reversed at approximately 0 mV was most commonly observed. In a proportion of cells this was followed by an outward current also associated with a conductance increase. Investigations are currently underway to determine whether postsynaptic factors such as these or presynaptic mechanisms underly high frequency depression at this synapse. (Supported by American Heart Association - Texas Affiliate and NSF PCM7823216).

160.6 INVOLVEMENT OF CENTRAL α-PRESSOR AND β-DEPRESSOR ADRENOCEPTORS IN THE CARDIOVASCULAR RESPONSES TO INTRACEREBROVENTRICULAR ADMINISTRATION OF CATECHOLAMINES IN THE RAT. F.M.A. Corrêa and V.L. Peres-Polon*. Dept. Pharmacology, Sch. of Medicine and Dept. Physiology, Sch. of Pharmacy and Dentistry of Ribeirão Preto, Univ. São Paulo. 14100, SP, Brazil.

The effect of the intracerebroventricular administration of catecholamines on the arterial blood pressure of anesthetized and awaken rats and its modification by pharmacological antagonists were studied.

One hundred seven animals were used in acute experiments. The rat were anaesthetized with urethane and intraventricular guide cannulas were implanted according to Corrêa, F.M.A. and Graeff, F.G. (Neuropharmac. 13: 65-74, 1974). Arterial blood pressure was recorded by means of a PE-50 polyethylene catheter introduced into the right carotid. A group of fifty three rats was prepared for chronic blood pressure recording under unanesthetized conditions. Intraventricular cannulas were implanted under sodium pentobarbital anaesthesia. Three days later the animals were anaesthetized with ether and a chronic polyethylene cannula implanted into the femoral artery, according to Krieger, E.M. (Circ. Res. 15: 511-521, 1964). Blood pressure recording was undertaken six days after the initial surgery.

Intracerebroventricular injection of isoprenaline (1-20 µg) and normetanephrine (20-100 µg) produced, respectively, depressor and pressor responses in both anesthetized and unanesthetized animals. Epinephrine (1-20 µg) produced predominantly depressor effects of greater intensity in anesthetized than in awaken animals, while norepinephrine (1-20 µg) caused depressor responses in anaesthetized animals and a predominant pressor response in the awaken rat. Intracerebroventricular pretreatment with propranolol (40-200 µg) markedly potentiated pressor responses to norepinephrine and epinephrine and only partially blocked the depressor response to isoprenaline. α-blocking agents such as phenoxybenzamine and phentolamine potentiated depressor responses to epinephrine and isoprenaline and reduced pressor responses to norepinephrine. The involvement of central α-pressor and β-depressor adrenoceptors, in the blood pressure responses to the intracerebroventricular administration of catecholamines, is proposed. Otherwise is also evidenced the involvement of a propranolol-insensitive mechanism in the depressor response to the intracerebroventricular injection of isoprenaline in the rat.

(Supported by grants from FAPESP, CAPES, FINEP)

160.7 CENTRAL NERVOUS SYSTEM ACTIONS OF BOMBESIN ON CARDIOVASCULAR REGULATION IN THE RAT. Laurel A. Fisher and Marvin R. Brown. Peptide Biology Laboratory. The Salk Institute, La Jolla, CA 92037.

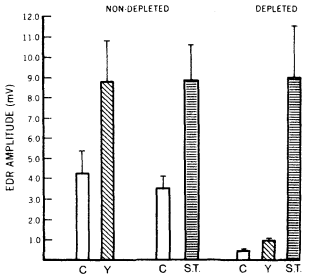
The tetradecapeptide bombesin (BOM) acts within the central nervous system (CNS) to elevate plasma levels of catecholamines, glucagon and glucose and to inhibit regulatory heat production. The hyperglycemic effects of BOM are secondary to stimulation of adrenal epinephrine secretion, whereas the thermoregulatory effects of BOM are independent of its activation of adrenomedullary sympathetic outflow. The following studies indicate that BOM also acts in the CNS to modulate vagal outflow. The CNS actions of BOM to stimulate adrenomedullary sympathetic outflow and vagal activity underlie separate effects on blood pressure and heart rate.

All experiments utilized conscious, freely-moving rats instrumented with lateral cerebroventricular cannulae, and jugular venous and femoral arterial catheters. Intracerebroventricular (icv) administration of BOM (17.1 pmoles-5.7 nmoles) produced dose-related elevations of mean arterial pressure (MAP). Maximal increases in MAP averaged 15 mm Hg and the duration of the hypertensive effect increased with dose of BOM. Heart rate (HR) decreased dramatically (50-130 beats/min) following icv injection of BOM. Reductions of HR occurred by 5-20 min post injection and remained significant for at least two hours post injection. BOM-induced bradycardia was not secondary to increased MAP as acute adrenalectomy blocked BOM-induced elevations of MAP but failed to prevent BOM-induced decreases of HR. The cardiovascular effects of icv BOM administration were not altered by intravenous (iv) injections of [1-deaminopenicillamine, 2-(O-methyl)tyrosine]-AVP (50 µg), a selective antagonist of the pressor actions of vasopressin. Methyl atropine (1 mg/kg, iv; injected 5 min prior to and 30 min following BOM) antagonized BOM-induced bradycardia but did not modify BOM-induced increases in MAP. The cardiovascular effects of BOM were not due to leakage of the peptide into the peripheral circulation as iv administration of a wide range of doses of BOM (570 pmoles - 57 nmoles) elicited transient pressor effects (lasting 1-5 min) and slight tachycardia.

Taken together, these results indicate that BOM acts in the CNS to stimulate vagal outflow to heart resulting in bradycardia, whereas the effects of BOM on MAP appear to be secondary to adrenal epinephrine secretion. Recent experiments demonstrate that icv administration of BOM suppresses cold-induced tachycardia, suggesting that BOM may inhibit regulatory heat production by its effects on cardiac output.

160.8 EVIDENCE FOR TWO DISTINCT SYMPATHOINHIBITORY BULBO-SPINAL SYSTEMS. P.J. Bernthal* and M.C. Koss. Dept. of Pharmacology, Univ. of Oklahoma, College of Medicine, Okla. City, Okla. 73190.

Yohimbine hydrochloride (0.5 mg/kg, i.v.) caused a long lasting potentiation of electrodermal (sympathetic-cholinergic) reflexes in intact anesthetized and decerebrate non-anesthetized cats. Transection of the cervical spinal cord also resulted in increased sudomotor reflex amplitude in non-anesthetized decerebrate preparations. Depletion of CNS monoamines by pretreatment with reserpine (5 mg/kg, i.p.) and α-methyl-p-tyrosine (2 x 300 mg/kg, i.p.) reduced the concentrations of norepinephrine, dopamine and serotonin to less than 93% of control levels in the thoracic spinal cord. In monoamine depleted preparations, yohimbine no longer facilitated reflex amplitude whereas the effect of spinal transection was not altered. These results suggest that there are two distinct sympathoinhibitory systems in the lower brain stem that converge on spinal sympathetic neurons, one of which is monoaminergic and one of which is not. Evidence for the baroreceptor independent nature of these descending inhibitory systems will also be presented.



The figure illustrates the effect of yohimbine (Y) hydrochloride (0.5 mg/kg, i.v.) and/or cervical spinal cord transection (S.T.) on electrodermal reflex amplitude in depleted or non-depleted non-anesthetized decerebrate cats. The first two groups demonstrate the mean increase (+ S.E.M.) in EDR amplitude following yohimbine administration (N=5) or cervical cord transection (N=13) in non-depleted cats. The third group shows the lack of effect of yohimbine (N=5) on the EDR following catecholamine depletion and the facilitation that occurs in the same preparations subjected to subsequent cervical cord transection. Measurements are of the maximal effect observed in the first 5-10 min after the procedure. (Supported by USPHS grants MH-25792, NS-14039 and a grant from the Oklahoma Affiliate of the American Heart Association)

160.9 ELECTROPHYSIOLOGICAL PROPERTIES OF SPINALLY-PROJECTING A5 NORADRENERGIC (NE) NEURONS IN THE RAT. Christopher E. Byrum* and Patrice G. Guyenet. University of Virginia, School of Medicine, Department of Pharmacology, Charlottesville, Virginia 22908.

A5 neurons projecting to the thoracic spinal cord (SC) were mapped using the sequential Faglu-HRP technique of Furness et al. (Histochem. J. 9, 745). An "A5 area" was arbitrarily defined in coronal sections as a 760 x 420 µm area of ventrolateral pons centered on the densest accumulation of NE cells. This area contained 74% of all NE cells commonly included in the A5 group (total of 870 ± 111 cells/rat). Large HRP injections in SC resulted in the labelling of up to 93% of NE-fluorescent cells in the "A5 area"; these represented 80% of all HRP + cells (N=4). Prior icv injections of 6-OHDA (2 x 200 µg, N=3) resulted in a 47% reduction in the number of A5 NE neurons and in the total loss of NE + HRP + cells; by contrast the number of NE - HRP + cells in the "A5 area" remained virtually unchanged.

Single unit recordings were made in the "A5-area" in urethane-anesthetized, paralyzed and respirated rats. The study was restricted to cells antidromically (AD) activated from thoracic SC. The vast majority of the cells encountered in the A5 area (N=45) apparently belonged to the same type (conduction velocity ca. 2-3 m/sec; slow regular discharge rate correlated neither with cardiac nor respiratory rates; total inhibition by i.v. clonidine, partial inhibition by i.v. desmethylimipramine, activation by i.v. α2 antagonists). These neurons (N=18) were inhibited by increases in mean blood pressure (mBP) beyond 110-120 mm Hg and generally silenced by mBP increases into the 150-180 mm Hg range. Identical results were obtained with NE, Angiotensin II, or Arg-Vasopressin as pressor agents. The cells were unaffected by lowering mBP 25-45 mm Hg below normal resting levels (typically 60-100) with a vasodilator (SCN^-) or a ganglionic blocker (trimethaphan).

The second type of AD-activated cell found in the "A5 area" consisted of a few (N=4) fast conducting units (10-15 m/sec) with a regular high discharge rate which was unaffected by either clonidine or mBP elevation up to 180 mm Hg. Finally the "A5 area" contained numerous other types of units not AD-activated from SC and therefore not characterized in this study.

In agreement with a previous study by Andrade and Aghajanian, (Brain Res. 202, 125) it is concluded that the slow-firing, clonidine-sensitive units most commonly found in the "A5 area" represent somatic or dendritic action potentials generated by the NE neurons. These cells are subject to feedback inhibition by BP which suggest that they may normally contribute to centrally mediated pressor responses. HL 28785

160.10 EFFECT OF BLOOD PRESSURE AND CLONIDINE ON THE ACTIVITY OF A2 NORADRENERGIC NEURONS. Scott D. Moore* & Patrice G. Guyenet (SPON: J.A. Wilson) Dept. of Pharmacology, Univ. of Virginia School of Medicine, Charlottesville, VA 22908

We have previously established that the hypotensive agent clonidine administered systemically to rats in doses of 5 to 40 µg/kg inhibits the discharges of A2 noradrenergic neurons. The present series of experiments were undertaken to investigate the hypothesis that clonidine inhibits A2 neurons by a direct action on the NTS. In addition the effect of pharmacological manipulations of blood pressure (BP) on the firing of A2 neurons was determined.

Single unit recordings were obtained in chloral-hydrate anesthetized rats. A2 neurons located in nuc. commissuralis were identified by antidromic activation from the median forebrain bundle as previously described (Moore & Guyenet, Brain Res., 263: 211). Iontophoretic electrodes were used for the application of neurotransmitters and drugs. All the A2 neurons sampled (n=26) were inhibited by epinephrine (EPI) or by the α2 agonist clonidine (CLO). This inhibition was antagonized by the α2 antagonist piperoxane (PIP) (n=12). PIP alone occasionally excited the neurons and in one case activated a silent cell. The β antagonist sotalol had no effect by itself and did not block the inhibition produced by EPI (n=4). In addition, A2 neurons were inhibited and excited by GABA and glutamate, respectively, but were unresponsive to serotonin.

Increases in BP were elicited by intravenous administration of norepinephrine (10-25 µg/kg) or Arg-vasopressin (2-6 µg/kg). Increases in mean BP to between 120 and 150 mm Hg decreased spontaneous activity in 7 out of 9 A2 neurons. Increases in mean BP to between 150 and 180 mm Hg decreased activity in 5 out of 5 neurons and silenced 3 cells. By contrast, a control sample of six unidentified units (3 in nuc. gracilis and 3 in nuc. commissuralis) were unaffected by elevations in mean BP in the 120-150 mm Hg range.

These experiments suggest that A2 neurons receive an inhibitory input from peripheral baroreceptors. Since these cells directly project onto the hypothalamic paraventricular nucleus, the present data suggest that BP feeds back on vasopressin-releasing cells via A2 neurons and that by inhibiting A2 cells clonidine may enhance the gain of this feedback. Supported by NIH grant HL-28785.

160.11 A GABAERGIC MECHANISM AT THE VENTRAL MEDULLA: DIFFUSION STUDIES USING ^{3}H-BICUCULLINE METHYLCHLORIDE. K.A. Yamada & A.D. Loewy. Dept. of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We have previously reported blockade of the baroreceptor reflex, in cats, by topical application of bicuculline on the ventral medullary surface (Fed. Proc. 42:1121, 1983). This effect was specific for GABAergic antagonist drugs, and was dose-dependent and reversible at lower doses. It is not known, however, exactly what anatomical substrate mediates this and other evoked responses elicited by topical drug application. Electron micrographic studies of the ventral surface (Dermietzel, R. In: H.H. Loeschcke (ed.), Acid-Base Homeostasis of the Brain Extracellular Fluid and the Respiratory Control System, George Thieme, Stuttgart, 1976, pp. 52-65) have revealed an extensive pore and vascular system by which drugs could easily diffuse dorsally to a site of action deeper than the suggested superficial site at the ventral surface. In order to determine what possible structures had been reached by bicuculline to cause blockade of the baroreceptor reflex, we followed the diffusion of ^{3}H-bicuculline methylchloride (BMC) in chloralose-anesthetized cats. 120-940 nCi of ^{3}H-BMC was topically applied to the intermediate area of the ventral surface via cottonoid pledgets. After 6.5-11 min of drug exposure, the pledgets were removed and the brainstem was blotted, dissected out, and frozen in freon on dry ice. 20 µm horizontal sections were cut from each of three 7-mm thick brainstem pieces in a cryostat at -20°C, weighed, solubilized, and scintillation counted. 4-6% of the radiolabel applied was recovered in the tissue sections. The results revealed a gradient of ^{3}H-BMC diminishing dorsally from the highest concentration (HC=2.15-2.33 nCi/mg tissue) found at the ventral surface, to 50% of HC 0.5-0.9 mm below the surface, to 10% of HC 1.8-2.5 mm below the surface, to no detectable ^{3}H-BMC 3.5-3.7 mm below the surface. In other experiments, 20 µm coronal sections were cut at -20°C, dried, fixed, and exposed to ^{3}H-sensitive LKB film. Preliminary results indicate that ^{3}H-BMC reached a depth of approximately 2 mm from the surface. However, it is still not known whether the concentrations reaching these deeper structures could account for the effects seen after topical drug application. In summary, although ventral surface application of various drugs elicits profound cardiovascular and respiratory responses, an anatomical substrate for these effects has never been demonstrated. It is therefore important to consider the possibility of drug diffusing to other ventral medullary structures (e.g., the A1 and C1 cell groups, the inferior olivary nucleus, etc.) in effecting their responses. (Supported by USPHS grants HL-25549 and HL-07275.)

160.12 ENDOGENOUS GABAERGIC MECHANISMS IN THE VENTROLATERAL MEDULLA AND THE REGULATION OF BLOOD PRESSURE. R.N. Willette, P.P. Barcas*, A.J. Krieger* and H.N. Sapru. Department of Neurological Surgery and Department of Pharmacology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

The ventrolateral medulla contains a caudal group of vasodepressor neurons (VLDA) and a rostral group of vasopressor neurons (VLPA). GABA receptor stimulation in the VLDA (A1 area) and VLPA (C1 area) cause neurogenic hypertension and hypotension, respectively. In the present study, the GABA receptor antagonist, bicuculline methiodide, was used to determine if GABAergic mechanisms were tonically active in both sites.

In urethane anesthetized rats vasodepressor and vasopressor neuron pools were functionally identified by microinjecting (100 nl) of the neuroexcitatory amino acid, L-glutamate (300ng/site). The bilateral microinjection of bicuculline into the VLDA caused a dose-related (0.1-100.0ng/site) decrease in blood pressure, pulse pressure, and heart rate. Maximum decreases in blood pressure and heart rate were - 51±2 mmHg and - 245±9bpm, respectively. Pretreatment with the alpha adrenergic receptor antagonist, phentolamine (3mg/kg, i.v.), completely blocked the bicuculline induced hypertension; the tachycardia persisted. Bicuculline microinjections in the VLPA also caused a 61% reduction in the fall in blood pressure evoked by stimulation of the aortic depressor nerve (2v,25Hz,3msec) and a 40% increase in the blood pressure response elicited by bilateral carotid occlusion. These results suggest that GABAergic mechanisms in both the caudal and rostral ventrolateral medulla are tonically involved with the maintenance and reflex regulation of blood pressure. This medullary GABAergic system may provide a reciprocal inhibition between the rostral vasopressor and caudal vasodepressor areas.

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160.14 EFFECTS OF L-TRYPTOPHAN ON BLOOD PRESSURE: ROLE OF SEROTONIN. W.A. Wolf* and Donald M. Kuhn* (SPON: M. Billingsley). Sect. Biochem. Pharmacol., NHLBI, NIH, Bethesda, MD 20205.

Studies were performed to elucidate the role of serotonin (5-HT) in mediating the cardiovascular effects of systemically administered L-tryptophan, the amino acid precursor for 5-HT. Various doses of L-tryptophan were administered intraperitoneally in spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKY). Systolic blood pressure measurements were obtained at various time points in conscious rats using the indirect tail cuff method with photoelectric sensing of pressure pulses. In the SHR, low doses of L-tryptophan (25-50 mg/kg) raised blood pressure by 20 mm Hg while the highest dose tested, 100 mg/kg, significantly lowered blood pressure. In the WKY, only hypertensive effects were seen at all doses (25-100 mg/kg). Concomitant analysis of tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels by HPLC in specific brain regions implicated in cardiovascular regulation (hypothalamus, dorsal raphe, nucleus tractus solitarius, medulla) suggested that the hypertensive effect, seen most prominently in the WKY, is mediated by increases in brain 5-HT while the hypotensive effect seen in the SHR is unrelated to changes in brain 5-HT levels. Furthermore, the cardiovascular and neurochemical effects of an equimolar dose of the dextrorotary isomer of tryptophan were compared to L-tryptophan in the SHR at a dose and time when the hypotensive effect of L-tryptophan is maximal. While D-tryptophan elevates brain 5-HT and 5-HIAA levels to the same extent as L-tryptophan, no hypotensive action was observed after 100 mg/kg of D-tryptophan. The significance of these results with respect to brain 5-HT and blood pressure regulation and possible alternative mechanisms for the antihypertensive action of L-tryptophan will be discussed.

160.15 ATTENUATION OF SALT-INDUCED HYPERTENSION IN THE DAHL RAT MODEL OF HUMAN ESSENTIAL HYPERTENSION BY CHRONIC CHOLINERGIC BLOCKADE. James A. McCaughran, Jr. and Richard Friedman *, Department of Psychiatry and Behavioral Science, SUNY @ Stony Brook, Stony Brook, NY 11794.

Recent studies from this laboratory suggest that enhanced central cholinergic neurotransmission in the Dahl salt-sensitive rat (DS rat) is an important contributing factor to the pathogenesis of hypertension. Therefore, the present experiment was done in order to determine the effect of chronic cholinergic blockade with atropine on either the development or maintenance of salt-induced hypertension in DS rats.

To investigate the effect of cholinergic blockade on the development of salt-induced hypertension, DS and DR rats were maintained on a high salt diet (8.0% NaCl w/w) and treated with atropine sulfate for 4 weeks via indwelling miniosmotic pumps. To examine the influence that atropine might also exert on rats with established hypertension, DS and DR rats were first maintained on a high salt diet for 3 weeks. Atropine, at the same dose as above, was then chronically infused over a 4 week period.

DS rats treated with atropine displayed a mean systolic BP that was significantly lower than DS rats treated with the vehicle (156 vs 200 mmHg). BP in DS rats that received atropine also failed to increase over a 9 week period following the termination of the treatment. As a result, 8 of 10 drug-treated rats survived 13 weeks on the high salt diet whereas by week 6 of the diet all control rats had died. DS rats maintained on a high salt diet for 3 weeks prior to atropine treatment developed BPs of 160 mmHg. During the 4 weeks of atropine administration, BP in the experimental group rose to 185 mmHg while BP in the control group rose to 247 mmHg. By the sixth week on the diet, all control rats had died. In contrast, 8 of 10 experimental rats survived to the ninth week and 2 of 10 to the thirteenth week of the diet. BP in DR rats was not affected by the administration of atropine.

The results of the present study clearly suggest that the cholinergic system in the DS rat is in part responsible for the development and maintenance of salt-induced hypertension. Previous studies from this laboratory have found that DS rats have a significantly greater density of cholinergic receptor sites and higher choline acetyltransferase and acetylcholinesterase activity in the forebrain and brainstem. Furthermore, normotensive DS rats display a greater pressor response to physostigmine than normotensive DR rats. Therefore, the present effects on BP likely reflect interference with a central cholinergic mechanism.

160.16 GABA INHIBITION OF CENTRAL SODIUM-INDUCED AVP-DEPENDENT PRESSOR MECHANISMS. Timothy J. Brennan* and J.R. Haywood. Dept. of Pharmacology, UTHSCSA, San Antonio, TX 78284.

We have previously demonstrated that intraventricular (IVT) gamma-aminobutyric acid (GABA) inhibits the pressor effect of artificial cerobrospinal fluid made hypertonic by the addition of sodium (Na). The present studies were undertaken to investigate the mechanism of the hypertonic CSF pressor effect and determine its sensitivity to GABA.

In conscious rats, IVT administration of 0.64, 1.07, and 1.92 microequivalents (μEq) of Na increased mean arterial pressure (MAP) 8±1, 13±1, and 19±1 mmHg, respectively. IVT injection of artificial CSF (0.42 μEq of Na) did not change MAP. Autonomic blockade with chlorisondamine augmented the pressor effect of Na so that 0.64, 1.07, and 1.92 μEq of Na increased MAP 13±3, 24±3, and 42±7 mmHg, respectively. After ganglionic blockade, pretreatment with 100 μg of GABA reduced the pressor effect of hypertonic CSF to 7±2, 11±2, and 18±4 mmHg. In addition, following chlorisondamine pretreatment, the pressor effect of 1.92 μEq of Na was reduced from 41±5 to 8±1 mmHg by $d(CH_2)_5Tyr(Me)AVP$, a vascular antagonist of arginine-vasopressin (AVP).

In another series of experiments, rats were pretreated with $d(CH_2)_5Tyr(Me)AVP$. The pressor effect of 0.42, 1.07, and 1.92 μEq of Na was reduced approximately 50% of control to 2±1, 8±1, and 11±1 mmHg, respectively. Similarly, hypophysectomy reduced these hypertonic CSF pressor responses to 1±1, 3±1, and 8±1 mmHg.

We conclude that IVT hypertonic CSF increases arterial pressure largely through the pressor effect of AVP. Following elimination of the sympathetic nervous system with ganglionic blockade, the central hypertonic CSF pressor response is mediated by AVP. This AVP-dependent pressor response is sensitive to low doses of IVT GABA. (Supported by American Heart Association, Texas Affiliate, and NIH HL29993.)

160.17 THE EFFECT OF CENTRALLY ADMINISTERED ARGININE VASOPRESSIN ON BLOOD PRESSURE IN THE CONSCIOUS RABBIT. S.M. Martin, T.J. Malkinson*, W.L. Veale and Q.J. Pittman. Departments of Medical Physiology and Pharmacology and Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

The peptide, arginine vasopressin (AVP), is known to influence reuptake of water in the kidney and peripheral vasomotor tone. Recent studies using rabbits and rats have indicated that AVP may also act within the central nervous system to influence blood pressure (Martin et al., Can. J. Physiol. Pharmacol., in press, 1983; Pittman et al., Endocrinology, 110: 1058, 1982). The present experiments provide further evidence that the increase in blood pressure evoked by intracerebroventricular (icv) administration of AVP is mediated centrally and not due to a peripheral action of AVP following diffusion across the blood brain barrier.

Male, New Zealand White rabbits were implanted with headplates, permitting access to a lateral cerebral ventricle, and a chronic intracarotid catheter. Following 5 days recovery from surgery, arterial pressure was recorded in conscious, lightly restrained rabbits given intracerebroventricular injections of AVP (10-1000 $ng \cdot 100\ \mu l^{-1}$). Within 1 min, blood pressure was noted to rise in a dose-dependent fashion to reach a peak approximately 1.6 min following the injection. Similar injections of AVP, given iv, caused a blood pressure increase only after the infusion of the highest dose. A further group of eight rabbits was given an iv infusion of chlorisondamine HCl (5-10 $mg \cdot kg^{-1}$) after which AVP (1000 $ng \cdot 100\ \mu l^{-1}$) was given icv. In these animals, whereas injection of AVP without the blocking agent initiated a rise in blood pressure of 17.5 ± 2.50 mm Hg, the response following ganglionic blockade was abolished in 7 animals. Four of these animals were given an icv injection of AVP, 3 h later. In three animals there was still no change in blood pressure but in one rabbit, an attenuated response was seen.

These results indicate that the short latency increase in blood pressure evoked by central administration of AVP is neurally mediated and not due to an effect on the vasculature after leakage of the peptide out of the brain. (chlorisondamine HCl provided by Ciba-Geigy).

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160.18 **FREQUENCY-DEPENDENT CARDIOVASCULAR CHANGES ELICITED FROM THE AMYGDALA.** L. K. Clarke*, R. A. Galosy, and C. D. Barnes. Texas Tech Univ. Health Sciences Center, Sch. of Med., Lubbock, Texas.

Alterations of heart rate and systolic and diastolic blood pressure in response to basolateral amygdala stimulation were studied in 25 acutely prepared, chloralose anesthetized cats. The blood pressure wave was recorded from the common carotid artery on a Narco Systems physiograph and digitized and stored on an Apple 2e computer. Bipolar electrode cannulae (Plastic Products) were stereotaxically placed in the basolateral amygdala and in the ventromedial hypothalamus and the amygdala was electrically stimulated for 15 seconds at 300-500 μA and at frequencies of 10 Hz, 30 Hz, 50 Hz, and 100 Hz. Blood pressure and heart rate were computed on a beat-to-beat basis for 15 seconds prior to each amygdaloid stimulation and for the 45 seconds during and following the stimulus period.

The changes in cardiovascular parameters from pre to post stimulus were analyzed using an analysis of variance and subsequent post hoc t-tests. The results demonstrate a pronounced frequency effect ($F_{3,810} = 24.8$ $P < .001$) for systolic and diastolic pressure. Depressor responses predominated at all frequencies. Pre to post stimulus changes in pressure at 10 Hz were significantly greater (-9.2 mmHg) than changes at all other frequencies. Pressure changes at 30 Hz and 50 Hz were not statistically different (-6.2 mmHg, -6.3 mmHg) and pre to post stimulus changes in pressure at 100 Hz were significantly smaller (-2.4 mmHg) than those at all other frequencies. Stimulation at 100 Hz in some animals resulted in slight pressor responses. Heart rate changes were not significant at any frequency. These results indicate the stimulation frequency effects may be responsible for the varied cardiovascular effects elicited from the amygdala in previous studies. Additional evidence suggests the involvement of certain putative neurotransmitters, specifically GABA, in the modification of these frequency dependent cardiovascular changes from the amygdala. This research was supported by NIH grant HL07474.

161.3 ELECTROLYTIC LESIONS OF THE SUBSTANTIA INNOMINATA ABOLISH THE LOCOMOTOR RESPONSE TO APOMORPHINE RESULTING FROM DENERVATION OF THE NUCLEUS ACCUMBENS. N.R. Swerdlow[1]*, L.W. Swanson[2], and G.F. Koob[2]. [1]School of Med., Univ. of Calif. San Diego, La Jolla, CA 92093 and the [2]Salk Institute, San Diego, CA 92138.

Apomorpine-stimulated locomotion in the rat is greatly enhanced following destruction of dopamine terminals in the nucleus accumbens (N.Acc.) with 6-hydroxydopamine(6OHDA). While this augmented response is ascribed to the action of the dopamine stimulant apomorphine on supersensitive receptors within the N.Acc., little is known regarding the mechanism by which increased receptor stimulation within the N.Acc. influences lower motor circuitry to effect changes in locomotion. Efferent projections of the N.Acc. have been described, however, and in this study we examined locomotor responses in 6OHDA-infused rats following destruction of the terminal region of first-order N.Acc. efferent fibers within the substantia innominata (SI).

Animals (n=40) were divided into two g oups that received stereotaxic injections of either 6OHDA or vehicle into the N.Acc. One week later, these groups were each divided into two subgroups with similar mean locomotor response to 0.1 mg/kg apomorphine. Within each group, one subgroup received electrolytic lesions of the SI and adjacent parts of the lateral preoptic area and the other received sham lesions. The following week, we examined locomotor activity after injection of 0.05 and 0.1 mg/kg apomorphine in these animals. Lesions of the SI region greatly diminished the locomotor response to apomorphine in 6OHDA-infused animals, but did not alter locomotion in vehicle-infused animals.

Destruction of dopamine terminals within the N.Acc. has also been reported to enhance the place-preference response to apomorphine in rats. In order to test the effects of lesions of the SI on this augmented response, animals from the above groups were trained on four consecutive days to associate apomorphine and saline with two distinct environments, as described previously. One day later, they were tested for their place-preference for these environments during a 10 min. test. Lesions of the SI significantly decreased the place-preference for apomorphine-paired environments in 6OHDA-infused animals, but did not alter place-preference responses in vehicle-infused animals.

Our results indicate that the efferent pathway from the N.Acc. to the SI serves as an important output of mesolimbic activity into motor circuitry involved in the expression of apomorphine-stimulated locomotion and place-preference.

161.4 BEHAVIORAL RELATIONSHIPS OF NIGROSTRIATAL UNIT ACTIVITY IN FREELY MOVING CATS. Robert E. Strecker, George F. Steinfels and Barry L. Jacobs. Dept. Psychol., Princeton Univ., Princeton, NJ 08544.

We recently reported on the behavioral relationships of dopaminergic (DA) neuron unit activity in the substantia nigra of awake, freely moving cats (Brain Res. 258:217, 1983). Although the discharge rate of these cells was 20% faster during active waking than during quiet waking (3.68 vs 3.07 spikes/sec), the rate and pattern of activity of these neurons did not vary significantly across quiet waking, slow-wave sleep, and REM sleep. The firing rate of these neurons also displayed no correlation with gross motor activity. They did respond, however, to the repeated presentation of a click or light flash with excitation followed by inhibition, with no evidence of habituation. The most impressive change in DA unit activity was the long suppression (1-10 seconds) of firing seen during orienting and the subsequent fixation which followed the presentation of a novel or meaningful stimulus. We concluded that these DA neurons appear to provide tonic background activity for the neostriatum which is lifted only under specific conditions such as orienting.

In order to evaluate the functional relationship between DA unit activity and the activity of neurons in the terminal regions of DA neurons, we are examining the activity of neurons in the caudate nucleus under the same conditions that we have characterized changes in DA unit activity. Single unit activity was recorded by means of low impedance movable microwires. Despite the large variation in baseline discharge rate across caudate neurons (range - 4 to 19 spikes/sec), as a group, their activity tends to vary in a similar manner across the sleep-waking cycle. They displayed their highest discharge rate during active waking which was approximately 50% greater than their discharge rate during quiet waking. While discharge rates decreased during both slow-wave sleep and REM sleep, to about 60% of quiet waking baseline rate, the variability between cells was much greater during REM sleep. During REM sleep the discharge rates of several cells increased somewhat, while other cells showed dramatic suppressions in firing rate relative to quiet waking baseline. Caudate units are known to exhibit a variety of responses to polysensory stimuli. Under our conditions, caudate neurons responded to light flash and auditory click, typically with an excitation followed by inhibition of activity. In response to stimuli producing orienting behavior, some caudate units appeared to exhibit a slight increase in rate, especially during the first few trials. This increase habituated with repeated stimulus presentation. Since changes in caudate unit activity are often associated with specific movements it is possible that the observed changes in firing rate may be related to the phasic movements rather than the changes in arousal or attention associated with orienting behavior.

161.5 MODIFICATION OF APOMORPHINE-INDUCED BEHAVIORAL EFFECTS BY DESTRUCTION OF LIMBIC DOPAMINE NEURONS, Robert J. Carey, V.A. Medical Center, Syracuse, NY 13210.

In rats, activation of postsynaptic dopamine receptors by high doses of apopmorphine can have two distinctive behavioral effects: locomotion or stereotypy. Some evidence suggests that locomotion reflects activation of limbic dopamine receptors whereas stereotypy is a manifestation of striatal dopamine receptor stimulation. In the present series of studies, limbic dopamine neurons were destroyed in rats pretreated with desmethylimipramine (25 mg/Kg) by injections of 6-hydroxydopamine (6 OHDA) (2 µl of 3 µg/µl) into either the ventral tegmental area or the nucleus Accumbens. The effects of 6-ODHA injections were compared with vehicle injections (1.0 mg/ml) ascorbic acid) and the effects on brain monoamines determined by HPLC with electrochemical detection. By destroying limbic dopamine neurons, limbic dopamine receptors would presumably be increased thereby increasing the likelyhood of locomotion rather than stereotypy behavior being elicited by apomorphine. In one set of experiments, rats given 0.5 mg/Kg apomorphine exhibited intense sniffing and stereotypy with a cessation of locomotion and a complete suppression of self-stimulation. Following destruction of limbic dopamine neurons, however, the same dose of apomorphine failed to elicit stereotypy and the animals exhibited typical non-drug levels of both locomotion and self-stimulation. In another experiment, unilateral destruction of nigral striatal dopamine neurons produced intense contralateral turning in response to apomorphine (0.25, 0.5 and 1.0 mg/Kg) and complete suppression of self-stimulation. Subsequent destruction of limbic dopamine neurons abolished turning at the high 1.0 mg/Kg apomorphine dose level and the rats exhibited locomotion as well as self-stimulation. Thus, these studies demonstrate that the destruction of limbic dopamine neurons can profoundly alter the behavioral responses induced by high doses of apomorphine and suggest that limbic and striatal dopamine systems may reciprocally interact such that one system at a time predominantly affects behavior.

161.6 SEROTONERGIC SUBSTRATES OF HYPERACTIVITY IN RAT. S.K. Sobrian and W.R. King*. Dept. of Pharmacology, Howard Univ. College of Medicine, Washington, D.C. 20059.

Altered metabolism of one or several monoamines in the CNS has been postulated as the possible etiology of the hyperactive child syndrome. Attempts to model the human hyperkinetic syndrome have centered around altering catecholamine metabolism by administering the neurotoxin 6-hydroxydopamine to neonatal rats and observing the development of activity in the dopamine-depleted preparation. This model has met with varying success in mimicing the cardinal features of hyperkinesis (e.g., overactivity and impaired learning); however, the paradoxical calming effect of stimulant drugs has been difficult to demonstrate. The present experiment was designed to determine if depletion of central serotonin (5-HT) would induce hyperactivity in the developing rat, and if this hyperactive could be attenuated by pharmacological or environmental manipulation.

On postnatal days 1-2 Sprague-Dawley rat pups received an intraventricular injection of 50 µg of 5,7-dihydroxytryptamine (5,7-DHT) either alone or in combination with 50mg/kg of pargyline, i.p.: GI: 5,7-DHT, and GII: P+5,7-DHT, respectively. Spontaneous motor activity (SMA) was monitored at 5 day intervals from 5-30 days of age; pups were tested either in isolation or in a familiar environment (home cage shavings), both before and after i.p. injections of d-amphetamine (1 mg/kg) or fenfluramine (2 mg/kg).

Depletion of central 5-HT alone (GII: P+5,7-DHT) did not induce hyperactivty in any of the test conditions. In contrast, pups in which both 5-HT and norepinephrine (NE) were reduced (GI: 5,7-DHT) were hypoactive. The presence of home cage shavings in the testing environment significantly attenuated the activity of only the P+5,7-DHT group. The ontogeny of SMA was examined by utilizing a curve fitting procedure to determine the age at which peak activity occurred. In general, depletion of 5-HT alone delayed the onset of peak activity; depletion of both 5-HT and NE accelerated the age at which maximum activity was observed.

The psychomotor stimulant d-amphetamine preferentially increased motor activity in the 5,7-DHT and P+5,7-DHT pups. In controls, drug-induced changes varied as a function of age. Fenfluramine decreased activity in all groups.

The results indicate that alterations in central serotonin do not induce hyperactivity in the developing rat; pups with reduced levels of this amine were hypoactive. However, changes in the characteristic ontogenetic pattern of SMA following treatment with 5,7-DHT indicate that serotonin is involved in the development of motor activity. (Supported by NSF Grant #80-26765).

161.7 UNIT ACTIVITY OF SEROTONERGIC NEURONS IN N. RAPHE MAGNUS IN FREELY MOVING CATS. C. Fornal, S. Auerbach, J. Heym and B. L. Jacobs. Prog. in Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

The activity of serotonin-containing neurons in the dorsal raphe nucleus (DRN), n. centralis superior (NCS) and n. raphe pallidus (NRP) has been examined across different behavioral states in freely moving cats (Brain Res. 163:135-150, 1979; Brain Res. 251:259-276, 1982; Soc. Neurosci. Abstr. 8:896, 1982). In general, these neurons display similar activity patterns across the sleep-wake cycle but differ in other important aspects. We now report the characteristics of neurons recorded in the region of n. raphe magnus (NRM) of freely moving cats. Single unit activity was recorded using a microwire technique previously described in detail (Brain Res. 163:135-150, 1979). Briefly, movable electrode bundles consisting of 32 and 64 μm dia, insulated, nichrome wires were implanted in the NRM at an angle 25° posterior to vertical. Stereotaxic coordinates for the NRM target were P 5.5, L 0.0, H -8.5. Electrodes were also implanted for recording the EEG, EOG, PGO waves and dorsal neck EMG. Serotonergic neurons were initially identified by their slow and regular spontaneous activity which was similar to that previously reported for serotonergic cells in the DRN, NCS and NRP. Discharge rates of NRM serotonergic neurons were highest during active waking (5.5 ± 1.0 spikes/sec, mean ± SEM, n=19), decreased during slow-wave sleep (2.4 ± 0.5 spkes/sec) and were slowest during REM sleep (0.3 ± 0.1 spikes/sec). The activity of these neurons appeared unrelated to PGO waves or sleep spindles. These cells responded to administration of the specific serotonin agonist 5-methoxy-N, N-dimethyltryptamine (5-MeoDMT, 250 μg/kg, i.m.) with a mean decrease in activity of about 60%. Serotonergic neurons in the NRM were either unaffected or only slightly excited by phasic auditory and visual stimuli. Non-serotonergic cells in the region had either much faster or irregular firing rates, did not display their lowest rates during REM sleep and their activity did not decrease after 5-MeoDMT. These data indicate that serotonergic neurons in the NRM more closely resemble another medullary raphe nucleus, NRP, than the two mesencephalic raphe nuclei, DRN and NCS. Thus, as compared to DRN and NCS, serotonergic neurons in the medulla have higher discharge rates during waking and slow-wave sleep, are less responsive to administrations of serotonin agonist drugs, and are generally unresponsive to phasic light and auditory stimuli. However, unlike cells in the NRP, NRM serotonergic neurons show a large decrease in discharge rate between waking and slow-wave sleep. In addition, most NRM serotonin-containing neurons increase their activity in response to strong arousing stimuli such as mildly painful pressure (see next abstract). Supported by NIMH grant MH 23433 and postdoctoral fellowship MH 08869.

161.8 UNIT ACTIVITY OF SEROTONERGIC NEURONS IN N. RAPHE MAGNUS OF FREELY MOVING CATS: RESPONSE TO MORPHINE AND NOXIOUS STIMULI. S. Auerbach, C. Fornal, J. Heym and B.L. Jacobs. Prog. Neurosci., Dept. Psychol., Princeton Univ., Princeton, NJ 08544

Analgesia produced by opiates may involve activation of descending pathways from n. raphe magnus (NRM) which inhibit discharge of nociceptive neurons in the spinal cord. Since depletion of serotonin antagonizes the analgesia induced by morphine, activation of serotonergic cells within the NRM might be important in mediating the analgesic effects of opiates. To test this hypothesis, we studied the response to morphine of identified serotonergic neurons recorded in the NRM of freely moving cats.

Recordings of single unit activity were made from the NRM as previously described (Fornal et al; this meeting). All units in the area of the NRM displaying slow and regular discharge patterns, suppression of activity during REM sleep, and a decrease in discharge in response to the specific serotonin agonist, 5-methoxy-N,N-dimethyltryptamine (250 μg/kg i.m.) were considered to be serotonergic. The unit activity of presumed serotonergic and non-serotonergic neurons in the NRM was studied before and after the systemic injection of morphine sulfate (2 mg/kg, i.p.). This dose of morphine produced profound analgesia in cats within 30 min as determined by the tail-flick test and response to pressure. Unit activity was monitored for 60 min post-injection. Most serotonergic units responded to morphine with either no change or a decrease in unit discharge. This was also true of control cells within the NRM.

We also tested the response of serotonergic units in the NRM to two types of painful stimuli: 1) radiant heat was focused on a distal shaved portion of the tail. The intensity of the heat was adjusted to induce baseline tail-flick latencies of 2-4 sec; and 2) a clamp was applied to various body regions (tail, back, ear). The force of the clamp was adjusted to induce mildly painful pressure as judged by observation of the cat's reactions (i.e. withdrawal from the stimulus but absence of rage and vocalization). Most serotonergic neurons in the NRM discharge maximally in response to mildly painful pressure but responded less consistently to radiant heating of the tail. When heat stimulation induced increases in unit discharge, the response seemed to be correlated with periods of increased behavioral arousal as indicated by increases in tonic and phasic muscle activity. Similarly, the increases in unit discharge induced by pressure seemed unrelated to application of the painful stimulus per se, but best correlated with the period of behavioral arousal.

Our results do not support the hypothesis that opiate-induced analgesia is dependent upon activation of serotonergic neurons in NRM. However, activation of NRM cells in response to arousing stimuli could be an important aspect of an analgesic system.

161.9 ASSESSING IMPULSIVITY IN AN ANIMAL MODEL OF THE ATTENTIONAL DEFICIT DISORDER. G. K. Hodge, A. E. Martin*, M. A. Foster*, and J. Salinas*. Dept. of Psychology, University of New Mexico, Albuquerque, NM 87131.

The Attentional Deficit Disorder (ADD), as described in the DSM III, is characterized by inappropriate inattention, impulsivity, and hyperactivity. Various attempts have been made to develop an animal model of the disorder focusing on motor activity as the measure of interest. Neonatal administration of 6-hydroxydopamine (6-OHDA) induces a transient period of hyperactivity in rats, reminiscent, perhaps, of the transient period of increased motor activity in children with ADD. d-Amphetamine, however, which attenuates hyperactivity in children, does not consistently attenuate the 6-OHDA-induced hyperactivity in rats (see Hodge, Neuroscience Abstr, 7:41, 1981).

Gordon (J of Abnorm Child Psych, 7:317, 1979) has assessed impulsivity in children using a DRL schedule requiring children to temporarily suppress a reinforced response. Gordon (1979) reports that the DRL schedule successfully discriminated between ADD and normal children. We have developed an animal model which employs a modified DRL as a measure of impulsivity in rat pups.

Following desipramine pretreatment, 5-day-old rats were injected intracisternally with either 100 μg/25 μl of 6-OHDA or 25 μl of the ascorbic acid/saline vehicle. Testing was conducted on postpartum days 15, 19, 22, 26, and 30, with an 18-h maternal deprivation period prior to each testing session. Pups were placed at one end of a polypropylene breeding cage with the dam restrained at the opposite end. Pups were required to refrain from approaching the dam for 15 sec on each of 40 trials. Impulsivity was operationalized as the mean number of commission errors (approaches) per trial. Thirty min prior to the start of each testing session, pups received saline or either .5 or 1 mg/kg i.p. of d-amphetamine.

Neonatal administration of 6-OHDA results in a transient deficit in impulsivity control, beginning at Day 19, ending at Day 26. The impulsivity impairment was attenuated with the administration of d-amphetamine, paralleling the response of similar pharmacological intervention with ADD children.

Because ADD is being increasingly considered as an attention rather than a motor disorder, animal models focusing solely on motor activity levels are of limited usefulness. An animal model of impulsivity, as described here, may be of value in the development of a better model of childhood hyperactivity.

(Supported by NIH-MBRS grant 3506-RR08139-0751)

161.10 INFUSION OF A MONOAMINE OXIDASE INHIBITOR INTO THE LOCUS COERULEUS CAN PROTECT AGAINST STRESS-INDUCED DEPRESSION. P. A. Goodman, J. M. Weiss, M. J. Ambrose*, K. A. Cardle*, W. H. Bailey and J. M. Charry*. Laboratory of Behavioral Biology, The Rockefeller University, New York, NY 10021.

This experiment demonstrated that micro-infusion of a monoamine oxidase inhibitor pargyline into the region of the locus coeruleus (LC) of rats could protect against stress-induced behavioral depression. Such depression can be produced by exposing animals to a stressor which they cannot control. Previous studies in our laboratory have demonstrated that stress-induced behavioral depression is correlated with large depletions (<20%) of norepinephrine (NE) in the LC of shocked rats and is accompanied by increased floating behavior in a swim test when compared to rats not exposed to shock (No-Shock). Furthermore, evidence suggests that such NE depletion in the LC may be an important mediating step in causing the depression. The present study determined whether behavioral depression could be prevented by pharmacologically reducing the catabolism of NE in the LC so as to eliminate the NE depletion that normally occurs after acute uncontrollable shock.

Animals were exposed to uncontrollable tail-shock on a schedule shown to produce behavioral depression. Immediately following shock and 90 minutes prior to the 15-minute swim test, animals were infused bilaterally in the region of the LC with pargyline or vehicle. (Drug concentration was 100 μg/μl and was infused at a rate of 0.1 μl/min for 10 minutes.) The results were as follows: (1) Shocked animals infused with vehicle exhibited significantly more floating behavior than No-Shock animals infused with vehicle. (2) Infusion of pargyline into No-Shock animals had no significant effect on their activity when compared with No-Shock vehicle-infused animals. (3) Infusion of pargyline into shocked animals prevented the increase in floating behavior usually seen in animals exposed to uncontrollable shock. As a consequence, the behavioral profile of these shocked animals was not significantly different from that of No-Shock animals infused with vehicle.

Measurement of monoamines (NE, DA, and 5-HT) in seven brain regions after the swim showed that infusion of pargyline into the LC eliminated the large depletion of NE in the LC seen in shocked animals infused with vehicle (27% depletion relative to No-Shock vehicle-infused animals), while having no effect on NE levels in other brain regions. Pargyline infusion also raised the level of 5-HT in the LC region, but again had no effect on 5-HT levels elsewhere.

In conclusion, the depressant effect of uncontrollable shock on behavior was prevented by infusion of pargyline into the LC. This is consistent with the hypothesis that large stress-induced depletions of NE in the LC are an important mediating event in producing stress-induced behavioral depression.

161.12 PLASMA EPINEPHRINE IS A SENSITIVE INDEX OF STRESS IN RATS
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For a physiological variable that is altered by stress to be clinically useful as an index or "barometer" of stress, it must be both reliable and sensitive. "Reliable" means that the variable changes whenever the animal is stressed. "Sensitive" means that changes in the variable co-vary with changes in magnitude of stress. Using groups of rats, we have shown that plasma catecholamines meet these criteria (Phys. Behav. 26:1049, 1981). But, for a test to be useful clinically, such relations must also be seen in individual animals. To test this, we subjected 8 chronically catheterized rats to a 30 sec, scrambled, constant current foot shock every other day. For this group, shock was delivered in an ascending order (0 mA on day 1, .25 mA on day 3, 1 mA on day 5 and 4 mA on day 7). Similarly, 7 rats were subjected to the same procedure, except shock was delivered in reverse order beginning with 4 mA on day 1. Blood was sampled remotely without disturbing the rat before the shock and immediately thereafter. Both norepinephrine and epinephrine increased reliably after foot shock stress: levels of both increased from baseline in 39 of 41 shock sessions. In the ascending series, norepinephrine showed monotonic increases as shock intensity increased in 7 of 8 rats. Similar epinephrine increases were seen in 6 of 8 rats. Assuming each increase occurred with a probability of 0.5, the binomial probability of seeing these distributions was significant ($p < .001$). Since a tendency was seen in norepinephrine data for baseline levels to increase over the duration of the experiment, the descending series was run to evaluate the possibility that learning was interacting with the stress response to produce the results found. Baseline levels again tended to increase. Nonetheless, monotonic decreases with decreasing shock were seen in 3 of 7 rats for norepinephrine and 6 of 7 rats for epinephrine. The binomial probability of seeing this distribution for the norepinephrine data was not significant, but the probability of seeing it for the epinephrine data was significant ($p < .001$). The responses varied greatly from one animal to another; post-shock hormone levels varied from as little as 2-fold at 1 current intensity to 10-fold at another. The data suggest that part of the monotonic response seen in our ascending norepinephrine series is due to an apparent interaction with learning. However, this was not the case for epinephrine. Thus, plasma epinephrine meets our minimal criteria of "reliability" and "sensitivity." Despite the problem of variability, this hormone appears to be the best visceral index of stress currently available. (Supp. by VA med. res. funds).

161.13 ANTERIOR CEREBELLAR VERMAL STIMULATION AND LESION: EFFECT ON CONDITIONED FEAR AND BASAL FOREBRAIN NEUROCHEMISTRY IN RAT.
T.J. Albert*, C.W. Dempesy*, A.E. Schlein*, and C.A. Sorenson (spon: R.G. Heath). Neuroscience Program, Amherst College, Amherst, MA. 01002.

Electrophysiological, anatomical, behavioral, and clinical studies suggest that the anterior cerebellar vermis (ACV) modulates forebrain emotional systems, particularly those controlling fear and anxiety, perhaps via monoaminergic pathways. This study evaluates the effects of both acute electrical stimulation and lesion of the ACV on conditioned fear in rat and assesses the associated changes in monoamine neurotransmission.

The behavioral model employed is the "potentiated startle effect" (PSE) in which anxiety is inferred from an enhancement of response to a startle-eliciting tone presented during a light cue previously paired with footshock. The PSE is sensitive to drugs with anxiogenic or anxiolytic properties in man (Davis, Psychopharm, 1979, 62:1).

ACV stimulation applied for one hour before and 45 minutes during testing produced a statistically significant increase in the PSE without affecting startle baseline. Associated reliable chemical changes in the nucleus accumbens (nAcc) consisted of a decrease in DA, an increase in DOPAC, and a decrease in 5HIAA in AMPT pre-treated rats. In contrast, RF lesion of the ACV produced a statistically significant decrease in the PSE at 12 days post-lesion without affecting startle baseline, but no reliable chemical changes in the nAcc at 21 days post-lesion, although a reliable increase in 5HIAA was found in the septal area.

The present data suggest that acute ACV stimulation increases both conditioned fear and nAcc DA release, consistent with the previous finding (Iversen, in Psychobiology of the Striatum, Amsterdam, 1977:99) that nAcc DA application is associated with hypersensitivity to threatening cues. The lesion data suggest the ACV exerts a tonic facilitatory influence on this same behavior. Serotonergic involvement is also implicated.

These data cannot be readily integrated with clinical reports of mood - and affect - normalizing changes with chronic ACV stimulation (Heath, Jour. Nerv. & Ment. Disease, 1977, 165:300). However, such stimulation is typically reported to enhance responsiveness to the environment, possibly consistent with increased transmission of DA in the mesolimbic pathway.

161.14 CHOLINE EFFECTS IN ANIMALS WITH HIPPOCAMPAL DAMAGE.
J. E. Springer and R. L. Isaacson. Sponsored by: Carolyn Ware. Department of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

In rats the behavioral similarities between bilateral hippocampal destruction and the systemic administration of muscarinic antagonists include increased activity in a novel environment, difficulty in learning a successive discrimination, slowness in habituating, and in inability to inhibit a previously acquired response. These similarities suggest that hippocampal damage may produce effects on remaining neural systems that are also influenced by cholinergic systems. We examined this by giving rats hippocampal or control lesions of the neocortex, and observing their spontaneous behaviors in a novel open field 10 or 60 min following the systemic administration of choline chloride (60 mg/kg) or saline vehicle.

Choline administration reduced locomotion and increased the duration of exploratory bouts in animals with hippocampal damage but only at the shorter injection-test interval. The behavior of the control animals was unaffected at either time interval. In another experiment we found that similar injections of choline chloride ameliorated some of the behaviors induced by administration of the muscarinic receptor antagonist scopolamine but, again, only 10 min after the choline injection. It is suggested that choline administration can benefit, in a time-dependent fashion, animals which exhibit behaviors that occur following hippocampal damage. This effect may occur through changes in acetylcholine systems since choline administration also eliminates similar behaviors seen after muscarinic antagonism.

161.15 ATROPINE INCREASES SENSITIVITY TO STRESS IN RATS
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We have previously reported that various stressors increase levels of pituitary cyclic AMP in vivo in the rat [Kant et al., Pharmacol. Biochem. Behav., 17, 1067-1072 (1982)]. During a pilot experiment we observed that rats pretreated with 60mg/kg atropine sulfate prior to footshock demonstrated an enhanced pituitary cyclic AMP response to footshock. We therefore initiated experiments to further characterize the interaction between stress and atropine.

In the first experiment, rats were pretreated with 60 mg/kg atropine sulfate or saline 15 min prior to exposure to 15 min of either footshock, forced running, immobilization or conditioned psychological stress (rats were placed into a footshock chamber where footshock had been received the previous 4 days). Control rats were sacrificed immediately upon removal from their home cages. In a separate experiment, rats were pretreated with saline or 5, 10, 30 or 60 mg/kg atropine sulfate 15 min prior to 15 min of footshock.

All animals were sacrificed immediately following each stressor by high power microwave irradiation to prevent post-mortem changes in cyclic AMP levels. The pituitaries were dissected, weighed and sonicated in sodium acetate buffer and the supernatants were assayed for cyclic AMP by radioimmunoassay. Trunk blood was collected in the second experiment and the plasma assayed for prolactin.

I.P. injection, conditioned psychological stress, forced running, immobilization and footshock all increased levels of pituitary cyclic AMP as compared to controls. Atropine-pretreated rats demonstrated higher pituitary cyclic AMP levels than saline-pretreated rats after all five stressors with statistically significant differences found after injection, immobilization and shock stressors.

Rats pretreated with 5, 10, 30 or 60 mg/kg atropine sulfate showed an increased pituitary cyclic AMP response to footshock with statistically significant differences observed between the 30 and 60 mg/kg groups vs saline-pretreated rats subjected to footshock. Prolactin response to footshock was also increased in all atropine-pretreated rats with statistically significant differences observed between the 5, 30 and 60 mg/kg groups vs saline-pretreated rats subjected to footshock.

In a follow-up experiment examining the analgesic properties of atropine, atropine-pretreated rats exhibited a significantly decreased latency between application of heat source and tail flick as compared to saline-pretreated rats.

These data suggest that atropine potentiates neurochemical and neuroendocrine responses to stressors, and that this effect is partially explained by the lowering of pain threshhold by atropine.

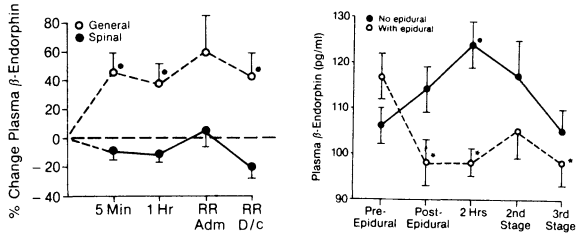
161.16 PLASMA BETA-ENDORPHIN IMMUNOREACTIVITY IN CLINICAL STRESS. J.L. Weiss, S.R. Hameroff, R.C. Cork, J.J. Misiaszek, Depts. Anesthesiology & Psychiatry, Univ. Arizona Health Sciences Center, Tucson, AZ 85724.

Plasma beta endorphin (β-end) has been implicated in physiological responses to stress. Peripheral β-end affects heart, lung, immunocytes, gut, autonomic ganglia, microcirculation and other hormones. Acutely, stress responses can have both beneficial and deleterious effects in clinical medicine. We present 3 studies using plasma β-end immunoreactivity (New England Nuclear) as an index of patients stress response.

Methods. Informed consent and Human Subjects Committee approval were obtained from patients. 1. Spinal vs. general anesthesia 15 males undergoing cystoscopic transurethral prostate resection included 6 patients selecting general anesthesia and 9 selecting subarachnoid local anesthetic (spinal). Anesthetic techniques were appropriately standardized and blood samples (5ml) withdrawn from an indwelling catheter (Fig). 2. Epidural analgesia for labor pain. 15 term pregnant women in labor participated. 10 received local anesthetic (.25% bupivacaine) via epidural catheter. 5 women declined the epidural, but served as controls. 3. Electroconvulsive therapy (ECT) 7 psychiatric inpatients meeting DSM III criteria for major depressive disorder participated who were scheduled for ECT under general anesthesia with methohexital and succinylcholine. β-end was also correlated with heart rate, blood pressure, and plasma catecholamines.

Results. Results from studies 1 and 2 are shown. Study 3 (not shown) revealed a sharp rise (from 30 to 60 pg/ml) in mean β-end immunoreactivity after ECT.

Discussion Studies 1 and 3 demonstrate that β-end stress respones are not ablated (and may be provoked) by general anesthesia. Study 2 (and the spinal group in study 1) shows that pain related stress may be inhibited by regional blockade of painful stimuli. β-end immunoreactivity will be correlated with other stress markers.



161.17 MUSCLE PROTEASE ACTIVITY IN NORMAL AND IMMUNOSUPPRESSED SEPTIC RATS. D. Secrist* and R.L. Ruff (SPON: F. Bahls). Departments of Physiology and Biophysics and Medicine (Neurology), University of Washington, Seattle, WA 98195.

Recent *in vitro* studies (*N. Eng. J. Med. 308*:545, 553, 1983) suggest that sepsis induces muscle proteolysis by a prostaglandin (PG) mediated activation of lysosomal proteases. Rats were immunosuppressed (I) by daily subcutaneous injections of dexamethasone (DEX) 1.5 mg/kg. This treatment produced muscle wasting associated with increased cathepsin (CAT) D activity but no increase in CAT B or calcium activated protease (CAP) activity. A lower dose of DEX 0.3 mg/kg did not activate CAT D. Pepstatin, a CAT D inhibitor, could partially prevent the atrophy associated with DEX 1.5 mg/kg treatment. After four weeks, I rats had reduced lymphocyte counts and spontaneously developed systemic bacterial infections. Normal rats were inoculated with *Streptococcus pneumoniae* to produce spontaneous sepsis (SS). Both SS and I rats developed fever, accelerated muscle wasting, and depletion of intracellular potassium with increased intracellular sodium in muscle associated with a marked increase in muscle CAT B activity without activation of CAT D or CAP. Muscle wasting, but not electrolyte changes, could be partially prevented by leupeptin. Indomethacin is a potent PG inhibitor while acetamenophen is a weak peripheral PG inhibitor. Both acetamenophen and indomethacin reduced fever in SS and I rats, but only indomethacin prevented muscle wasting and electrolyte changes. The data indicate that muscle wasting and electrolyte changes with sepsis are PG mediated with PG activation of CAT B leading to accelerated proteolysis. Supported by NIH grants NS00498, NS16696, and NS18544.

MONOAMINES AND BEHAVIOR I

162.1 LEARNED HELPLESSNESS: EFFECTS ON BRAIN MONOAMINES. D.H. Hellhammer, M. Bell*, M. Ludwig* and M.A. Rea. Dept. Clinical Psychology and Max Planck Clinical Research Unit for Reproductive Medicine, Westf. Wilhelms-University, D-4400 Münster, F.R. Germany.

We have recently discussed a biphasic model of learned helplessness (Hellhammer, D.H., in: The Origins of Depression, ed. by J. Angst, Springer, Berlin-Heidelberg-New York 1983), suggesting a functional imbalance of low noradrenergic and high sertonergic activity in response to uncontrolable aversive events. This study was designed to measure levels of monoamines and metabolites by HPLC/EC in nine discrete brain areas in response to helplessness training.

The triadic yoked control paradigm was used, and ten triplets of adult, male rats were trained for one hour on each of five consecutive days. In each triplet one rat could escape a tail-shock (FI 20, 0.7 mA) by turning a wheel (HE-animals), while it's yoked counterpart received the same amount and frequency of shock but could not end the shock by turning the wheel (HY-animals). This treatment provokes in HY-animals an increase in freezing responses and a deficit in a later shuttlebox escape-learning (FR 2). The third rat of each triplet was handled under the same experimental conditions, but did not receive any shock (HC-animals).

After the last training session rats were killed by decapitation, and brains were dissected at -10 °C. Levels of DA, DOPAC, HVA, NE, MHPG, 5-HT and 5-HIAA were determined in each brain part. For both the HE-group and the HY-animals higher levels of 5-HIAA were found in the midbrain, suggesting an enhanced functional activity at serotonergic receptor sites in response to shock-induced stress. In the pons/medulla levels of 5-HT decreased in the HE-group, possibly reflecting a reduced metabolism of serotonin. On the other hand, we found a decrease of NE in the yoked animals in this brain area. These results support the idea that low noradrenergic activity in the pons/medulla is a consequence of behavioral suppression induced by uncontrolable shock, while serotonin is less active in the HE-animals who coped actively with the aversive event. Moreover, levels of DA were higher in the HY-rats when compared with their HE-counterparts in the pons/medulla and the hypothalamus, and we consider this a consequence of enhanced motor activity in the HE-animals. (Supported by the Deutsche Forschungsgemeinschaft).

162.2 ELECTROLYTIC MEDIAN RAPHE LESIONS REDUCE THE ABILITY OF HALOPERIDOL TO SUPPRESS AN INSTRUMENTAL RESPONSE. A.Kozlowski, D. Wirtshafter and K.E. Asin.(SPON:J.D.Davis).Dept.Psychol.Univ. Illinois at Chicago, Chicago, Il. 60680.

A number of reports have suggested the existence of some sort of interaction between dopaminergic neurons and the median raphe nucleus. For example, electrolytic median raphe lesions have been found to increase dopamine turnover in the nucleus accumbens (Herve et al., 1981) and to potentiate amphetamine-induced hyperactivity (Lucki & Harvey, 1979). Furthermore, electrolytic raphe lesions have been reported to reduce the ability of dopamine antagonists to produce catalepsy (Kostowski et al., 1972) and impair active avoidance (Kostowski & Plaznik, 1982).

The current experiment was designed to examine the generality of the conclusion that raphe lesions are able to antagonize the ability of dopamine antagonists to disrupt behavior. Rats with electrolytic median raphe lesions, or sham operated controls, were trained to traverse a straight alley for food reward. Following eight days of training, running speeds did not differ between groups. On the next two days rats were then tested 30 min following an injection of haloperidol (0.15 mg/kg). Rats with median raphe lesions ran significantly faster than controls following haloperidol treatment. The effect of the lesions was largest in the initial segment of the alley, suggesting that median raphe damage may antagonize the ability of haloperidol to slow movement initiation.

Although it is often assumed that the behavioral effects of electrolytic raphe lesions result from serotonin depletion, recent work has indicated that many of these effects may result from damage to nonserotonergic elements within the raphe. We will, therefore, also present data of the effects of haloperidol on running speeds following specific serotonin depleting lesions produced with the neurotoxic agent 5,7-dihydroxytryptamine.

162.3 HALOPERIDOL CATALEPSY SHOWS SENSITIZATION WHICH DEPENDS ON THE PASSAGE OF TIME RATHER THAN REPEATED TREATMENT, S. M. Antelman*, D. Kocan*, D. J. Edwards, M. Fraser*, J. Perel* and S. Knopf*. Depts of Psychiatry and Pharmacology-Physiology, Univ. of Pittsburgh, Schools of Medicine and Dental Medicine.

Most animal studies of the influence of repeated neuroleptic administration have shown that tolerance develops to the initial effects of these agents. Yet the neuroleptic effects of greatest clinical interest, i.e., the antipsychotic influence and tardive dyskinesia, take weeks and months, respectively, to develop. That is, they show sensitization rather than tolerance. The same agent is often capable of displaying tolerance or sensitization depending on whether treatments are massed or high dose on the one hand or relatively low dose or intermittent on the other. Since "low" doses and intermittent treatments favor the development of sensitization, we examined whether such regimes could result in sensitization of haloperidol-induced catalepsy, a response typically associated with tolerance.

First, we administered a single injection 0.4 mg/kg, (i.p.) of Haldol® to adult male Sprague-Dawley rats daily for 28 days and tested for catalepsy once a week. Catalepsy was defined as remaining immobile for at least 25 sec. with forepaws elevated on a platform. Two min. tests were conducted at 5,10,20,30,40, 50 and 60 min. after injection. In virtually every instance the incidence of catalepsy was greater after 7,14,21 or 28 days of treatment than it was following a single injection of Haldol ($p<.002$). Thus, clear evidence of sensitization was obtained.

Since we have previosuly shown that sensitization after amphetamine or antidepressants depends on the passage of time rather than repeated treatments, we inquired whether sensitization of Haldol catalepsy is similarly independent of repeated treatment. We now report that a single injection of 0.4 mg/kg Haldol is sufficient to significantly sensitize the cataleptic response to a second injection of the same dose when this is tested 1,2,3 or 4 weeks later ($p<.001$ all second injections, week 1-4 relative to all first injections). The same results have been obtained whether animals are tested for catalepsy in novel surroundings or their home cages and are therefore unlikely to be due to conditioning factors. Also radioimmunoassay of Haldol levels in the striatum indicates that drug buildup cannot account for our results.

These findings provide the first indication that at least some of the effects of neuroleptics may grow merely with the passage of time independent of repeated treatment. As such they raise the very provocative questions of 1) whether daily treatment with these agents is necessary for clinical efficacy in schizophrenia and 2) whether the development of tardive dyskinesia may be more a function of the passage of time rather than repeated treatment.

162.4 STRESS EFFECTS ON SENSITIZATION AND EXPLORATION: PRIOR EXPOSURE TO D-AMPHETAMINE OR HALOPERIDOL. H. Schreiber, M. Warren*, L. Nez*, J. Kubota*, B. Zito*, R. Lopez-Gaston*, K. Wanya*, W. Ogle*. Neurosci. Lab., Div. Behav. Sci., Highlands Univ., Las Vegas, N.M. 87701.

A single administration of d-amphetamine (AMPH) can sensitize the response to a subsequent injection of AMPH for up to 6 days (Browne & Segal, Pharmac Biochem Behav 6, 545, 1977). The present experiment determined (a) whether repeated exposure to an inescapable stressor would potentiate the sensitization produced by prior exposure to AMPH or haloperidol (HAL) and (b) whether the decrement in exploration in a novel setting seen following repeated exposure to a stressor would be influenced by a prior injection with AMPH or HAL. Adult male rats received AMPH (12 mg/kg X 2, IP), HAL (5 mg/kg X 2, IP) or saline (SAL, IP) and were left undisturbed for the next 9 days. Then, half of each group received 3 days of cold water immersion (3 min/day, 4.5°C). On the following day, rats were observed for 5 min in a small novel chamber housed in a larger sound-attenuated environmental chamber, injected with apomorphine hydrochloride (APO, .8 mg/kg) and, 10 min later, again observed for 5 min in the small chamber by a blind observer, who recorded stereotypical and other behaviors at 5 sec intervals in a time-sampling fashion. Before APO injection, SAL-history stressed rats showed less sniffing than SAL-history non-stressed rats, $t(48)=3.96$, $p=.01$, and less sniffing than AMPH-history stressed rats, $t(48)=2.24$, $p<.02$. Previous work in our laboratory has shown that sniffing is the predominant exploratory behavior seen in this apparatus upon the first few exposures. After APO injection, stressed rats showed more stereotypical gnawing/licking, $F(1,48)=4.7$, $p=.04$, and more mouthing/licking movements, $F(1,48)=5.6$, $p=.02$, than nonstressed rats. Thus, stress sensitized (i.e. potentiated) stereotypy with little evidence of an interaction with prior drug treatment. In contrast, the exploration decrement following stress was abolished by AMPH or HAL treatment from days earlier. The results are discussed in relation to the interaction of stress and sensitization (Antelman, et al., Science 207:329, 1980) and the potential involvement of stimulus filtering in sensitization (Kokkinidis & Anisman, Psychol. Bull. 88:551, 1980). Supported by NIH-MBRS grant 2-S06-RR-08066-12.

162.5 Effects of mesolimbic dopamine lesions on hoarding, exploratory behavior and alimentary patterns in the rat. A.E. Kelley * and L. Stinus* (SPON: F.Moody-Corbett) Lab. Neurobiologie des Comportements, Université de Bordeaux , Bordeaux, France.

The ascending mesolimbic dopamine (DA-A10) system, arising in the ventral tegmental area (VTA), is thought to be involved in adaptive behavior and motivational processes. We have carried out a behavioral study of animals with 6-hydroxydopamine (6-OHDA) lesions of this neuronal system. The study included the following groups: VTA lesion, Nucleus accumbens lesion (N.Acc.), and control. In a fourth group, for purposes of comparison, the forebrain noradrenaline system was lesion at the level of the superior cerebellar peduncle (PCS). Three weeks after surgery, rats were selected or discarded on the basis of their amphetamine and apomorphine responses, to retain only completely lesioned rats. In a test of hoarding behavior, in which controls hoarded nearly all 60 biscuits from an open field to the home cage in a 2-hour test, hoarding was severely diminished or abolished in VTA and N.Acc. lesioned animals.Hoarding in PCS rats was not significantly affected. A series of alimentary tests were then conducted. In a 24-hr. food-intake test with no prior deprivation, parameters such as food consumption, food spillage, no. of biscuits partially eaten or untouched, were recorded. In a 30 min. test with prior deprivation, additional parameters included no. of eating bouts and duration of eating. Although no major difference in food consumption was found among groups, DA-lesioned rats showed significantly different patterns of food intake. No differences were noted in water intake or patterns of drinking. Two further experiments which tested consummatory responses to physiological challenge revealed no differences among groups. In separate tests of exploratory behavior in an 8-hole and 4-hole box, PCS and control rats displayed similar behavior. DA-lesioned rats showed either marked exploratory deficits (VTA) or changes in patterns of investigatory behavior (N.Acc.).Hoarding is a highly organized intrinsic behavior in rodents, elicited by appropriate external stimuli. Locomotor exploration is also elicited by external cues, primarily novelty. The mesolimbic lesioned animals are deficient in the expression of such behavior; our data tentatively suggest that such animals do respond appropriately to internal physiological stimuli. It is concluded that such lesions of the mesolimbic DA system alter the mechanisms by which salient environmental stimuli elicit appropriate, goal-directed responses.

162.6 CONDITIONED PLACE PREFERENCE FROM INTRA-ACCUMBENS BUT NOT CAUDATE OR AMYGDALA AMPHETAMINE INJECTIONS Geoffrey D. Carr* and Norman M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Canada, H3A 1B1.

Amphetamine administration has been demonstrated to be rewarding in rats using both the self-administration paradigm and the conditioned place preference paradigm (conditioned reinforcement). Some studies have suggested that amphetamine-induced stimulation of dopaminergic activity in the nucleus accumbens mediates the reward. Selective lesions of the dopamine terminals in the accumbens using the neurotoxin 6-hydroxydopamine attenuate amphetamine self-administration and place preference. Further, rats are reported to lever press for amphetamine injections into the accumbens. The present study was designed to determine if injections of amphetamine directly into the accumbens are rewarding by using the conditioned place preference paradigm. We further examined whether the possibly rewarding effects are specific to the accumbens or could be obtained from other dopamine terminal areas (caudate nucleus; amygdala). Rats received an injection of amphetamine (10ug in 0.5ul) through a cannula aimed at one of the three brain sites and were then placed (randomly assigned) into one of two distinctive environments for 30 minutes. The following day, they were injected with saline and placed into the other environment. After twelve such pairing days, they were given a free choice between the environments (via an open shuttle box) and the time spent in each was recorded. The rats that had received the accumbens injections showed a significant preference for the amphetamine-paired environment over the saline-paired environment ($t=2.58$, $df=17$, $p<.01$) (the environments were counterbalanced across drug conditions). In contrast, there was no preference (or aversion) induced by either the intra-caudate ($t=.57$, $df=8$, $p>.05$) or intra-amygdala ($t=.20$, $df=17$, $p>.05$) injections. The data demonstrate that stimuli paired with amphetamine injections in the accumbens become conditioned reinforcers themselves. This is interpreted as suggesting that amphetamine-induced stimulation of dopaminergic activity in the accumbens produces some type of rewarding effect. The failure of the amygdala and caudate rats to show a preference suggests that the effect is specific to the accumbens.

162.7 **STRESS PROVOKED ENHANCEMENT OF AMPHETAMINE ELICITED PERSEVERATION** H. Anisman, D. Hoffman* and R. Zacharko (SPON: P.E. Wainwright). Dept of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Reexposure to a stressor or cues associated with a stressor will increase the utilization of brain norepinephrine and dopamine in selected brain regions, suggesting that the mechanisms subserving the neurochemical alterations are subject to conditioning or sensitization. The behavioral effects of amphetamine were assessed in order to determine whether previous application of stressors would likewise influence the response to the drug treatment. Following administration of d-amphetamine mice exhibit a perseverative tendency in which they tend to visit successively two arms of a symmetrical Y-maze. Exposure to acute shock immediately prior to testing resulted in an appreciable enhancement of the perseverative tendency engendered by the drug. Such an effect occurred irrespective of whether or not the shock was administered in the presence of cues similar to those present in the environment in which perseveration was assessed. Moreover, it appeared that the organism's ability to cope with the stressor through behavioral means was fundamental in provoking the subsequent perseveration. In particular, escapable shock did not enhance the perseverative tendency, while a comparable amount of inescapable shock, applied in a yoked paradigm, augmented the amphetamine-elicited perseveration. Exposure to an uncontrollable stressor was also found to enhance the amphetamine elicited peseveration when testing was conducted 72 hr following the stress session. Such an effect, however, was evident only when the environment in which shock was administered was similar to the test environment. Long-term effects of the stressor were not evident when the stress and test environments were dissimilar. It is suggested that stressful events influence the subsequent response to amphetamine owing to conditioning factors, although a potential role for sensitization cannot be definitely excluded.

162.8 A BEHAVIORAL ANALYSIS OF THE EFFECTS OF AMPHETAMINE ON PLAY AND LOCOMOTOR ACTIVITY IN THE POST-WEANING RAT. L.A. Raskin and Mary F. Sutton*. Dept. Psychology, Amherst College, Amherst, MA 01002.

Amphetamine has been shown to canalize or direct the activity of a young rat towards ethologically relevant stimuli. In the five-day-old, amphetamine increases the speed of approach to and attachment to the nipple of an anesthetized dam, in the 15-day-old drug-induced activity is directed towards an anesthetized adult, however in the juvenile rat amphetamine reportedly disrupts species-specific behaviors such as huddling and play. The present experiments further assessed the effects of amphetamine in the post-weaning rat by measuring drug-induced behaviors in the presence of an alert and anesthetized companion. In Experiment I subjects were videotaped in the presence of an alert non-treated, same-age rat and components of play, a predominant behavior of the post-weaning rat, were recorded. Results confirmed previous reports that low doses of amphetamine (0.5 mg/kg) disrupt play behavior, however in the present experiment higher doses of amphetamine (1.0 mg/kg) did not disrupt play. Further analysis of drug-induced behavior revealed that the 1.0 mg/kg amphetamine-injected rat did not exhibit non-directed activity around the cage but rather engaged in play with the companion, however the flexibility in motor patterns of the drug-treated animal were markedly different than controls. The second experiment confirmed that amphetamine-induced activity is directed towards ethologically relevant stimuli by observing drug-treated 32-35-day-olds either in the presence of an anesthetized same-age rat, or in isolation. Following drug treatment all subjects were active nearly 100% of the observation period, however, subjects that were tested with the anesthetized same-age rat exhibited their activity around that stimulus. The present experiments suggest (a) that flexible motor patterns are disrupted following amphetamine treatment and (b) that ethologically relevant stimuli influence amphetamine-induced behavior in the post-weaning rat.

162.9 PHARMACOLOGICAL MANIPULATIONS OF DOPAMINERGIC AND SEROTONERGIC TRANSMITTER SYSTEMS ALTER MATERNAL AGGRESSIVE BEHAVIOR OF CD-1 MICE. J. R. Ieni and J. B. Thurmond. Neuropsychopharmacology Program, Univ. of Louisville, Lou. KY 40292.

Dopamine (DA) and serotonin (5-HT) have been implicated in the regulation of several variants of male aggression. However, little is known about the involvement of these transmitters in female aggression. During the postpartum period, lactating animals display intense fighting behavior toward conspecifics (Svare, B. in D. Gubernick and P. Klopfer (Eds.) Parental Care In Mammals, 1981). This study was conducted to examine the roles of DA and 5-HT on the aggressive behavior of postpartum mice.

Both p-chlorophenylalanine (PCPA) (200 & 400 mg/kg) and 5-hydroxytryptophan (5-HTP) (100 mg/kg) inhibited fighting of postpartum mice; increasing attack latencies and reducing total number of attacks. Combined treatment with PCPA and 5-HTP also suppressed fighting behavior. In addition, mothers treated acutely with 5-HT receptor antagonists displayed less aggressive behavior than control mothers. Mianserin (2 & 4 mg/kg) and methysergide (4 mg/kg) significantly reduced number of attacks emitted by postpartum mice, but failed to alter attack latency. Methiothepin (0.25 & 0.5 mg/kg) also lowered the number of attacks emitted. Biochemically, PCPA depleted brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), whereas 5-HTP increased brain 5-HT and 5-HIAA. The receptor antagonists had minimal effects on brain 5-HT and 5-HIAA levels, but methiothepin significantly elevated brain dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).

Catecholamine (CA) synthesis inhibition with alpha-methyl-p-tyrosine (AMPT) or DA receptor antagonism with haloperidol reduced postpartum aggression. Conversely, L-dihydroxyphenylalanine (L-DOPA) (50 mg/kg) elevated maternal aggressive behavior. This L-DOPA effect could be blocked by pretreatment with haloperidol. AMPT disrupted CA synthesis; indicated by decreased brain norepinephrine, DA, DOPAC and HVA. L-DOPA elevated brain DA, DOPAC and HVA, while haloperidol elevated brain DOPAC and HVA, as well as 5-HT and 5-HIAA.

The data suggest a facilitatory role for DA, and a complex, possibly facilitatory role for 5-HT in the regulation of maternal aggressive behavior. Pharmacologic manipulations of brain DA and 5-HT affect maternal aggressive behavior and forms of male aggressive behavior (e.g., isolation and territorial) in a similar manner. Thus, transmitter regulation of aggressive behavior in males and females may be similar, although the stimuli and environmental conditions which evoke the behavior are different.

162.10 NEUROLOGICAL DEFICITS INDUCED BY 2-DEOXYGLUCOSE IN 6-HDA-TREATED RATS: EXAMINATION OF THE CRITICAL LOCUS OF DOPAMINE DEPLETION. Abigail M. Snyder*, Michael J. Zigmond and Edward M. Stricker (SPON: David J. Kupfer). Psychobiology Program, Department of Psychology, University of Pittsburgh, Pittsburgh, PA 15260.

The intraventricular administration of large doses of 6-hydroxydopamine (6-HDA) to rats pretreated with systemic desmethylimiparmine (DMI, 25 mg/kg) produces a permanent and extensive (>90%) depletion of dopamine (DA) throughout the brain. Although these animals often recover from the initial akinesia induced by the lesion, neurological deficits are temporarily reinstated by exposure to such stressors as the severe glucoprivation produced by systemic administration of 2-deoxyglucose (2DG) (Snyder, Zigmond & Stricker, Soc. Neurosci. Abstr. 8: 362, 1982). The present experiments examine the locus of DA depletion responsible for this disruptive effect of stress.

Adult rats (220-280 g) received 6-HDA (200 or 250 ug in 20 ul, ivt) after treatment with DMI. Several weeks later, when neurological function appeared normal under basal conditions, they were injected with 2DG (500 mg/kg, ip) and were found to become akinetic and cataleptic. These deficits could be reversed acutely either with l-dopa (60 mg/kg, ip) given after RO4-4602 (50 mg/kg, ip), or with apomorphine (0.1 mg/kg, sc). If prior to the administration of 6-HDA rats were pretreated with bupropion (125 mg/kg, ip), a drug which blocks catecholamine uptake, the depletion of DA was reduced and 2DG-induced neurological deficits were prevented.

Rats showing 2DG-induced akinesia had marked DA depletions in striatum, olfactory tubercle, hypothalamus and frontal cortex. When 6-HDA (1 or 2 injections of 8 ug in 4 ul, bilaterally) was administered directly into the DA-containing cell body regions of the midbrain, or into terminal areas in striatum, nucleus accumbens or olfactory tubercle, we found the loss of DA from striatum was predictive of 2DG-induced behavioral effects while the loss of DA from mesolimbic areas was not. Finally, when 6-HDA was placed bilaterally into various portions the striatum, rats receiving lateral and central injections showed 2DG-induced neurological deficits while rats with medial injections did not. Collectively, these results suggest that the loss of DA-containing terminals in the central and/or lateral portion of the striatum may be responsible for the neurological deficits produced by 2DG in rats with 6-HDA-induced brain lesions. (Supported in part by MH-29670.)

162.11 RAPHE HYPERACTIVITY: EVIDENCE FOR A MEDIATIONAL ROLE FOR DOPAMINE. L. Wing, D. Wirtshafter, and K.E. Asin. Department of Psychology, University of Illinois at Chicago, Chicago, Illinois 60680.

Considerable evidence indicates that electrolytic lesions of the median raphe nucleus lead to pronounced hyperactivity and more recent studies suggest that dopaminergic mechanisms may be altered following these lesions. For example, Herve et al. (1981) have reported an increase in dopamine utilization in the nucleus accumbens following electrolytic median raphe lesions, and we have reported (Wirtshafter and Asin, 1981) that dopamine antagonists are able to abolish lesion-induced hyperactivity. The current study was designed to investigate in more detail the role of dopamine in the hyperactivity produced by median raphe lesions.

Male, Sprague-Dawley derived rats were administered either the catecholamine neurotoxin 6-hydroxydopamine (-OHDA) (8 ug/4 ul) in 0.2% ascorbate vehicle, or the vehicle alone. Infusions were made into the anterolateral hypothalamus, c udal to the nucleus accumbens. Twelve days following surgery, rats were habituated to a photocell activity cage and two days later, their response to an isotonic saline injection was recorded. Two days subsequent to this, rats were injected with 1.5 mg/kg d-amphetamine, and their locomotive response to the drug was measured for 1 hour.

Animals were tested for activity in the open field 2 days later, and were then either sham operated or given an electrolytic median raphe lesion. Post-operatively, rats were again tested in the photocell cage for spontaneous levels of locomotor activity and also following an injection of 0.3 mg/kg apomorphine (s.c.).

Preliminary results indicate that 6-OHDA infusions into the anterolateral hypothalamus do indeed abolish the hyperactivity induced by the administration of amphetamine and, in addition, attenuate the hyperactivity produced by electrolytic median raphe lesions. These results are compatible with a dopaminergic involvement in the median raphe hyperactivity syndrome.

162.12 ASYMMETRY AND SEX DIFFERENCES IN THE EFFECTS OF UNILATERAL DOPAMINE DEPLETION ON BODY WEIGHT REGULATION. Terry E. Robinson, David M. Rock* and Jill B. Becker. Psychology Dept. and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

The aphagia, adipsia and other regulatory deficits so commonly seen after bilateral lateral hypothalamic lesions or brain dopamine (DA) depletion also occurs in a less severe form following unilateral lesions or DA depletion. However, because there is an endogenous asymmetry in the nigrostriatal DA system we suspected that the effects of unilateral DA depletion on regulatory behaviors may depend on which side of the brain is damaged. To investigate this hypothesis we screened intact rats for amphetamine (AMPH)-induced rotational behavior and then operationally defined one hemisphere as "dominant" (D) for rotational behavior (the side contralateral to the preferred direction of rotation), and the other as non-dominant (ND). The animals were then pretreated with desipramine and injected with 6-OHDA (3.5-8 µg/4 µl) into either the D or ND substantia nigra. Both male and female rats were tested. After surgery every animal was weighed daily for 25 days. At least 30 days after surgery the response to two regulatory challenges (2-deoxy-d-glucose and hypertonic saline) was assessed, as was open field behavior and AMPH-induced rotational behavior. The effects of unilateral DA depletion on body weight regulation depend on at least 3 factors: (1) whether the D or ND substantia nigra is damaged; (2) the extent of the DA depletion; and (3) the sex of the animal. For example, male rats with partial DA depletions on the D side ($\bar{X}$ = 59%) showed a chronic reduction on body weight relative to sham-operated controls, whereas those with ND-sided lesions ($\bar{X}$ DA depletion of 65.7%) did not. The effects of partial D vs. ND-sided 6-OHDA lesions on body weight were very different ($F=7.76$, $p<0.02$). More complete DA depletions ($\bar{X}$=98%) resulted in a greater chronic reduction in body weight following both D and ND-sided lesions, but again, the effects of ND-sided lesions were less severe than D-sided lesions. The effects of similar lesions in females were quite different. Neither D nor ND-sided partial DA depletions (<95%) produced chronic body weight changes, and although complete DA depletions (>95%) resulted in a chronic weight reduction the tendency was for ND-sided lesions to produce a greater effect than D-sided lesions (i.e. opposite the males). These results are striking, not only because of the demonstration of an asymmetry and sex differences in the neural control of body weight regulation, but because partial DA depletions produced a chronic decline in body weight. It is often stated that nearly complete DA depletion is required to produce regulatory deficits. Our results suggest this is not true, if the side of the lesion and sex of the rat are taken into consideration.

162.13 INFLUENCES OF ESTROGEN ON AMPHETAMINE-INDUCED ROTATIONAL BEHAVIOR Jill B. Becker and Terry E. Robinson. Psychology Department and Neuroscience Laboratory, The University of Michigan, Ann Arbor, MI 48109.

Endogenous ovarian hormones modulate striatal dopamine (DA) activity in the female rat. In behavioral and biochemical studies we have found that striatal DA activity is greatest on estrus and attenuated by ovariectomy (OVX). From these studies we have concluded that estrogen (and/or progesterone) potentiates striatal DA activity in the female rat. However, others have reported that estrogen inhibits striatal DA mediated behaviors (Bédard et al, Neurosci Lett, 17, 89, 1980), or that the effect of estrogen is initially inhibitory, followed by potentiation (Gordon, Brain Res Bull, 5, 679, 1980). In this study we report that the length of time between estrogen treatment and the behavioral test [amphetamine (AMPH)-induced rotational behavior] is critical to the behavioral response observed. Perhaps of greater importance, we also find that different estrogen administration schedules differentially influence the subsequent behavioral response to a second AMPH injection (i.e. sensitization; see Robinson et al, Brain Res., 253, 231, 1982).

Female rats received unilateral 6-OHDA lesions of the substantia nigra and were ovariectomized 2 weeks later. Two weeks after OVX hormone injections began. One group received oil injections (OIL). The other 3 groups received 5 µg estradiol benzoate (EB) daily for 4 days. One of these was tested 4 h after the last EB injection (EB:4h), another 24 h after the last EB injection (EB:24h), and the third 4 days after the last EB injection (EB:4d). For the behavioral test, each animal received 2.5 mg AMPH/kg and turning was recorded for 2 h. The entire procedure was repeated 2 weeks later.

During the 1st test, there was no difference between OIL and EB:24h. However, rats with EB:4h or EB:4d turned more than animals with OIL or EB:4h. This suggests that there is a very rapid potentiation of AMPH-induced rotational behavior 4 hours after EB, as well as a long-term potentiation seen 4 days later. During the 2nd behavioral test all groups turned significantly more than they did during the 1st test, except for EB:24h. Therefore, the apparent inhibitory effect seen 24 h after EB exposure may be due to an interaction between estrogen and the effect of repeated drug injections. We conclude that estrogen can potentiate striatal DA activity and caution that the behavioral paradigm used, and especially the use of repeated drug injections, should be very carefully considered when evaluating the effects of estrogen on striatal DA activity.

162.14 NEONATAL 6-HYDROXYDOPAMINE ATTENUATES THE NEURAL AND BEHAVIORAL EFFECTS OF ENRICHED REARING IN THE RAT. L. O'Shea*, M. Saari*, B.A. Pappas, R. Ings* and K. Stange*. Depts. of Psychology, Nipissing U., North Bay and Carleton U., Ottawa, Ontario.

Critical periods during which the brain is shaped by experience may require intact functioning of the noradrenergic system originating from the locus coeruleus (Kasamatsu and Pettigrew, Science, 194:206, 1976). Since the raising of rats in enriched environments increases their cortical weight, alters a number of measures of brain morphology and chemistry and facilitates maze learning, we determined here if the neonatal loss of forebrain norepinephrine (NE) would attenuate the effects of such rearing.

Newborn male rats were administered subcutaneous 6-hydroxydopamine (6-OHDA) so as to deplete forebrain NE. After weaning at 25 days, half of the rats were raised for 35 days in an enriched environment and the other half were raised under normal isolated conditions. Subsequently the 6-OHDA rats and their vehicle controls were trained in a Lashley type III maze and then sacrificed for assay of regional brain weights and brain catecholamines. These procedures were carried out by experimenters who were blind to the treatments to which the rats had been assigned. Enriched rearing (as compared with the normal rearing) was found to affect several variables for the vehicle injected rats. None of these effects were observed, however, in the 6-OHDA rats. Specifically, we found for the vehicle but not the 6-OHDA rats (whose cortical and hippocampal NE levels were reduced to 24 and 8% of normal, respectively) that enriched rearing: 1) enhanced acquisition of the maze habit, 2) increased the weight of the telencephalon, 3) decreased the weight of the hypothalamus, 4) elevated hypothalamic dopamine levels and 5) elevated dopamine levels in a posterior cortical sample which also contained the amygdala.

In general our findings for the effects of enriched rearing in the vehicle injected rats are consistent with previous reports of the consequences of environmental enrichment. The striking insensitivity of the neonatal NE depleted rats to such enrichment leads us to conclude that forebrain NE plays a permissive role in the behavioral, neurochemical and neuromorphological alterations induced by environmental manipulations early in life. This conclusion is congruent with the results of Kasamatsu and Pettigrew (op. cit.), who demonstrated that NE was essential for monocular eye occlusion to reduce binocular responsiveness of visual cortical neurons in the cat. Thus, as they suggest for the cat, the rat apparently also requires the integrity of forebrain NE innervation for at least some aspects of the shaping of the brain by early experience.

162.15 METHOD FOR THE IDENTIFICATION OF SEROTONIN-CONTAINING NEURONS IN SINGLE UNIT STUDIES IN FREELY MOVING CATS. V.M. Trulson and M.E. Trulson. Dept. of Pharmacol., Marshall Univ., Sch. of Med., Huntington, WV 25701.

Recent studies have examined the physiological and pharmacological properties of single units in various raphe nuclei of freely moving cats. These cells are presumed to be serotonin (5HT)-containing neurons, since the raphe nuclei contain a large number of serotonergic units. The serotonergic nature of these units, however, has not been conclusively demonstrated. The present report describes a method for identifying these neurons. A movable microdrive, consisting of 4-32μ dia. Formvar coated nichrome wires glued to a center glass micropipette (tip dia., 2-4 μ) was stereotaxically positioned above the nucleus raphe dorsalis (RD), nucleus raphe pallidus (RPA), or nucleus centralis superior (NCS), and cemented to the skull. The micropipette was filled with a solution of L-tryptophas (10^{-1}M). Gross electrodes for recording the EEG, EMG and EOG were also implanted. After recovery from the anesthesia, the microelectrode bundle was advanced until a unit was isolated. Unit activity was recorded across the sleep-waking cycle, its response to phasic auditory and visual stimuli was tested, and then an injection of 5-methoxy-N,N-dimethyltryptamine (5MeODMT, 50-250 μg/kg, i.m.) a direct-acting 5HT agonist, was given, and unit activity was recorded for 1 h post-injection. The cat was anesthetized with chloral hydrate (400 mg/kg, i.p.), a tube was connected to the top of the micropipette, and for 20 min. 4 Kg/cm^2 of pressure was applied using a pneumatic pump, in order to eject tryptophan. A 20 μA direct current was passed through the recording electrode to mark the recording site, and the brain was removed and processed for 5HT fluroescence histochemistry according to standard techniques. Coronal brain sections containing the recording loci were examined under a fluorescence microscope at 400 nm excitation and 510 nm emission wavelengths. These studies revealed that recording loci located adjacent 5HT fluorescing neurons had the following properties: They discharged in a rhythmic pattern at a rate of 2-6 spikes/sec during waking; their activity decreased during slow-wave-sleep and showed at least a 70% decrease during REM sleep; neurons in the RD and NCS were driven by phasic auditory and visual stimuli, while RPA neurons were unresponsive to these stimuli; and the activity of RD and NCS neurons was suppressed by low doses (50 μg/kg) of 5MeO-DMT, while RPA neurons were inhibited only by high doses (250 μg/kg) of the drug. Interestingly, some neurons in the NCS displayed slow, rhythmic discharge rates and were inhibited by low doses of 5MeOMDT, but were not excited by auditory or visual stimuli, nor was their activity significantly decreased during REM sleep. These neurons showed no 5HT fluroescence, indicating that they were non-serotonergic cells. Thus, not all NCS neurons that display a slow, rhythmic firing rate and are inhibited by low doses of 5HT agonists are serotonergic neurons.

162.16 STATE DEPENDENT EFFECTS OF PERIPHERAL EPINEPHRINE ADMINISTRATION ON THE EXTINCTION OF LEARNED FEAR. M. A. Downen and R. A. Jensen. Developmental Biopsychology Laboratory, Southern Illinois University, Carbondale, IL 62901.

Systemic administration of epinephrine (E) and other agents that cause peripheral arousal reliably produce facilitation of the acquisition and retention of avoidance behavior. However, the effects of these agents on the extinction of avoidance responses has not been extensively studied. Although there is a considerable body of literature dealing with the effects of ACTH and other peptides on the extinction of avoidance responding, information on the effects of catecholamines on the forced extinction of learned fear is lacking.

Earlier findings from our laboratory indicated that peripheral E administration impairs the extinction of an active avoidance response. Conversely, administration of E enhanced the extinction of an inhibitory avoidance response (Neurosci. Abs., 1982, 8, 148).

To further study these effects and to determine whether state dependency may account for some of these findings, E or saline was administered prior to training and also before the extinction trials. In the present experiment, five groups of 90 - 140 day-old male Long-Evans rats were injected with saline or 0.1 mg/kg E immediately prior to training in an inhibitory avoidance task with a 0.75 mA, 1.0 sec foot-shock. Twenty-four and 48 hours later, animals were again injected with either saline or E immediately prior to a forced extinction trial. This extinction trial consisted of being placed for 6 minutes in the portion of the training apparatus in which shock had previously been delivered. Retention was measured 24 hours after the second extinction trial.

On test day, the saline-treated extinguished control animals showed significantly shorter latencies to re-enter the area of the apparatus in which they previously had been shocked than did the saline-treated non-extinguished control rats. This verified the effectiveness of our extinction procedure. Animals that were treated with E prior to both the training and the extinction trials exhibited significantly longer retention latencies as compared to saline-treated controls. Animals that received either saline before training and E before extinction, or E before training and saline before extinction did not differ significantly from saline-treated extinguished controls.

The pattern of results seen here suggests that some of the effects of epinephrine administration on the extinction of learned fear may be due to state dependency rather than through a direct effect of peripheral catecholamine systems on learning and memory processes. Current investigations are aimed at assessing the effects of amphetamine administration and adrenal medullectomy on the extinction of learned fear.

162.17 TRANSPLANTATION OF FETAL NORADRENERGIC NEURONS INTO DORSAL BUNDLE-LESIONED ADULT RATS: EFFECTS ON HABITUATION, LEARNING AND MEMORY. Timothy J. Collier, Don M. Gash, and John R. Sladek, Jr. Dept. Anatomy, Univ. Rochester Sch. of Med., Rochester, NY 14642.

Transplantation of fetal neurons as a form of replacement therapy for behavioral and physiological deficiencies produced by brain lesions in adult animals has yielded promising results in initial studies. One area of interest is whether neuronal grafts can influence complex cognitive functions such as learning and memory. Central noradrenergic projections to hippocampus and cortex represent one class of neurons believed to influence behavioral plasticity, possibly via modulation of arousal and attention. We studied the effects of bilateral 6-hydroxydopamine lesions (6 μg in 1.5 μl) of the dorsal norepinephrine system, and compensatory grafts of fetal (16 days gestation) noradrenergic neurons into the lateral ventricle/dorsal hippocampus, upon habituation, learning and memory of a T-maze rewarded alternation habit. An initial series of dissections was undertaken to verify the location of locus coeruleus neurons in the 16 day gestation fetus. Using the maps of Seiger and Olsen (Z. Anat. Entwickl.-Gesch. 140, 281-318 (1973)), blocks of tissue roughly 1.5 mm on a side were dissected from the dorsal brainstem at the level of the pontine flexure. The presence of monoamine neurons in this area was verified by immersion fixation with a formaldehyde-glutaraldehyde solution and examination with fluorescence microscopy. With reliable location and dissection of noradrenergic neurons available, the study of behavioral effects of grafts commenced. Behavioral testing began 5 months post-surgery. Latency to eat a novel food in the novel T-maze environment was used as an index of habituation. Food-deprived subjects were exposed to the situation for a limited time each day and had to meet a criterion of immediate ($\leq$ 5 sec.) food consumption. Unoperated controls (n=3) habituated rapidly (median =5 days) compared to lesioned animals (n=3; 12 days). Lesioned animals hosting grafts (n=3) required an intermediate length of time (8 days) to habituate to the novel circumstances. Likewise, when acquisition of the alternation habit was examined, animals with grafts fell between the behavioral extremes represented by lesioned and control groups (median days to acquisition: lesioned=7, lesioned + graft=11, controls=14). No significant difference in memory performance was detected. Following completion of ongoing behavioral studies subjects will undergo histofluorescence analysis of lesion-induced denervation and the extent of reinnervation by grafted neurons. The results suggest that noradrenergic neurons transplanted near the dorsal hippocampus can partially compensate for the behavioral deficits produced by dorsal bundle lesions. Furthermore, this paradigm suggests that the dorsal noradrenergic system may participate in the behavioral adjustments associated with habituation and learning, with less influence on processing of recent memory. Supported by Fellowship MH 08829 (T.C.), and Grants NS 15109 (D.G.) and AG 00847 (J.S.)

162.18 LESIONS IN ASCENDING NORADRENERGIC PATHWAYS AND WHEEL-RUNNING ACTIVITY. W. E. Gladfelter, Dept. of Physiology, West Virginia University Medical Center, Morgantown, WV 26506.

The placement of bilateral electrolytic lesions in the ventrolateral hypothalamus (LH) of rats produces a marked decrease in wheel-running activity. Ascending noradrenergic fibers may be damaged by these lesions. An experiment was done to determine whether extra-hypothalamic damage to these fibers can produce a decrease in wheel-running activity similar to that observed after placement of electrolytic LH lesions.

Male Sprague-Dawley rats (24) were housed individually in activity cages, consisting of a small living compartment, and an activity wheel with a mechanical counter. The cages were kept in a room in which temperature was maintained at 21 ± 2°C and the room lights were on a 12 hour on-off cycle. Water was present at all times but food was restricted to maintain body weight at 280 ± 5 grams throughout the experiment. After weekly wheel-running activity was measured for a control period of 8 weeks duration, midbrain electrolytic or chemical lesions were placed in either the ascending ventral or dorsal noradrenergic pathways, or in both ascending pathways. Electrolytic lesions were made by passing an anodal current of 1 ma. for 5 seconds through an insulated (except for the tip) nichrome electrode. Chemical lesions were made by infusing, over a period of 1½-2 minutes, 2 μl of a solution of 6-hydroxydopamine (5 μg/μl in 0.9% NaCl solution containing 0.2 μg ascorbic acid/μl) through a cannula made from 27 gauge stainless steel tubing by a Harvard Apparatus infusion pump. After the placement of the lesions, the rats were returned to their activity cages and weekly wheel-running activity was measured for another 8 weeks. Then the rats were killed by decapitation and their brains were removed. The hypothalamus, caudate nucleus, and cerebral cortex were removed separately from each brain and these tissues were prepared for analysis of endogenous noradrenalin and dopamine content. The midbrain of each brain was placed in 10% formalin solution and prepared for histological verification of the lesion sites.

Postoperative wheel-running activity was not significantly decreased below that of controls in any of the lesion groups. But a few individual rats in each group did have a decrease in wheel-running activity that was greater than that of any control rat. However, there was no significant correlation (Spearman's rank correlation) between the magnitudes of the changes in postoperative wheel-running activity and the level of hypothalamic noradrenalin or dopamine, or caudate dopamine content. (Supported by WVU Med. Corp.and NIH Biomed. Res. Grant 5 SO7-RR05433-18.)

162.19 CENTRAL NORADRENERGIC NEURONS: CORRELATION OF SPONTANEOUS ACTIVITY WITH SYMPATHETIC TONE. P.B. Reiner and A.R. Morrison, Labs of Anatomy, School of Veterinary Medicine, University of Pennsylvania, Phila., Pa., 19104

A subpopulation of neurons scattered throughout the dorsolateral pontine tegmentum, and especially within the noradrenergic cell-rich nucleus locus coeruleus (LC), are virtually silent during paradoxical sleep (PS). We have investigated two hypotheses which might explain this phenomenon. The first is that the silence of these neurons during PS is functionally linked to the antigravity muscle atonia which characterizes that state. To test this, we have recorded LC neuronal activity in cats in which muscle tone persists during PS, the PS without atonia preparation. We have found 2 cells in the LC which are silent during PS without atonia and are otherwise indistinguishable from our larger group of 29 PS-off cells in normals. Although pontine lesions which release muscle tone during PS may alter the firing of some neurons during PS, these data indicate that at least some central noradrenergic neurons are silent while muscle tone persists. Furthermore, while attemting to characterize these neurons pharmacologically with the alpha-adrenergic agonist clonidine, a potent inhibitor of central noradrenergic neurons, we noted that muscle tone persisted throughout the period of inhibition of LC neuronal activity. Thus we have identified at least two instances in which we can dissociate the silence of these neurons from muscular atonia.

We have also tested the hypothesis that the silence of LC neurons during PS is functionally linked to the reduction of sympathetic tone (SYM) which has been reported to occur during that state. We have confirmed, via tripolar nerve cuff recordings, that there is indeed a tonic reduction of cervical SYM during normal PS. Although we have no direct recordings of SYM during PS without atonia, our indirect evidence is consistent with the notion that SYM is reduced in this state as well. For example, during both normal PS and PS without atonia, the nictitating membrane is prolapsed and the pupil is miotic. As noted above, clonidine, which is thought to act as an anti-hypertensive agent at least partly through sympatholytic effects, inhibits the spontaneous activity of central noradrenergic neurons. Thus we have been unable to dissociate the silence of central NA neurons from reductions of SYM. These data tentatively suggest a functional link beteen the activity of central noradrenergic neurons and peripheral SYM.

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162.20 CENTRAL NORADRENERGIC NEURONS: KOLLIKER-FUSE NEURONAL ACTIVITY VARIES ACROSS BEHAVIORAL STATE. T.D. Parsons*, P.B. Reiner and A.R. Morrison (SPON: R. Miselis) Labs of Anatomy, School of Veterinary Medicine, Univ. of Penn., Phila., Pa.,19104

We have confirmed that neurons in both the locus coeruleus (LC) and the lateral parabrachial nucleus vary their firing rate across behavioral state, becoming virtually silent during paradoxical sleep (PS). These neurons have been presumed to be noradrenergic on the basis of physiological criteria coupled with their anatomical localization to an area containing a large, albeit heterogeneous, population of noradrenergic (NA) neurons. We now report that neurons located in the Kolliker-Fuse (KF) nucleus, at the far lateral edge of the cell group designated A7 by Dahlstrom and Fuxe, also vary their firing rate across behavioral state in a manner very similar to cells lying more medially in the pontine tegmentum.

We have recorded 10 cells in the KF of the freely-moving, unanesthetized cat. Four cells were classified as PS-off cells using criteria identical to those used for neurons in the LC. Mean firing rates across behavioral state were: AW=42.75, QW=24, SWS=8.25, PS=0.5 spikes/min. These neurons, like PS-off cells in the LC, were easily and consistently driven by auditory stimuli. In addition to these anatomical and physiological data, we have also begun to classify these neurons pharmacologically. Clonidine, a potent alpha-adrenergic agonist, is reported to silence central noradrenergic neurons in acute preparations, presumably through the activation of autoreceptors. In unanesthetized cats, we have found small doses of clonidine (3-5 ug/kg, I.V.) to inhibit the activity of 3/3 PS-off cells tested in KF.

These data lend credence to the notion that the PS-off cells found both within the LC and throughout the dorsolateral pontine tegmentum are indeed noradrenergic. Furthermore, anatomical studies have shown that different NA cell groups may have different terminal fields, thereby suggesting different functions. Nevertheless, our electrophysiological results indicate that at least with respect to behavioral state, there appears to be functional homogeneity among some central NA neurons.

(Supported by NIH grants NS-13110 and GM-07170)

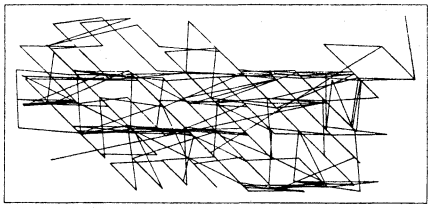
162.21 HYPERKINESIS IN RATS SELECTIVELY PRODUCED BY INTERMEDIATE SIZE RIGHT OCCIPITAL CORTICAL SUCTION LESIONS. K.L. Kubos*, T.H. Moran*, K.M. Saad*, P.R. Sanberg and R.G. Robinson. (SPONS: G.D. Pearlson) Dept. of Psychiatry and Behavioral Sciences, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

We have previously reported that right middle cerebral artery (MCA) ligation has profound effects upon behavior, as well as catecholamine concentrations (Robinson and Coyle, 1980) and produces a fronto-cortical infarction approximately 2mm in diameter. Conversely, left ligation, while producing a comparable 2mm ischemic lesion, was without effect. A subsequent investigation (Pearlson and Robinson, 1981) found that comparably placed right hemispheric (RH) suction lesions averaging 1.25mm in diameter also induced behavioral and biochemical effects similar to those produced by the ischemic lesions. Further investigation indicated that the ability of 1.25mm RH suction lesions to induce behavioral and biochemical alterations diminished as the distance from the frontal pole increased (Pearlson, et al., 1982). The efficacy of frontal compared with posterior lesions in producing bio-behavioral alterations has been postulated to be related to the relative anterior compactness of backward coursing cortical NE fibers and the consequent greater damage to these fibers by anterior ablations. We therefore examined the effects of producing 0.7, 1, 1.5 or 2mm diameter suction lesions in rat occipital cortex. The previous hypothesis predicted that posterior lesions would need to exceed the size of anterior lesions to produce comparable effects upon NE and therefore locomotor activity. In agreement with this hypothesis we found that the larger the area of aspirated tissue, the greater was the NE depletion from the ipsilateral frontal cortex. However, only the 1mm diameter lesion which produced the third largest NE depletion induced hyperactivity as measured in running wheels. Thus, while the largest (2mm) diameter lesion produced the greatest NE depletion, only the third largest (1mm) ablation studied induced hyperactivity. These results indicate that the ability of the posterior cortical lesion to produce hyperkinesis is independent of its effects on cortical NE.

162.22 HALOPERIDOL DISRUPTS THE SPATIAL PATTERNING OF EXPLORATION BY RATS. L.M. Adams* and M.A. Geyer. (SPON: E. Battenberg). Dept. of Psychiatry, Univ. of Calif., San Diego, CA 92093.

Despite numerous reports of the ability of haloperidol (HAL) to antagonize the behavioral effects of dopamine-agonist drugs, little is known of the effects of HAL alone, particularly in low doses.

Rats were treated with saline or 15 µg/kg HAL, returned to holding cages for 20 min, then injected with saline or 60 µg/kg of the dopamine agonist lisuride 10 min before being placed in a 12x24" behavioral pattern monitor where crossovers, rearing and holepokes were monitored. Routes of locomotion were reconstructed from stored sequences of X-Y position changes. Fifteen µg/kg HAL produced a slight increase in all activity measures (significant in the last half of the hour session), yet reduced by 50% the hyperactivity induced by lisuride. HAL also produced a marked disruption of the normal patterning of exploration. The normal progression from circling the perimeter to traversing through the center, and finally establishing a "home" corner from which excursions are made along a small set of preferred routes was not seen in HAL-treated rats. Beyond the first 10 min, rats treated with HAL exhibited complex, contorted routes of locomotion, with an apparent disregard for distinctions between center and periphery (see below). Additionally, they failed to establish a "home" corner or preferred route of locomotion. This effect of 15 µg/kg HAL was replicated in a subsequent dose-response study. Further, HAL's disruption of exploratory patterns was also seen at doses of 5 and 45 µg/kg, while 135 µg/kg HAL completely suppressed locomotion beyond the first 5 min of the session. Further data on the dose-dependency of HAL's effects on activity measures will be presented. The possibility that disruption of spatial patterns by low doses of HAL is related to nigral activation via DA autoreceptors will be discussed. Supported by NIDA02925.



15 µg/kg HAL 40-60 min from test start

163.1 THE ROLE OF NORADRENALINE IN TASTE AVERSION LEARNING IN THE RAT. L.T. Dunn*and B.J. Everitt* (SPON. European Neuroscience Association) Dept. of Anatomy, University of Cambridge, Cambridge, U.K.

Previous reports have inconsistently implicated cortical noradrenaline in the extinction but not the acquisition of learned taste aversions, as well as in extinction in certain other instrumental learning situations. However in these studies cortical noradrenaline was manipulated using either electrolytic lesions or, in those cases where neurochemical lesions were employed,have resulted in large depletions of hypothalamic as well as cortical noradrenaline.

Consequently we undertook a series of studies using 6-hydroxydopamine lesions of either the dorsal noradrenergic bundle (DNAB) innervating primarily cortex, or the ventral noradrenergic bundle (VNAB) innervating primarily hypothalamus. Typically the DNAB lesion produces a 95% depletion of cortical noradrenaline accompanied by a 50% depletion in the hypothalamus whilst the VNAB lesion produces 70% depletion of hypothalamic and 20% depletion of cortical noradrenaline, as measured by high performance liquid chromatography with electrochemical detection.

The effects of these lesions were studied in a variety of paradigms using 23 hour fluid deprived male hooded rats. 0.2% saccharin solution was used as the conditioning stimulus and 0.15 M LiCl (20ml/Kg) as the unconditioned aversive stimulus, given in a single i.p. administration immediately following the conditioning trial.

Using a single bottle no choice test no differences were observed in volume of fluid ingested following the DNAB lesion whilst a deficit in extinction was found in the VNAB lesioned animals. This effect was replicated using the more sensitive two bottle test where the animals are given a choice between saccharin and water.

These results may reflect a true extinction effect similar to that claimed to follow depletion of cortical noradrenaline. Alternatively, they could represent an enhanced acquisition of the association between the taste and subsequent illness, initially masked by a floor effect, due to some change in sensory processing or change in emotionality which have both been proposed to follow VNAB lesions.

163.2 BEHAVIORAL ALTERATIONS FOLLOWING REPEATED EXPOSURE TO UNCONTROLLABLE FOOT-SHOCK OR DESMETHYLIMIPRAMINE. R.M. Zacharko, N.J. Bowers*, C. Prince*, and Hymie Anisman. Dept of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

It has been postulated that following repeated application of uncontrollable stressors several adaptive neurochemical changes occur. Among other things, the synthesis and utilization of norepinephrine (NE) are increased, and subsensitivity of β-NE receptors and dopamine (DA) autoreceptors have been reported. These adaptive neurochemical changes have been proposed as being responsible for the elimination of the behavioral deficits attributable to acute uncontrollable stressors. In a similar fashion, following repeated treatment with tricyclic antidepressants, β-NE receptor subsensitivity ensues, and it is thought that the alterations of receptor sensitivity are responsible for the therapeutic efficacy of the drug. Parallels have been drawn between the effects of repeated uncontrollable stressor effects and those elicited by tricyclic antidepressants. That is, both represent a neuronal adaptation which results in blunting of negative affect. In the present investigation the effects of repeated uncontrollable foot-shock and chronic treatment with desmethylimipramine were evaluated on several behavioral paradigms in mice, which are thought to be adequate models of human depressive disorders. Following application of uncontrollable footshock (360 shocks of 2 sec duration, 150 uA) performance was disrupted in a shuttle escape task, a water-swim paradigm ("behavioral despair paradigm") and in responding for electrical stimulation from the nucleus accumbens. These disturbances were not eliminated among mice that received repeated application of the uncontrollable stressor over 14 successive days. Indeed, the behavioral disturbances in each of these paradigms were greatly exacerbated. In contrast, repeated application of desmethylimipramine (5 - 10 mg/kg bid) resulted in reduction of the performance deficits seen in each of these tasks. Chronic DMI has been reported to provoke both β -NE receptor subsensitivity, and subsensitivity of DA autoreceptors, whereas repeated footshock results in NE neuronal alterations, without appreciable adaptation with respect to DA neuronal activity. Accordingly, the differential effects of these treatments may be attributable to the alterations of DA autoreceptors provoked by DMI. Moreover, the results suggest that the effectiveness of DMI in antagonizing behavioral disturbances are due to adaptive changes beyond those which occur following repeated stressor application.

163.3 BEHAVIORAL AND NEUROCHEMICAL SEQUELAE OF MAGNESIUM DEFICIENCIES. K. M. Kantak, N. Hale*, S. Izenwasser*, A. Meyers* and D. Zucker*. Lab. of Behavioral Neuroscience, Dept. Psychology, Boston Univ., Boston, MA 02215.

In the present series of experiments, the effects of dietary magnesium (Mg), which was limited to 15%, 25%, 50%, 75% and 100% of the daily requirement, were examined in male and female adult mice for its influence on offensive aggression (using the resident-intruder paradigm) and brain catecholamine function.

The results demonstrated that after 2 weeks, there was a 50% decrease in offensive threat postures and a 55% decrease in offensive biting attack in male residents fed the 15% diet. After 6 weeks, these decreases in threat and attack behaviors were sustained. Additionally at this time, there was a 36% and 37% decrease in threat and attack behaviors in male mice fed the 25% diet. These behaviors in male mice fed the 50% and 75% requirements of Mg were within normal limits of the 100% control. The aggressive behavior of female residents was highly variable and thus analysis revealed no consistent dietary influences. That these different deficienceis were affecting female physiological processes was evident from data on reproductive sucesses. Female residents fed the 15% diet failed to get pregnant, or after doing so, spontaneously aborted the fetuses. Of females fed the 25% diet, only half delivered live young. However, the average litter size was reduced by one half and all pups died within 2 or 3 days of birth. In the 50%, 75% and 100% diet groups, all females delivered normal sized litters which survived through weanling.

An assessment of catecholamine function was made after 7 weeks on the various Mg deficient diets. To study dopamine function, the amount of stereotyped sniffing induced by 2 mg/kg apomorphine was compared in males and females across groups. A decrease in the amount of stereotyped sniffing was evident in both sexes fed the 15% and 25% diets. L-amphetamine (1.5 mg/kg) induced locomotion and rearing was measured to study norepinephrine function. A decrease in the amount of induced motor behavior was evident in males fed the 15% and 25% diets, and in females fed the 15%, 25% and 50% diets. The behavioral changes described above were not related to alterations in the amount of food ingested or body weight level. These measures remained within normal limits throughout the experiments for all groups examined.

In summary, a deficiency of Mg limited to 15% and 25% of the daily requirement reduced offensive aggression in male mice, reduced reproductive sucesses in female mice and reduced catecholamine function in both sexes. This reduction in catecholamine function may be the basis for the observed decline in offensive aggression.

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163.4 3H-SPIROPERIDOL BINDING IN DIFFERENTIALLY HOUSED MICE. C.A. Wilmot* and C. VanderWende (SPON: A.Feldstein). Dept. of Pharmacology, Rutgers Univ., Piscataway, NJ 08854.

The social isolation of male mice for 4-8 weeks produces a behavioral syndrome characterized by heightened reactivity, increased behavioral sensitivity to central stimulants and intense fighting behavior towards conspecific males. We have previously determined that significant differences in the behavioral responsiveness to amphetamine, apomorphine and phencyclidine of group-housed (GH) and individually-housed (IH) mice were most marked within the first 2 wks of differential housing(DH). To assess whether changes in central dopamine receptor number or affinity contributed to these behavioral differences, regional spiroperidol binding experiments were conducted.

Male CF-1 mice were differentially housed, individually or in groups of 20-25, commencing at 5 wks of age. After 0,14,35,40,45,50 and 60 days, both GH and IH mice were tested for fighting behavior towards an intruder male, olfactory bulbectomized, after which the mice were decapitated, and striatal(STR), limbic(LIMB) and frontal cortex(FC) tissues were taken and pooled from 8-10 mice according to housing condition and the presence or absence of fighting behavior. Tissue samples were frozen at -70C. until used. Saturation isotherms were derived with ligand concentrations ranging from 0.05 to 8 nM.

STR tissue consistently yielded a linear Scatchard plot with no significant differences among the GH from 0-60 days DH, Bmax= 613$\pm$14 fmoles/mg protein and Kd= 0.34$\pm$.06nM (mean$\pm$SEM), whereas the Bmax of IH STR declined to 87% of the GH by 50-60 days of DH with no change in Kd. A different trend was obtained from LIMB and FC tissues. At the onset of DH, Scatchard analysis was best fit by a single site model of lower affinity than the STR site: LIMB- Bmax 121 fmoles/mg prot., Kd 1.2nM; FC Bmax 304 fmole/mg prot., Kd 1.9nM. A curvilinear plot was consistently obtained from 14-60 days DH in both GH and IH with a high-affinity site Kd .1nM and low-affinity site Kd 2-3nM. A relative increase in the number of high-affinity sites in the FC of IH mice was noted. These results suggest that the development of different behavioral responses in GH and IH may be accompanied by changes in spiroperidol binding.

]Supported by NIMH Fellowship 5F31MH08873 to C.Wilmot and Busch Foundation Grant to C. VanderWende.

163.5 AFFINITY FOR THE DOPAMINE D_2 RECEPTOR PREDICTS NEUROLEPTIC POTENCY IN BLOCKING THE REINFORCING EFFECT OF MFB STIMULATION. Adam Davis* and C.R. Gallistel (Sponsor: Dr. M. R. Kare) Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104.

For each of nine neuroleptics, the dose required to block sustained responding for intracranial stimulation of the medial forebrain bundle was determined in the rat. To check whether the blocking of responding was due to effects on reinforcement as opposed to effects on performance factors, the rats were always tested for task-specific extinction of responding by transferring them to another testing box once they refused to respond in the first testing box. With all the neuroleptics, task-specific extinction was not seen in control tests with a general anaesthetic (Chloropent) nor with picrotoxin, a drug that can produce pseudo-extinction. Affinity for the dopamine D_2 receptor (from in vitro studies) predicted neuroleptic potency in blocking reinforcement, whereas affinity for other aminergic receptors (D_1, D_3, the α- adrenergic receptor, S_1, and S_2) did not.

163.6 PIMOZIDE AND CHLORPROMAZINE REDUCE REINFORCEMENT EFFICACY AND MOTOR CAPACITY IN THE RAT. G.M. Heyman*, D.L. Kinzie*, L.S. Seiden. (SPON: G. Gudelsky). Dept. Pharmacol. Physiol. Sci., University of Chicago, Chicago, IL 60637.

In two experiments, rats (8 subjects per group) responded for water on a series of 5 different variable-interval reinforcement schedules. The relationship between response rate and reinforcement rate was accurately described by the equation for a rectangular hyperbola (e.g., the Matching Law, the Michaelis-Menten equation). This equation has two parameters. One is equivalent to the asymptotic response rate, and the other is equivalent to the rate of reinforcement that maintains half-asymptotic responding. Experimental evidence indicates that the asymptotic response rate measures motor capacity and that the rate of reinforcement necessary for half-asymptotic responding measures reinforcement effectiveness. Pimozide and chlorpromazine selectively and independently changed both parameters. A 0.1 mg/kg dose of pimozide increased the rate of reinforcement necessary for half-asymptotic responding by about 86%, and a 0.75 mg/kg dose of chlorpromazine increased this parameter by about 46%. However, at these dose levels, neither drug changed the asymptotic response rates. At higher doses, the neuroleptics changed both parameters. A 0.20 mg/kg dose of pimozide decreased the asymptotic response rate by about 20%, and it increased the reinforcement parameter by an additional 23%. A 1.5 mg/kg dose of chlorpromazine decreased the asymptotic response rate by about 15% and it increased the reinforcement parameter an additional 63%. Higher doses of both drugs either eliminated responding or disrupted it to the extent that it was not possible to fit a rectangular hyperbola to the response rates. Since an increase in the rate of reinforcement necessary for half-asymptotic responding is equivalent to a decrease in the proportion of responding maintained by a given rate of reinforcement, the lowest doses of pimozide and chlorpromazine selectively decreased reinforcement efficacy, and the higher doses decreased both efficacy and motor capacity. Using standard deviation scores to measure changes in the parameters, the ratio of equally effective doses was about 1.0 and 7.5 (pimozide to chlorpromazine). This ratio approximates the ratio of ED_{50} values for two different behavioral tests: inhibition of shock avoidance and antagonism of amphetamine induced stereotypy. Comparisons with other experiments in our laboratory support the hypothesis that catecholamines contribute to the physiological processes that mediate reinforcement. For example, amphetamine's effect on the parameters of the matching equation is just the opposite of those of the neuroleptics. Supported by USPHS MH-14274, Training Grant; PHS MH-011191; RSA MH-10562.

163.7 EFFECTS OF INESCAPABLE SHOCK ON CEREBRAL BLOOD FLOW AND CEREBROVASCULAR PERMEABILITY IN RATS. C.W. Hughes, T. Kent*, J. Campbell*, H. Croskell*, and S.H. Preskorn. Depts. of Psychiatry & Pharmacology, Univ. of Kansas Sch. of Med., Kansas City, KS 66103.

Various inescapable shock (IS) paradigms have been suggested as animal models of human neuropsychiatric disorders. The behavioral deficits observed in these animals have been reproducibly related to an approximately 20% reduction in norepinephrine (NE) in the locus coeruleus (Weiss et al., Brain Res. Rev., 1981,3:167). What physiological consequences a depletion of this magnitude causes is uncertain. We therefore sought to examine this issue by testing the effects of IS on the responsivity of the cerebral microcirculation to metabolic demand. This responsivity appears to be in part regulated by the locus coeruleus and related adrenergic neurons.

A duel label isotope procedure (Irwin and Preskorn, Brain Res., 1982) was used to simultaneously measure CBF and PS to water. Three groups were tested: IS studied one hour after exposure, IS controls, and untreated animals. Both IS and IS control rats were individually confined in a 15 cm(w) X 46 cm(l) X 50 cm(h) chamber with a 0.3 cm dia grid (spaced 1.5 cm center to center) floor for sixty minutes. The IS treatment consisted of alternating periods of 0.8ma scrambled, constant-current shock (10 secs.) followed by ten seconds of no shock. Following treatment, rats were removed and placed in a holding cage until PS and CBF measurements were made.

	PS vs CBF	CBF vs $PaCO_2$	N
IS Shock	y= .8 + .54x F=119	y= -.56 + .06x F=90	11
Control*	y= .97 + .51x F= 44	y= .05 + .05x F=42	19
*Pooled:	no difference between naive and IS controls		

The table above is based on the average value obtained for the rostral and caudal telencephalon and diencephalon. Examination for regional changes in these individual areas as well as in the medulla-pons and cerebellum are in progress. Preliminary analysis of brain levels of the biogenic amines suggest that alterations in central catecholamines do occur with this treatment.

The PS and CBF findings suggest that IS treatment which produces approximately a 20% depletion of NE does not alter the regulation of the cerebral microcirculation. These parameters appear to be regulated by the central adrenergic system and such neuromodulation appears to be resistant to a depletion of this magnitude. However, such regulation is undoubtably only one of many functions for this system. (Supported in part by MH-00272 and NS-17252 and the PMAF.)

163.8 REGIONAL BRAIN MONOAMINE LEVELS AND TURNOVER IN AUDIOGENIC SEIZURE SUSCEPTIBLE AND NON-SUSCEPTIBLE MICE. N. Mogharreban* and C. E. Lints. Dept. of Psychology, Northern IL Univ., DeKalb, IL 60115.

DBA/2J mice are genetically prone to audiogenic seizures (AGS) and show a peak susceptibility between 19-21 days of age. Although several studies have reported that these mice have lower brain levels or norepinephrine (NE) and serotonin (5-HT) than seizure resistant strains at the age of peak AGS susceptibility, we have been unable to replicate these findings for whole brain (Lints, C.E., Willott, J.F., Sze, P.Y. and Nenja, L.H., Pharmac. Biochem. & Behav., 12:385, 1980). Because whole brain assays can mask important regional differences, this study compared seizure prone DBA with seizure resistant C57Bl/6J mice for both levels and turnover of NE and 5-HT in six discrete regions of the brain.

19-21 day old DBA and C57 littermates of both sexes served as subjects. Each subject was sacrificed by decapitation and the brain was quickly removed and dissected into brain stem (pons and medulla), cerebellum, caudal midbrain, rostral midbrain, diencephalon, and telencephalon. The tissue was then weighed, frozen in liquid nitrogen, and stored in a freezer until assay within two weeks. Each sample consisted of tissue pooled from the brains of six littermates except for the telencephalon, where tissue from only two littermates was included. Following a butanol extraction the amine levels were measured fluorometrically. Turnover was determined by measuring the accumulation of NE and 5-HT two hours after an i.p. injection of pargyline (400 mg/kg), a monoamine oxidase inhibitor.

At the age of peak AGS susceptibility, no statistically significant differences between the seizure prone DBA and seizure resistant C57 strains of mice were found for either levels or turnover of NE and 5-HT in any of the six brain regions studied. These data support our previous observations made upon whole brain assays.

163.9 AGGRESSION AND BRAIN MONOAMINES IN DOMINANT AND SUBORDINATE PIGEONS. Irving Goodman, Rosalind Burns*, Albert Azzaro & David Smith
Departments of Psychology, Neurology & Anesthesiology, West Virginia University, Morgantown, WV 26506.

Pairs of male pigeons, when placed in a restricted environment, tend to settle the problem of territorial control with an initial period of aggressive interaction. The resulting victor or dominant member goes on to display such behaviors as bow-cooing, chasing, shoving, wing-slapping and pecking attacks. The subordinate member typically displays avoidance and escape behavior with no apparent vocalization. These roles, once established, are maintained over days of testing, although specific behaviors may vary in intensity and frequency. The present study tried to determine if the concentrations of monoamines (dopamine (DA), norepinephrine (NE) and serotonin (5-HT) within the telencephalon, diencephalon, and brain stem varied in relation to aggressive role and number of days subjected to aggressive interaction.

Video monitored male pairs of prescreened dominant and subordinate pigeons were tested in a 43 x 90 x 52 cm enclosure over 2 - 8 daily sessions, each lasting 15 min. Birds were sacrificed within 1 hr following the last observation and high performance liquid chromatographic (HPLC) assays of brain monoamines were carried out soon thereafter. Statistical comparisons of monoamine concentrations in different brain regions within dominant and subordinate individuals with those from randomly selected controls (handled but neither screened nor aggression-tested) revealed the following significant comparisons. Subordinate groups tested for 2, 4 or 8 days had higher DA concentrations in the brain stem and higher 5-HT concentrations in the telencephalon. On the other hand, dominant birds had elevated NE concentrations in the telencephalon and lowered 5-HT concentrations in the diencephalon.

These findings suggest that, depending upon the aggressive role expressed, (1) monoaminergic concentration shifts affect different brain regions, (2) different combinations of monoamines show concentration changes and (3) both dominant and subordinate role experiences are associated with significant concentration changes in in more than one monoamine. Whether the observed levels of monoamines in dominant and subordinate pigeons represent causal or associational relationships is unanswered by this study.

163.10 SHORT AND LONG TERM EFFECT OF LITHIUM ON BRAIN CATECHOLAMINE. D. Ghoshdastidar* and M. K. Poddar.
Department of Biochemistry, University College of Sciences, University of Calcutta, Calcutta- 700 019, INDIA.

The efficacy of lithium chloride in the therapy of mania is now known. During the last few years much speculation about its(lithium ion) mode of action has raised its effect on brain catecholamine regulation.

In vivo intraperitoneal administration of lithium chloride (100 mg/kg) to adult male albino rats(120 - 150 g) showed that short term exposure for different time intervals(15, 30, 60 and 120 min) produced a slight but significant decrease in endogenous dopamine (DA) at 120 min(23%) and noradrenaline(NA) at 30 min (34%). Long term exposure for 8 and 14 days at a dosage 100 mg/kg/day significantly decreased only DA (53% and 26% respectively). No significant change was noted in endogenous NA concentration under similar condition of treatment. The measurement of turnover rate of DA and NA, using non-isotopic method, showed that lithium increased the turnover rate of both DA and NA both under short and long term exposures. These results suggest that lithium induced changes in central catecholamine metabolism may have some role at least in part to explain the psychomotor action of lithium in mania.

(supported by Indian Council of Medical Research, New Delhi, India.).

163.11 NEUROCHEMICAL ADAPTATION FOLLOWING CHRONIC STRESS. J. Irwin and H. Anisman. Dept Of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Acute exposure to physical or psychological insults results in increased utilization of brain norepinephrine, and may reduce concentrations of this amine in some brain regions. Following repeated exposure to a stressor a series of adaptive changes occur, including a compensatory increase in NE synthesis, such that the amine reduction is no longer evident. A series of experiments assessed amine utilization and concentrations with repeated exposure to stressors, and determined the persistence of the NE variations following termination of the stressor. A single session of inescapable shock (360 shocks of 2 sec duration, 150uA) increased utilization of NE in hypothalamus, hippocampus, cortex and locus coeruleus, and reduced the concentrations of this amine in some brain regions (primarily hypothalamus). Serotonin (5-HT) concentrations, in contrast, increased in most brain regions examined among mice that received acute shock. Following 14 sessions of shock administered on successive days, adaptation was observed, such that the NE reductions were eliminated, while the 5-HT concentrations approached those of nonstressed animals. Amine concentrations were found to vary with time following chronic stressor termination. Immediately after chronic stressor termination NE concentrations were comparable to those of nonstressed animals. However, within several hours of stressor termination NE concentrations in all regions examined significantly exceeded those of nonstressed mice. The enhanced NE concentrations persisted for as long as 168 hr in hypothalmus, but in the locus coeruleus, hippocampus and cortex the increased NE concentrations were evident for only 24 hr following stressor application. Evaluation of NE utilization determined immediately after chronic stressor application revealed increased utilization of the amine. However, several hours following stressor termination NE utilization rates were reduced relative to nonstressed mice. The enhanced amine concentrations are likely attributable to increased synthesis coupled with reduced utilization of the amine. These data suggest that several adaptive changes occur following repeated stressor application. Upon exposure to the stressor among chronically shocked mice utilization of NE increases in order to contend with the immediate environmental demands. Upon termination of the stressor utilization rates decline, while increased synthesis persists, thereby resulting in greater concentrations of the amine. As a result the organism may be better prepared to deal with impending stresses.

163.12 RAPHE UNIT ACTIVITY IN FREELY MOVING CATS: LACK OF EFFECT OF DIETARY FACTORS. M.E. Trulson. Dept. of Pharmacol., Marshall Univ. Sch. of Med., Huntington, WV 25701.

Several studies have indicated that central serotonin (5HT) metabolism can be influenced by dietary factors. Brain tryptophan, and consequently 5HT metabolism, is regulated by the ratio of plasma tryptophan/competitive neutral amino acids (tyrosine, phenylalanine, leucine, isoleucine and valine). Ingestion of a standard laboratory diet results in no change in brain tryptophan or 5HT metabolism, because this diet contains all of the neutral amino acids. Ingestion of a diet lacking the competitive neutral amino acids but containing tryptophan results in increased brain tryptophan levels and increased 5HT metabolism, while a tryptophan-free diet containing the competitive neutral amino acids decreases brain tryptophan and 5HT metabolism. All of these findings are based on neurochemical studies. The present report examines this issue more directly, by investigating the effects of dietary manipulations of the activity of single 5HT-containing neurons in the dorsal raphe nucleus of freely moving cats. Unit activity was recorded by means of movable 32 or 64 μ dia. insulated nichrome wires (see Brain Res. 163, 1979, 135-150 for complete methodology). Unit activity was recorded across the complete sleep-wakefullness-arousal continuum, and the cats were then food deprived for 24 h (water was available ad lib). At the end of the 24-h food deprivation period, the cat was presented with one of the following diets: (A) standard laboratory cat chow; (B) a diet containing normal amounts of tryptophan but lacking the competitive neutral amino acids; or (C) a tryptophan-free diet containing normal amounts of the competitive neutral amino acids. Unit activity was minitored for 4 h after the meal. The data revealed that unit activity did not change significantly (2.46 ± 0.13 spikes/sec) during the food deprivation period, as compared to baseline levels (2.59 ± 0.22 spikes/sec). Presentation of food resulted in an arousal state, which significantly, but transitly, increased unit activity (3.03 ± 0.10 spikes/sec). Unit activity was not significantly changed from baseline levels following meal ingestion, for any of the three diets tested (Diet A, 2.63 ± 0.18; Diet B, 2.39 ± 0.20; Diet C, 2.66 ± 0.19 spikes/sec). Neurochemical analyses revealed the following: Diet A resulted in no significant changes in brain tryptophan (-2.7%), 5HT (+3.8%) or 5HIAA (-3.2%) from control levels. Diet B resulted in a significant increase in tryptophan (+56.4%), 5HT (+12.9%) and 5HIAA (+30.7%), while Diet C produced significant decreases in brain tryptophan (-48.6%), 5HT (-19.1%) and 5HIAA (-34.6%). These data confirm that dietary manipulations produce significant changes in brain tryptophan and 5HT metabolism, but the altered 5HT metabolism apparently does not change the functional activity of serotonergic neurons, since no changes in raphe unit activity were observed.

163.13 THE RELATIONSHIP BETWEEN ELECTRICALLY ELICITED SNIFFING AND SELF-STIMULATION IN THE RAT. J. Rossi III and J. Panksepp. Department of Psychology, Bowling Green State University., Bowling Green, OH 43403

Sniffing behavior has frequently been observed to be an unconditional consequence of electrical stimulation of hypothalamic circuits which support intra-cranial self-stimulation (ICSS). The present studies analyzed the relationship between elicited sniffing and ICSS obtained from the same electrodes, and assessed how thresholds of the two measures co-varied after food deprivation and various pharmacological manipulations.

Sniffing thresholds were determined by video analysis of restrained rats administered computer-controlled 2 sec, 5uA (sine wave) current steps. Thresholds were determined by 2 successive current steps eliciting sniffing responses. ICSS thresholds and rates were determined for touch responses to an intermittently illuminated aluminum tea-ball in a darkened chamber, during 10 min intervals which were initiated following 5 rewarded contacts (.5 sec current trains/contact @ 5uA). At the end of each 10 min period the current was incremented by 5uA. Thresholds were defined by 80 or more contacts (i.e., pre-determined 1% confidence limit) at two successive current intensities.

The average sniffing threshold current for 31 rats with electrode placements in the posterior lateral hypothalamus was 14.2uA, and their mean self-stimulation threshold was 16.8uA. These thresholds were reliably correlated [r=.93, p<.001]. For 16 rats with mid-lateral hypothalamic placements the mean sniffing threshold was 17.5uA, the average ICSS threshold was 21.8uA, and these thresholds were also highly correlated [r=.87, p<.001]. There was no reliable difference between the placements for sniffing thresholds, although ICSS thresholds from the posterior sites were found to be reliably lower.

Sniffing thresholds were reliably reduced by 24 hrs food deprivation, but this maneuver affected neither ICSS thresholds nor rates. Both sniffing and ICSS thresholds were reliably reduced by 48 hrs deprivation, and ICSS rates were reliably elevated.

Haloperidol (0.1,0.25,0.5 mg/kg) failed to reliably alter sniffing thresholds. ICSS thresholds were reliably elevated by the .25 mg/kg dose and no ICSS behavior was observed at any current tested after the 0.5 mg/kg dose.

D-amphetamine failed to reliably effect either sniffing or ICSS thresholds at any dose tested (0.5,1.0,2.0 mg/kg) although ICSS rates were reliably increased after the two highest doses.

These results suggest that there is a relationship between electrically elicited sniffing and ICSS, and that the commonality of the circuits involved in the expression of each does not extend to the brain dopamine system.

163.14 NEURAL SUBSTRATES OF CONDITIONED EMOTIONAL RESPONSE (CER) J.D. Lane, D.R. Cherek+ and J.E. Smith+. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107; and +Department of Psychiatry, LSU Medical Center, Shreveport, LA 71130.

To evaluate the neurochemical changes associated with CER and its reversal by acute diazepam, groups of littermate rats were conditioned to associate a CS with footshock. These groups, which included controls for shock history, etc. received nothing, vehicle or 5 mg/kg i.p. diazepam, and radiolabelled precursors to neurotransmitters of interest (to evaluate turnover). The binding of tritiated QNB and diazeapm was also assessed in cortical total particulate membrane preparations. On testday, the presentation of CS elicited CER, suppression and collateral behaviors in the conditioned animals, while diazepam-treated animals did not attend to the CS. After 15 min exposure to the CS, all groups were totally frozen, the brains removed, sectioned and microdissected into discrete regions. There were few changes in content of transmitters or metabolites, suggesting that small physiological functional pools were being utilized; however, there were many changes in the turnover of biogenic amines (DA, NE, 5-HT) and amino acids (Glu, Asp, Gly and GABA). In addition, QNB and BZ binding was reduced by CER, and only QNB was partially reversed by diazepam administration (these changes probably represent compensatory reactions to changes in turnover of ACh and endogenous BZ ligands) Changes in the CER paradigm were consistent with the activation of a neural circuit in which DA input from the ventral tegmentum and NE input from the locus coeruleus to the subiculum are depressed, releasing tonic inhibition of Glu efferents to the hippocampus, which in turn stimulates a Glu pathway to the septum. 5-HT input from the raphe to the frontal cortex is depressed which releases tonic inhibition of Glu efferents to the amygdala, caudate-putamen and preoptic-diagonal band region, which in turn stimulate cholinergic efferents to the cortex (inferred from the QNB binding data). Five changes were related exclusively to the CER paradigm, while the remainder are related to three categories of changes in the presence of diazepam--those unique to the drug, those which reversed the effects of CER, and those which enhanced the effect of CER. The changes due to acute diazepam administration were primarily activation of amino acids and mixed changes in the turnover of the biogenic amines. These cannot so easily be evaluated by circuits because of the potential interaction of the three categories of changes. Neurotransmitter-specific pathways are modulating the CER paradigm and reversal by benzodiazepines. [supported in part by NIMH MH 31835 (to JDL)]

TRANSMITTER UPTAKE, STORAGE, AND SECRETION I

164.1 [^{3}H]MAZINDOL BINDING ASSOCIATED WITH NEURONAL DOPAMINE UPTAKE SITES IN CORPUS STRIATUM MEMBRANES. Jonathan A. Javitch*, Robert O. Blaustein* and Solomon H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Drugs which inhibit biogenic amine neuronal uptake have been employed as ligands to label sites on brain membranes associated with uptake systems for norepinephrine and serotonin. Mazindol is a potent inhibitor of both norepinephrine and dopamine uptake and is clinically employed as an appetite suppressant. The binding of [^{3}H]mazindol to rat corpus striatum membranes appears associated with specific neuronal dopamine uptake sites. Scatchard analysis indicates a dissociation constant (K_D) of about 18 nM with a maximal number of binding sites (B_{max}) of 7.3 pmol/mg protein. The Hill coefficient of 1.0 suggests the absence of cooperative interactions.

The absolute and relative potencies of various drugs in competing for [^{3}H]mazindol binding to striatal membranes correlate closely with their potencies in inhibiting dopamine uptake into striatal synaptsomes. Mazindol and nomifensine are the most potent agents. By contrast desipramine and imipramine, which are highly potent inhibitors of norepinephrine and serotonin uptake respectively, are very weak inhibitors of both dopamine uptake and [^{3}H]mazindol binding sites. Dopamine inhibits [^{3}H]mazindol binding less potently than it inhibits dopamine uptake, resembling findings that norepinephrine inhibits [^{3}H]desipramine binding less potently than it inhibits NE uptake (Lee, Javitch and Snyder, J. Neuroscience, 2:1515, 1982). In other brain areas, levels of [^{3}H]mazindol binding are less than 15°/° of those in the corpus striatum. As a further finding, in rat cerebral cortex membranes the potencies of drugs at inhibiting [^{3}H]mazindol binding correlate with their potencies at inhibiting [^{3}H]desipramine binding. This may indicate an association with norepinephrine as well as dopamine uptake sites.

[^{3}H]Mazindol binding to striatal membrane is dependent on sodium with no binding in the absence of sodium and an apparent Km of 250 mM. The sodium stimulation is due to a change in the affinity of the ligand for its binding site with no change in B_{max}. No other cations evaluated, including lithium, potassium, rubidium and Tris, can substitute for sodium.

The ability to label sites associated with dopamine uptake utilizing [^{3}H]mazindol provides a powerful tool for evaluating dopamine neuronal function. Further studies may clarify whether [^{3}H]mazindol interacts with the dopamine uptake recognition site or another allosterically linked site. The regulation of [^{3}H]mazindol binding by sodium should permit an analysis of how dopamine uptake is regulated by this ion.

164.2 BINDING OF THE DOPAMINE UPTAKE INHIBITOR ^{3}H-NOMIFENSINE TO STRIATAL MEMBRANES. M.L. Dubocovich and N.R. Zahniser. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611 and Dept. of Pharmacology, University of Colorado Med. Sch., Denver, CO 80262.

The tricyclic antidepressants desipramine (DMI) and imipramine (IMI) appears to exert their therapeutic action by inhibiting the neuronal uptake of norepinephrine (NE) and serotonin (5HT), respectively. Nomifensine, which is also an antidepressant, is more potent than either IMI or DMI to inhibit the neuronal uptake of dopamine in the striatum. Recent studies demonstrated that radiolabeled ^{3}H-DMI and ^{3}H-IMI have an unique high affinity for the neuronal uptake sites of NE and 5HT, respectively. In this study we have characterized in striatal membranes a binding site for ^{3}H-nomifensine (S.A.: 45 Ci/mmol, custom synthesized by New England Nuclear).

Binding was determined in washed membranes prepared from rat and rabbit striatum in Tris/ClH buffer (NaCl, 120 mM; KCl, 5 mM; pH 7.4) at 0°C. Samples were incubated for 1 hour, and the reaction was terminated by dilution with ice-cold buffer and rapid filtration. The binding of ^{3}H-nomifensine to striatal membranes is time dependent and increased linearly with tissue concentration. Benzotropine (100 μM), used to defined specific binding, inhibited 65% of total ^{3}H-nomifensine binding. Unlike ^{3}H-DMI the specific binding of ^{3}H-nomifensine was not augmented by pretreatment of the membranes with 300 mM KCl. In membranes from hippocampus, structure with poor dopaminergic innervation, benzotropine (100 μM) inhibited only 20% of total ^{3}H-nomifensine binding.

Competition experiments showed a monophasic reduction of ^{3}H-nomifensine binding by unlabeled nomifensine (IC_{50}: 0.06 μM) and benzotropine (IC_{50}: 0.6 μM). ^{3}H-Nomifensine labels a site in striatum with the following pharmacological profile (affinity): nomifensine > M-1 [4-OH-nomifensine] > benzotropine > M-2 [3 methoxy-4-OH nomifensine] > M-3 [3-OH-4-methoxynomifensine] > nortriptyline > 3PPP [3-(3-OH-phenyl)-N-n-propylpiperidine] > spiperone > phentolamine > dopamine > DMI > tyramine > IMI > norepinephrine > 5HT. A highly significant correlation (R:0.96, p < 0.001, n=9) was found between the potency of these drugs for the inhibition of ^{3}H-nomifensine binding and the inhibition of ^{3}H-dopamine uptake in striatal synaptosomes reported in the literature. These results suggest ^{3}H-nomifensine labels the recognition site for the neuronal uptake of dopamine in striatum. The regional distribution and the localization of this binding site is currently under investigation.

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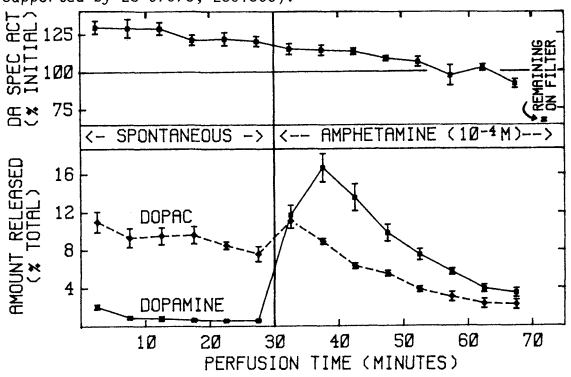
164.3 INTRANEURONAL DOPAMINE (DA) COMPARTMENTALIZATION AND AMPHETAMINE (AMPH) EVOKED RELEASE. D. Minnema* (SPON: I.A. Michaelson). Dept. Env. Hlth., Univ. Cinti. Coll. Med., Cinti., OH 45267.

Current thought is that DAergic nerve terminals preferentially release newly synthesized and/or recently acquired DA relative to long term stored DA, particularly when release is evoked by AMPH. This hypothesis was examined by comparing newly synthesized ^{14}C-DA (dpm) to the total (endogenous) DA released from perfused rat striatal synaptosomes (P_2). Following a 30 min incubation with ^{14}C-tyrosine, two aliquots of incubate were separately filtered (Millipore). The DA and DOPAC retained on one of the filters provided an initial DA specific activity (SA) value. The other filter was perfused with normal Krebs-Henseleit bicarbonate buffer (pH=7.4, 37°C, flow rate = 0.5 ml/min) for 30 min (spontaneous release) followed by a 40 min perfusion with 10^{-4}M AMPH in buffer. Perfusates were collected at 5 min intervals. Al_2O_3 isolated DA and DOPAC were separated by HPLC and quantified by electrochemical detection and LSC. The results show that the SA of DA released during the perfusion is greater than that contained in the P_2 on the initial filter (upper graph), indicating a preferential release of newly synthesized DA during spontaneous release which slowly declines over time despite a marked release of DA evoked by AMPH (lower graph). Preliminary studies with dual labels suggest that the recently taken up DA and the newly synthesized DA behave similarily. The results suggest that the DA released spontaneously or by AMPH is related to the geometry of this releasable pool, where DA closer to the membrane is released first. (Supported by ES-07073, ES01566).



164.4 EFFECTS OF DOPAMINE AND SEROTONIN UPTAKE BLOCKERS ON SOME NEUROCHEMICAL EFFECTS OF METHAMPHETAMINE. C.J. Schmidt* and J.W. Gibb, (SPON: W. Stevens).Dept. Biochem. Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT. 84112.

Recent evidence suggests that the uptake of indirect agonists such as amphetamine and drug-induced monoamine release may be mediated by a common carrier also responsible for neurotransmitter reuptake (Fischer and Cho, JPET, 208:203, 1979). Inhibitors of this uptake mechanism block many of amphetamine's effects both in vitro and in vivo. We have previously shown that specific dopamine (DA) and serotonin (5HT) uptake blockers can antagonize the effects of high doses of methamphetamine (METH) on the central dopaminergic and serotonergic systems of the rat (Hotchkiss et al., JPET, 214:257, 1980; Schmidt et al., Abs. Neurosci. 8:818, 1982). These effects are characterized by marked reductions in brain concentrations of both DA and 5HT as well as by their major metabolites, and their biosynthetic enzymes. The studies described here further examine the effects of inhibitors of monoamine uptake on the neurochemical effects of METH.

For in vivo studies, rats were coadministered either amfonelic acid (AFA, 0.15 mg/kg) or nomifensine (NOM, 0.50 mg/kg) with METH (15 mg/kg) every 6 hrs for 5 doses. As shown previously, AFA blocked the METH-induced decrease in striatal tyrosine hydroxylase (TH) activity. At the doses used NOM did not block this effect of METH. Tryptophan hydroxylase (TpH) activity, was significantly depressed by METH in the presence of either AFA or NOM.

Superfusion studies using striatal slices preloaded with [^{3}H]-DA examined the effects of DA uptake blockers on METH-induced release. Both AFA (10^{-5}M) and NOM (10^{-5}M) blocked the release of [^{3}H]-DA caused by METH (10^{-5}M).

The 5HT-uptake blockers, chlorimipramine (Chl-IMP) and citalopram (CIT), were used in in vivo experiments similar to those described above. Chl-IMP (4 mg/kg) blocked the decrease in both striatal and cerebral TpH activity seen after METH (10 mg/kg x 5) without altering the decrease in striatal TH activity. Similar results were observed at higher doses of METH (15 mg/kg x 5). CIT (2 mg/kg) also blocked the effects of both doses of METH on striatal TpH activity. However, at the lower dose, CIT potentiated the effect of METH on the DA system in spite of protecting the 5HT system. Striatal TH activity, DA, DOPAC and HVA were reduced to a significantly greater extent by CIT + METH compared to METH alone. These results suggest an inhibition of METH metabolism by CIT. (Supported by DA 00865 and GM 07579).

164.5 THE EFFECT OF ORGANIC CALCIUM ANTAGONISTS ON RELEASE OF (^{3}H) DOPAMINE FROM AGGREGATE CULTURES OF MIDBRAIN NEURONS. Ismail A. Shalaby, Stephen B. Freedman, * and Richard J. Miller* Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, Il.60637.

Organic calcium antagonists known to block voltage-sensitive Ca^{2+} channels, exhibit a greater sensitivity to cardiac and smooth muscle as compared to nerve terminals (Nachsen & Blaustein, Mol. Pharm.16:579,1979). Depolarization-induced release of dopamine (DA) has been shown to be a calcium-dependent process. In this study we report that the organic calcium antagonists nitrendipine, nisoldipine, D-600 and diltiazem all fail to inhibit the Ca^{2+}-dependent 70mM K^+-induced release of (^{3}H)DA from aggregate cultures of mesencephalic neurons. We (Shalaby et al., J. Neurosci. In press) have demonstrated a Ca^{2+}-dependent K^+-induced release of (^{3}H)DA from 3 week old cultures of neurons from the rostral mesencephalic tegmentum (RMT) that had been dissociated and allowed to reaggregate and grow in rotary culture with cells from the striatum (CS). 3 week old aggregates of the RMT-CS were exposed to 56uM (^{3}H)DA for 30 min at 37°C. After the excess (^{3}H) was washed off, aggregates were incubated with 1ml aliquots of buffer for 5 min at 37°C. After each incubation the supernatant was exchanged with fresh aliquots (a total of 20 5 min consecutive incubations). When basal efflux of (^{3}H)DA had stabilized a 70mM K^+ aliquot was introduced for 5 min. The 70mM K^+ incubation was repeated 3 times after basal efflux stabilization, with the 2nd stimulation containing the Ca^{2+} antagonists. Release of (^{3}H) per fraction was expressed as a % of tissue (^{3}H)/5 min fraction, and 70mM K^+ induced a release of 13.4% of tissue (^{3}H). To assess drug effects the ratio of release during the 2nd K^+ stimulation to the mean of the 1st and 3rd was obtained. This ratio was subtracted from 1 and multiplied by 100 to obtain % inhibition:

Percent Inhibition of 70mM K^+-induced Release of (^{3}H)DA

Drug	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-3}M
Nitrendipine	8	11		12		
Nisoldipine	10	7		-2		
D-600			-9	17	28	
Diltiazem		20	14	23	35	
$CoCl_2$(3mM)						82

Statistical analysis comparing aggregates stimulated with K^+ without drugs to aggregates exposed to drugs indicated that only $CoCl_2$ (3mM) significantly inhibited K^+-induced release of (^{3}H)DA.

While these organic Ca^{2+} antagonists block voltage-dependent Ca^{2+} channels and block depolarization-induced Ca^{2+} influx in muscle, their lack of effect of Ca^{2+}-dependent depolarization-induced transmitter release suggests that separate Ca^{2+} channels might exist in nerves.

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164.6 CHARACTERIZATION OF DOPAMINE AND NOREPINEPHRINE EFFLUX FROM A CRUDE PREPARATION OF STORAGE VESICLES ISOLATED FROM RAT STRIATUM. G.S. Takimoto*, B.R. Bianchi* and T.R. Miller* (SPON: J. Hirsch). Dept. of Biomedical Sciences, Univ. of Illinois Coll. of Med. at Rockford, Rockford, IL 61107-1897.

Evidence has been obtained from our laboratory indicating that synaptosome-stored ^{3}H-dopamine(^{3}H-DA) is more susceptible than ^{3}H-norepinephrine(^{3}H-NE) to depletion by sympathomimetic amines and agents such as reserpine that inhibit catecholamine(CA) transport into storage vesicles. This finding was further investigated in the present study by monitoring the effects of sympathomimetic amines, reserpine and ATP+Mg on the spontaneous efflux of ^{3}H-CAs from a crude preparation of storage vesicles isolated from rat striatum. ^{3}H-DA or ^{3}H-NE was incorporated into striatal vesicles by incubation of the vesicle fraction for 4 min at 20°C in the presence of phosphate buffer, pH 7.4, containing 0.05μM ^{3}H-CA, 1mM ATP+Mg and 5μM iproniazid. ^{3}H-CA uptake was terminated by a 50-fold dilution of the incubation mixture with phosphate buffer (20°C). Addition of the dilution buffer simultaneously initiated the efflux incubation period. A greater proportion of ^{3}H-DA (60%) than ^{3}H-NE (42%) was spontaneously depleted from the loaded striatal vesicles following a 5 min efflux incubation period. Tyramine (0.5μM) and metaraminol (0.5μM) when added to the dilution buffer had no effect upon the efflux of either ^{3}H-DA or ^{3}H-NE. Reserpine (0.1μM) and ATP+Mg (1mM) when added separately to the dilution buffer retarded the spontaneous efflux of ^{3}H-DA by 55% and 71%, respectively. If reserpine and ATP+Mg were combined in the dilution buffer, the effect on ^{3}H-DA efflux was additive, as the vesicular loss of ^{3}H-DA was completely prevented. The spontaneous efflux of ^{3}H-NE was completely inhibited when either reserpine or ATP+Mg was added to the dilution buffer, and combining these agents elicited no additional effect. If tyramine was combined with ATP+Mg in the dilution buffer, the efflux of ^{3}H-DA was greater than that obtained in the presence of ATP+Mg alone, whereas ^{3}H-NE efflux was similar in the presence of tyramine + ATP+Mg and ATP+Mg alone. Both tyramine (0.5μM) and reserpine (0.1μM) inhibited the vesicular uptake of 0.05 μM ^{3}H-DA and ^{3}H-NE by approximately 45%. These data indicate that DA is retained less effectively than NE by storage vesicles prepared from rat striatum. The spontaneous depletion of vesicle-stored DA and NE was differentially affected by reserpine, ATP+Mg and tyramine + ATP+Mg, which may reflect dissimilarities in the mechanisms of outward translocation and/or intravesicular stabilization of these CAs. Supported by Illinois Heart Association. Grant-in-aid #N-17.

164.7 RELATIONSHIP BETWEEN CYCLIC AMP LEVELS AND EVOKED DOPAMINE RELEASE IN PC-12 PHEOCHROMOCYTOMA CELLS IN CULTURE. L. Baizer* and N. Weiner. (SPON: J. Caldwell). Dept. Pharmacology, Univ. Colo. Health Sci. Center, Denver, CO 80262.

We have previously suggested (Fed. Proc. 42: 3788, 1983) that cyclic AMP (cAMP) may be involved in the process of dopamine release from PC-12 pheochromocytoma cells. Elevated potassium (56 mM) or nicotine (100 μM) produces increases in cAMP levels in PC-12 cells that are well correlated, with respect to time-course, magnitude, and dependence on external calcium, with dopamine release from the cells.

Forskolin causes a rapid (maximal in 5-10 minutes) and dose-related increase in PC-12 cellular cAMP levels. Increases in cAMP by forskolin are synergistic with those caused by nicotine or elevated potassium. Forskolin has differential dose-related effects on spontaneous and evoked dopamine release in PC-12 cells. At low concentrations (50-100 nM), forskolin produces approximately a two-fold increase in K^+-evoked release without a significant effect on spontaneous release. At higher concentrations (1 μM or greater) forskolin causes a significant increase in spontaneous release. However, at a concentration of 1 μM, forskolin produces a smaller increase in K^+-evoked release than at 100 nM; net evoked dopamine release is 30% above control levels.

Treatment of PC-12 cells with nerve growth factor protein (NGF) causes the cells to cease dividing and differentiate into cells showing many of the characteristics of cultured sympathetic neurons. We have found, as did Greene and Rein (Brain Res. 138: 521, 1977), that treatment of PC-12 cells with NGF increases their sensitivity to nicotine; net dopamine release in response to 100 μM nicotine is three-fold greater in NGF-treated cells than in control cells. Correspondingly, 100 μM nicotine causes a three-fold greater increase in cAMP levels in NGF-treated cells than in control cells.

The NGF-induced increase in sensitivity to nicotine is maximal after six days of NGF treatment, a similar time-course as that seen by Dumblatt and Tischler (Nature 297: 152, 1982) for the NGF-induced increase in muscarinic cholinergic receptors in these cells. The NGF-induced increase in sensitivity to nicotine suggests that NGF may also produce an increase in nicotinic cholinergic receptors in these cells in culture. Supported by USPHS grants NS 07927 and NS 09199 and GM 07635.

164.8 DIFFERENTIAL STIMULATION OF SYNAPTOSOMAL NOREPINEPHRINE UPTAKE BY HIGH SALT DIET IN DAHL RATS. J. Rho and K. Hough*. Dept. of Medicine, Schools of Medicine and Pharmacy, University of Southern California, Los Angeles, CA 90033.

Dahl salt-sensitive rats (S) become hypertensive when fed a high salt (8.94% NaCl) diet but remain normotensive on a low salt (0.94% NaCl) diet. Dahl salt-resistant rats (R) are normotensive on either diet. In order to study a possible alteration of the transmembrane norepinephrine (NE) uptake system in Dahl S rats, we have studied the uptake processes directly in the synaptosomes isolated from the hypothalamus of both Dahl S and Dahl R rats in two different dietary groups; Dahl S rats on both high and low NaCl diets and Dahl R rats on both high and low NaCl diets. The synaptosome-enriched subfraction was prepared by flotation, employing our modification of a discontinuous Ficoll-sucrose gradient (Booth and Clark, Biochem. J. 176:365, 1978). The NE uptake is highly sodium-dependent (70%) and ouabain sensitive (70%). The initial ^{3}H-NE uptake by the hypothalamic synaptosomal fraction of Dahl R and Dahl S rats on a low salt diet during the first 10 minute incubation period averages 1.04 ± 0.14 and 1.39 ± 0.21 p moles per mg protein respectively while those of Dahl R and Dahl S rats on a high salt diet were 1.92 ± 0.23 and 1.71 ± 0.20 respectively. There was no significant difference between sensitive and resistant rats in either the 0.94% or the 8.94% NaCl diets. A high salt diet, however, has a stimulatory effect on the uptake of ^{3}H-NE by hypothalamic synaptosomes by both strains of rats, and that the 8.94% NaCl diet differentially enhanced the reuptake of ^{3}H-NE in sensitive strain (23% increase, not significant) as compared to resistant strain (85% increase, $P < .05$).

164.9 ALPHA-2 ADRENERGIC REGULATION OF NOREPINEPHRINE RELEASE IN THE RAT SUBMANDIBULAR GLAND AS MEASURED BY LCEC. J.T. Turner*, D.L. Pierce* and D.B. Bylund. Department of Pharmacology, University of Missouri, School of Medicine, Columbia, MO 65212.

High performance liquid chromatography coupled with electrochemical detection (LCEC) was used to determine endogenous norepinephrine (NE) release in rat submandibular gland in vitro. Previous studies (Filinger et al., Arch. Pharmacol. 304:21, 1978) of NE release from salivary glands have utilized preloading of the gland with tritium-labeled NE which may not fully equilibrate with endogenous NE pools. Our modification of a plasma catecholamine assay (LCEC Note No. 14, Bioanalytical Systems, W. Lafayette, IN) overcomes this problem and allows the precise determination of NE levels down to at least fifty picograms. Using this method in conjunction with a minced gland preparation, we have examined the potassium-stimulated increase in NE release by the gland as well as the dose-dependent potentiation of the potassium stimulation by the alpha-2 antagonist, yohimbine. Yohimbine maximally potentiates K^+-induced NE release at 1.0 μM with an ED_{50} of 0.18 μM. The alpha-2 nature of this effect is further evidenced by the comparatively low potency (ED_{50} = 2 μM) of prazosin, an alpha-1 antagonist, in potentiating NE release in response to K^+. These data are consistent with the concept that these drugs exert their effects by binding to presynaptic alpha-2 receptors and thus block the inhibition of NE release by NE. Further, the ED_{50} ratio for yohimbine compared to prazosin (about 0.1) is in agreement with the IC_{50} ratio for these drugs in inhibiting [^{3}H]yohimbine binding in rodent tissues. The ED_{50} and IC_{50} ratios of about 0.1 are markedly different from the yohimbine/prazosin IC_{50} ratios of 0.003-0.03 vs. [^{3}H]yohimbine seen in non-rodent tissues and cells, including human platelets (Bylund and U'Prichard, Int'l Rev. Neurobiol. 24:343, 1983). These results suggest species differences in alpha-2 adrenergic receptors. The experimental system described herein promises to be an effective tool with which to study the role of other neuromodulators, including peptides, in the regulation of neurotransmitter release.

164.10 PRESYNAPTIC MODULATION OF RELEASE OF [^{3}H]NORADRENALINE EVOKED FROM VARICOSITIES ISOLATED FROM GUINEA-PIG MYENTERIC PLEXUS. W. MacDonald*, D. Fitzpatrick* and T. D. White. Department of Pharmacology, Dalhousie Univ., Halifax, N.S., Canada, B3H 4H7.

Extrinsic noradrenergic fibres innervate the myenteric plexus of the intestine, where they exert presynaptic inhibitory actions on ACh release, possibly at reciprocal axo-axonic synapses with cholinergic terminals. In the present study, we have investigated the effects of various cholinergic and adrenergic agonists on [^{3}H]NA release from myenteric varicosities (synaptosomes) isolated from ileal longitudinal muscles of 2 adult male guinea pigs. The isolated varicosities were incubated with $2x10^{-7}$M [^{3}H]NA for 15 min, washed 3 times and finally incubated in Krebs-Henseleit medium with various agents. Release of [^{3}H]NA as dpm/mg protein/10 min was detected in the supernatant following centrifugation in a Beckman microfuge for 3 min. Veratridine ($5x10^{-5}$M), KCl (increased by 24mM), nicotine (10^{-4}M) and ACh (10^{-4}M) all evoked Ca^{2+}-dependent release of [^{3}H]NA, release by veratridine being much greater (5-10x) and less Ca^{2+}-dependent than release by the other agents. The muscarinic agonist, bethanechol (10^{-4}M), and antagonist, atropine (10^{-7}M), had no effects on resting efflux or on release evoked by veratridine, KCl, nicotine or ACh. However, the α_2 agonist, clonidine (10^{-5}M), reduced ACh-, nicotine-and KCl-evoked release of [^{3}H]NA to 24%, 43% and 57% of control values respectively, but had no effect on veratridine-induced efflux of dpm's. The following conclusions can be drawn: 1) Isolated myenteric varicosities, presumably those which are noradrenergic, can take up and release [^{3}H]NA by a Ca^{2+}-dependent mechanism when exposed to KCl, nicotine or ACh. Veratridine evokes a large efflux of radioactivity which is only partially Ca^{2+}-dependent. 2) These isolated myenteric varicosities possess presynaptic nicotinic receptors but no evidence for presynaptic muscarinic receptors was obtained. Either the muscarinic receptors which modulate NA release are located on portions of the sympathetic axon remote from the varicosities, or they are destroyed during formation of the isolated varicosities. 3) The isolated varicosities possess presynaptic α_2 receptors which, when stimulated, inhibit [^{3}H]NA release. These receptors may play a role in modulating NA release in vivo.
(Supported by the MRC of Canada).

164.11 HALOPERIDOL ELEVATES EXTRACELLULAR CATECHOLAMINE METABOLITES IN RAT STRIATUM WITHOUT AFFECTING ASCORBIC ACID OR INDOLEAMINE METABOLITE LEVELS. R. D. Blakely*, S. A. Wages*, J. B. Justice, J. G. Herndon*, and D. B. Neill* (SPON:L. Walker). Depts. of Biology, Chemistry, and Psychology, Emory Univ., Atlanta, GA 30322.

In vivo oxidation currents from carbon paste electrodes chronically implanted in rat striatum increase following intraperitoneal haloperidol injections. It is unclear to what degree ascorbic acid, monoamine neurotransmitters, and their metabolites contribute to these signal increases. In order to elucidate the neurochemical components responsible for this effect, high-performance liquid chromatography with electrochemical detection (HPLC-EC) was employed to analyze perfusates passed through the striatum of unanesthetized rats. Push-pull perfusion was conducted at a rate of 4uL/min. between two pulled glass micropipettes, sealed within the lumen (200um dia.) of a hollow, semipermeable cellulose fiber (Spectrapor HF, pore cutoff=5000 amu). Thus, all exchange of compounds between the extracellular fluid and artificial CSF occured across the dialysis membrane, permitting the collection of a perfusate suitable for direct, on-line chromatographic analysis without pre-column modification of the collected fractions. 100uL samples were obtained every 30 minutes and immediately injected into a Dupont Zorbax C8 reverse phase column, with mobile phase conditions appropriate for the separation of the monoamine neurotransmitters, their metabolites, and ascorbic acid. DOPAC, HVA, 5-HIAA, and ascorbic acid could be consistently identified in the perfusates of all animals. Fractions (5 to 6) were collected until a stable baseline condition had been achieved. Following administration of haloperidol (1mg/kg, i.p.) perfusate concentrations of DOPAC and HVA showed significant increases ($p<.001$) above predrug levels, while no alterations in ascorbic acid and 5-HIAA levels could be detected. Each of the catecholamine metabolites rose to over 200% of baseline levels. The rise in extracellular DOPAC occured 30 minutes prior to the increase in HVA. Saline administration failed to alter the concentrations of any of these compounds. These data support the contention that increases in striatal oxidation currents observed following neuroleptic administration are dependent upon increased extracellular efflux of catecholamine metabolites and demonstrate an insensitivity of extracellular ascorbic acid and 5-HIAA to dopamine receptor blockade.

164.12 ON-LINE CHROMATOGRAPHIC ANALYSIS OF STRIATAL DIALYSATE IN BEHAVING ANIMALS: COMPARISON WITH VOLTAMMETRY. Joseph B. Justice, Jr., Sherry A. Wages , Adrian C. Michael , Randy D. Blakely and Darryl B. Neill , Departments of Chemistry and Psychology, Emory University, Atlanta, GA, 30322

In order to monitor the extracellular chemistry of the striatum in behaving rats we have assembled a system consisting of a high performance liquid chromatograph with electrochemical detection and a push-pull perfusion system in which the perfusate flows through a micro dialysis tube implanted in the striatum. The dialyzed perfusate passes through a fluid swivel and continuously flows through the sample loop of the HPLC, where it is automatically injected at preset intervals. The perfusate is analyzed for ascorbic acid (AA), the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). At a flow rate of 4 ul/min, each 100ul sample of dialysate contained approximately 40 ng of AA, 5 ng of DOPAC and HVA and 1 ng of 5-HIAA. A comparison is also made of chronoamperometric data recorded from the striatum with carbon epoxy and carbon paste electrodes and chromatographic data obtained from extracellular striatal perfusate. It is shown that the decline in oxidation current during the initial sampling period in voltammetry is due primarily to a decrease in ascorbic acid. It is also shown that different electroactive components of the extracellular fluid in the striatum are the cause of changing oxidation currents under different stimulus conditions.

164.13 IN VIVO VOLTAMMETRIC RECORDING WITH NUJOL OR SILICONE OIL CARBON PASTE ELECTRODES. K. Mueller[a], C. D. Andrews and P. J. Knott. Dept. of Pharmacol., Marshall Univ. School of Med., Huntington, WV 25701

In vivo voltammetry is becoming an increasing popular tool for measuring catecholamine release from selected brain areas. Interpretation of results is often complicated by use of different electrodes by different labs. We compared carbon paste electrodes fabricated with either nujol or silicone oil and found major differences between the two.

In general, nujol and silicone oil electrodes produce a very similar response to 0.1mM dopamine and a very similar response to 0.2mM ascorbate in vitro. That is, for each substance, peak positions and heights are virtually identical for the two types of electrodes. In vivo, however, nujol and silicone oil electrodes are different in several respects.

After chronic implantation in the anterior caudate of rats silicone oil electrodes consistently record 3 peaks (during a linear sweep at 10mV/sec from -200 to +500mV) on initial testing (48 hr after implantation). Nujol electrodes, however, often do not record distinct peaks until 10 to 14 days after implantation; occasionally nujol electrodes do not record any peaks when tested intermittently up to 20 days after implantation. Furthermore, the signals (in $nAsec^{-\frac{1}{2}}$) recorded by nujol electrodes are consistently smaller than the signals recorded by silicone oil electrodes; peak heights produced by endogenous substances and ip acetaminophen are virtually always smaller for the former. Nujol electrodes often record only 2 peaks as a result of merging of peaks 1 and 2. Thus data interpretation is difficult, at best. In contrast, silicone oil electrodes record 3 peaks up to 20 days or more after implantation, although the signals tend to decrease slightly over time.

We are unable to explain why the two types of electrodes are so similar in vitro but so dissimilar in vivo. We conclude that carbon paste electrodes fabricated with silicone oil have several advantages over carbon paste electrodes fabricated with nujol.

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164.14 LONG TERM EFFECTS OF XYLAMINE (XYL) ON AMPHETAMINE (AMPH) INDUCED RELEASE OF NOREPINEPHRINE (NE) IN VIVO. C. D. Blaha*, S. Howard-Butcher and R. F. Lane. Institute of Neuroscience, University of Oregon, Eugene, OR 97403 and Dept. of Pharmacology and MRRC, UCLA, Los Angeles, CA 90024.

Xylamine (Xyl), a nitrogen mustard which is thought to specifically inhibit the NE carrier was examined with respect to its long term effects using in vivo voltammetry.

Male Sprague-Dawley rats were injected with Xyl (12.5 mg/kg i.p.) or saline 5 days prior to experimentation. On the 6th day, the rats were anethetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic instrument. Surface modified graphite electrodes, selective for catecholamines were inserted into the neostriatum and the contralateral motor cortex overlying the neostriatum.

Chronoamperometric measurements were made by applying a potential of +0.25V vs Ag/Ag Cl for 1 second to each electrode with an interval of 1 minute between measurements.

Baseline values of NE in the cortex and DA in the neostriatum were obtained. The values for NE release in the cortex were decreased by approximately 90% of normal values, while there was no effect on the release of DA in the neostriatum.

Animals were then treated with Amph (1 mg/kg i.v.). The ability of Amph to produce a central nervous system stimulation appears to be dependent upon the availability of newly synthesized DA or NE.

In control animals Amph induced a 25% increase in NE release from cortex and a 31% increase in DA release in the neostriatum. However, there was no effect on the release on NE from the cortex in Xyl treated animals, even though tissue levels of NE are only reduced by approximately 30%.

These data suggest that Xyl produces a long lasting effect on NE. Xyl has been shown to block NE uptake for at least ten days after administration (Dudley et al JPET 217:834, 1981). The results obtained in this study are consistent with the notion that the release mediated by AMPH is the result of an exchange diffusion mechanism involving the NE uptake system.

This work was supported by USPHS Grants HD 05615, NS 13556 and GM 07257.

164.15 DIFFERENTIAL EFFECT OF XYLAMINE ON NOREPINEPHRINE (NE) AND DOPAMINE (DA) RELEASE USING IN VIVO VOLTAMMETRY. S. Howard-Butcher, C. D. Blaha* and R.F. Lane. Department of Pharmacology and MRRC, UCLA, Los Angeles, CA 90024 and Institute of Neuroscience, University of Oregon, Eugene OR, 97403.

Xylamine (Xyl) is a new compound which selectively inhibits the NE carrier while leaving the DA levels unaffected. (Dudley et al JPET 217:834, 1981). Using in vivo voltammetry the release of both NE and DA were measured.

Male Sprague - Dawley rats were injected with chloral hydrate (400 mg/kg i.p.) and body temperature maintained with a heating pad. Electrode implantation followed standard stereotaxic techniques. Surface modified graphite electrodes, selective for catecholamines, were inserted into the neostriatum and the contralateral cortex overlying the neostriatum. Semi-differential voltammetric measurements were performed by scanning the potential linearly from -0.1V to +0.5V vs Ag/AgCl and measuring the peak current centered at +0.13V. Scans were repeated once every 5 min on alternate electrodes.

After a stable baseline was obtained, animals were injected with Xyl (12.5 mg/kg i.p.). There was an initial increase in NE release followed by a decrease in release of NE in the cortex of approximately 76% over a 2 hour period. The release of DA in the neostriatum was unchanged. This decrease in NE release was maximal 2 hours after Xyl injection.

In an attempt to block the effect of Xyl, in a second series of experiments, rats were pretreated with Desmethylimipramine (DMI 20 mg/kg i.p.).

Initially DMI produced a slight increase in measured NE (26%) and had no effect on DA. Xyl was injected 1 hour after DMI pretreatment. DMI blocked the Xyl induced decrease in NE release.

These data suggest that Xyl binds specifically to the NE carrier and that DMI protects the carrier from the blocking/depleting actions of Xyl.

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TRANSMITTER UPTAKE, STORAGE, AND SECRETION II

165.1 ANTIBODIES AGAINST BLOOD PLATELETS INFLUENCE 5-HT UPTAKE AND IMIPRAMINE BINDING IN SYNAPTOSOMES. M.E. Trukenmiller, I. Angel*‡, S.M.Paul‡ and J.H. Neale. Dept. of Biology, Georgetown University, Wash. D.C. 20057 ‡Clinical Neuroscience Branch, NIMH, NIH, Bethesda,MD 20205

Purified human blood platelet membranes were injected into BALB/c mice and the splenocytes subsequently fused with NS1 myeloma cells. A single fusion produced more than 900 hybrid cell colonies and of these, more than one hundred produced antibodies which reacted with platelet membranes in solid phase radioimmunoassay. These cell cultures were expanded and their antibodies assayed for effects on serotonin uptake and imipramine binding in freshly prepared human platelets. Antibodies which produced an effect in either or both of these assays were further tested for their ability to react with rat brain synaptosome membranes in RIA and to influence serotonin uptake and imipramine binding in fresh rat brain synaptosomes. We have found that several hybridoma cell cultures produce antibodies which react with brain synaptosomes and which influence the binding of imipramine and/or the uptake of serotonin. The specificity and mechanism of action of these antibodies are of interest inasmuch as the imipramine receptor in blood platelets and brain has been implicated in certain affective disorders and the action of tricyclic anti-depressant drugs in humans.

165.2 REGULATION OF THE RELEASE OF SEROTONIN (5 HT) BY AN AUTORECEPTOR ON SYNAPTOSOMES FROM RAT SPINAL CORD TISSUE. P. J. Monroe and D. J. Smith. Depts. Pharmacology/Toxicology and Anesthesiology, West Virginia University Med. Ctr., Morgantown, W.V. 26506.

Two high affinity binding sites (5 HT_{1a} + 5 HT_{1b}) for ^{3}H-5-HT have recently been demonstrated in the rat spinal cord (Monroe and Smith, J. Neurochem., in press). A possible functional role of these sites may be the regulation of 5 HT release. Therefore, preliminary studies were done to evaluate the effect of serotonergic drugs on the release of 5-HT from synaptosomal tissue. A drug induced alteration in the K^+ evoked release of 5-HT should suggest the existence of an autoreceptor.

Synaptosomes were prepared from spinal cord tissue and were suspended in a Tris-buffered Besson's medium. After 2-10 min. incubations (37°C) in the absence then in the presence of 100 nM ^{3}H-5HT, an aliquot (0.4 ml) of tissue suspension (50 mg/ml) was loaded onto a bed of Sephadex G-15 and superfusion was begun. The standard procedure was to wash the tissue for 50 min., then to change to a medium containing drugs 20 min. prior to exposure of the tissue to medium containing high K^+ (15 mM). Tissue was lyzed at the termination of the experiment with 1N HCl. 5 HT release was expressed as a percentage of total ^{3}H available.

Initial experiments were done to examine the degree of metabolism of ^{3}H-5HT that might occur during the course of the experiments. A significant amount of ^{3}H-5HIAA forms during the initial incubation of the tissue, as ^{3}H-5HT is preloaded, but is washed from the tissue in an exponential manner. During superfusion, extrasynaptosomal metabolism was minimal. Pargyline (10 µM) did not alter the rate of appearance of ^{3}H-5HIAA in samples collected during periods of basal or K^+-induced efflux. During basal efflux periods ^{3}H-5HIAA constitutes approximately 50% of total tritium and decreases to < 20% during K^+-evoked release. Intrasynaptosomal metabolism of ^{3}H-5HT was observed only when tissues were superfused with exogenous 5-HT. This was prevented with 1 µM fluoxetine present to block the uptake carrier and subsequent displacement of ^{3}H-5HT from vesicles by the amine.

Exogenous 5-HT in the presence of fluoxetine attenuated, in a dose-dependent manner, the K^+ evoked release of ^{3}H-5HT, demonstrating the existence of an autoreceptor. Concentrations of 1, 10, 30 and 100 nM 5HT reduced release to ($\bar{x}$ ± SEM) 91 ± 8, 78 ± 6, 66 ± 4, 69 ± 9 % of control, respectively.

Experiments, using spiperone (a serotonergic drug for which the receptors exhibit differing affinities), are currently underway to determine which 5HT receptor subtype is associated with autoregulation.

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165.3 PURIFICATION AND CHARACTERIZATION OF TWO FORMS OF SEROTONIN BINDING PROTEIN. Liu*, K.P., Ehrlich, Y., Gershon, M.D. and Tamir, H., N.Y. State Psych. Inst., Univ. Vermont Coll. Med. and Univ. Columbia Coll. Med.

Serotonin binding protein (SBP) is a soluble protein found in synaptic vesicles of central and peripheral serotonergic neurons. Serotonin (5-HT) is physiologically stored as a complex with SBP in situ. Two forms of SBP were detected: 45kd and 56kd. Newly taken up ^{3}H-5-HT preferentially labels 45kd SBP. In order to better understand the relationship between the two forms we purified the two proteins to homogeneity and partially characterized them. Steps of purification included: $(NH_4)_2SO_4$ cut, molecular sieve chromatography on Sepharose-4-B, affinity chromatography, Ca-P gel, gel electrophoresis and HPLC. Purity was established by 2 dimensional gel electrophoresis. The two forms of SBP were similar in their amino acid composition, the enhancement of 5-HT binding by Fe^{+2} and inhibition by -SH reagents, chelators, and solutions of high salt content. Antibodies raised against the 45kd SBP cross-reacted with the 56kd form. The two forms of SBP differed in the following properties: 1) Lower dissociation constants and binding capacity were observed for 45kd SBP; 2) isoelectric point, (45kd more acid); 3) binding enhancement by gangliosides and bicarbonate (45kd ten fold less sensitive than the 56kd form); 4) inhibition of binding by ATP-γ-S (45kd form is insensitive, 56kd form inhibited); 5) capacity to act as a substrate for protein kinase (45kd form does not get phosphorylated, 56kd form is phosphorylated)

We suggest that a precursor-product relationship may exist between 45kd and 56 kd SBP and that the two forms may be located within different parts of serotonergic neurons. Supported in part by NSF Grant 09335 and NIMH 37575 and NIH 12969.

165.4 PRELIMINARY CHARACTERIZATION OF THE SEROTONIN UPTAKE SYSTEM IN SPINAL CORD. K. Stauderman*, P. Lundy* and D. J. Jones. Depts. of Anesthesiology and Pharmacology, The Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284

Descending serotonergic pathways have been proposed to play a regulatory role in various somatosensory, autonomic and motor functions within the spinal cord. While quantitative studies have linked the synaptosomal uptake of [^{3}H]-serotonin ([^{3}H]-5HT) to a neurotransmitter role in regional brain areas, such studies have not been performed in spinal cord. We are conducting experiments on the [^{3}H]-5HT uptake system in rat spinal cord, a partial characterization of which is presented here.

5HT uptake was measured by a modification of the procedure of Kirksey and Slotkin (Br. J.Pharmacol., 67:387-391, 1979). Spinal cords were rapidly removed and a crude synaptosomal fraction (S_1) prepared. Aliquots of this fraction (representing 0.25-1 mg protein) were incubated in oxygenated Krebs-Ringer bicarbonate buffer (37°C) for 5 min prior to the addition of [^{3}H]-5HT (0.01-0.05 uM). Final reaction volume was 1 ml. The reaction proceeded for 5 additional min (except for time course studies) and was stopped by the addition of 3 ml of ice cold incubation buffer followed by rapid filtration through Gelman AG filters at reduced pressure, with two subsequent 3 ml washes. The filters were then dried and counted.

Inital studies using [^{3}H]-5HT concentrations of 0.01 uM and 0.05 uM demonstrated linear uptake for at least 8 min. Uptake was also concentration and temperature dependent. At a [^{3}H]-5HT concentration of 0.025 uM, uptake was 180 pmole/gm tissue/5 min (0°C blank corrected). Decreasing the Na^{+} concentration from 145 to 30 mM caused a 53% decrease in this value. Finally, the system appears to be differentially sensitive to uptake inhibitors like fluoxetine (10^{-4} M) and desmethylimipramine (10^{-4} M) which inhibited uptake 90% and 80%, respectively.

The above studies establish an active 5HT uptake system in spinal cord which is Na^{+} temperature dependent and is sensitive to pharmacological blockade.

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165.5 VESICULAR CALCIUM PUMPING MAY DRIVE PREFERENTIAL RELEASE OF NEWLY SYNTHESIZED NEUROTRANSMITTERS. A.F. Boyne and T.E. Phillips. Dept. of Pharmacol. and Exp. Therap., Univ. of Maryland School of Medicine, Baltimore, MD 21201.

Observation of quick-frozen and freeze-substituted torpedine ray electric organ has suggested to us how the well established preferential release of newly synthesized acetylcholine (ACh) may occur. (1) 15-20% of the resting synaptic vesicle population is attached to the presynaptic nerve terminal in apparent active zones. Conventional aldehyde fixation appears to drive the exocytotic discharge of these vesicles. They are then unavailable for quantitation to the extent that they are incorporated into the nerve terminal membrane. (2) EGTA sensitive Ca^{2+} deposits can be seen within synaptic vesicles. The proportion of Ca^{2+} loaded vesicles increase with 0.1 Hz stimulation (2 experiments).

This suggests that the vesicular Ca^{2+}-ATPase drives Ca^{2+} uptake by vesicles in vivo. Since the active zone vesicles will be the first to be exposed to incoming Ca^{2+} and will experience the highest ambient concentration, they would be expected to concentrate Ca^{2+} preferentially. Ca^{2+} facilitates displacement of ACh from cholinergic synaptic vesicles (Michaelson, A.M., et al., BBRC, 80(3):547, 1978). During the action potential undischarged active zone vesicles would gain some Ca^{2+} and lose some ACh. A prediction, which becomes a test of the hypothesis, is that Ca^{2+} unloading and ACh reloading would occur between action potentials so that active zone vesicles would gradually and preferentially become filled with cytoplasmic ACh, i.e. predominately newly synthesized ACh. Those vesicles which opened and closed to the extracellular space would saturate with extracellular Ca^{2+} and may achieve equilibrium with cytoplasmic ACh more quickly.

The predicted reversal of ACh/Ca^{2+} could occur directly once the Ca^{2+} of the marginal cytoplasm was lowered from its high point during the action potential, or it may occur indirectly, perhaps after Na^{+}/Ca^{2+} exchange and subsequent operation of the original ACh loading mechanism on the partially Na^{+} filled vesicles.

In summary, we propose that active zone vesicles undergo a Ca^{2+} driven, relatively selective, acceleration of ACh turnover which results in their preferential loading with newly synthesized ACh. If this is correct, it suggests that (1) the increase in specific gravity of recycling vesicles (Zimmermann and Denston, Neurosci., 2:695, 1977) is the result of an incompletely reversed Ca^{2+} load. And (2), the generality of preferential release of newly synthesized neurotransmitter may be causally related to the generality of Ca^{2+} pumping by the associated storage granules. (Supported by NINCDS Grant #16167).

165.6 HOW ATP PREVENTS LEAKAGE OF CATECHOLAMINE FROM SECRETORY VESICLES L. S. Kao* and E. W. Westhead. Biochemistry Department, Univ. of Massachusetts, Amherst, MA 01003

Secretory vesicles of the adrenal medulla (chromaffin granules) like those of parasympathetic neurons, contain very high catecholamine concentration. At 37°, leakage of catecholamines from isolated bovine chromaffin granules is rapid but is prevented by the presence of ATP in the suspending medium. A proton-pumping ATPase provides energy for catecholamine uptake but constant reuptake in vivo would be a very costly process. We have examined the mechanism of ATP inhibition of catecholamine depletion using intact isolated granules and resealed "ghosts"--vesicles that have been lysed and resealed with catecholamines inside but not nucleotides or soluble macromolecules. Within 4 hours at 37° isolated granules are depleted of catecholamines and nucleotides to about 50% and 30% respectively, with 30% of catecholamine release occurring within an hour. Epinephrine and norepinephrine are released in parallel but catecholamines are released significantly faster than nucleotides. Addition of tracer amounts of $^{34}SO_4^{=}$ to the suspension during depletion showed that leakage was not through non-specific pores. ATP-Mg at 10 mM inhibits 90% of the catecholamine release. Atractyloside (0.2 mM) which binds to the nucleotide carrier and inhibits ATP uptake does not decrease protection by ATP. Atractyloside alone inhibits catecholamine release, as does ADP. Neither AMP nor ADPNP inhibit release. The same phenomena are seen when "ghosts", preloaded with catecholamine, are used in place of intact granules. The mechanisms by which ATP prevents loss of catecholamine from the granules is suggested by the following facts: (1) Binding of ATP,ADP or atractyloside to the nucleotide carrier inhibits release. (2) Release is biphasic. (3) In the fast phase, catecholamine release is faster than nucleotide release. From these facts we deduce that the requirement for electrical neutrality within the granules is a determining factor. Catecholamines within the granules are cationic, so their release must be accompanied by equivalent release of anions. Blockage of the nucleotide carrier (which has broad specificity; Weber, A., Westhead, E. W. and Winkler, H., Bioch. J. 210, 789 (1983)) inhibits release of at least divalent anions and thus inhibits the coupled release of catecholamines. It has become apparent in recent years that the nucleotide concentration in chromaffin granules is insufficient to neutralize all of the catecholamine content. The biphasic release kinetics along with the high initial ratio of catecholamine to nucleotide released shows that anions other than nucleotides escape more easily from the granule.

165.7 MECHANISM OF GLUTAMATE UPTAKE INTO BRAIN SYNAPTIC VESICLES: INVOLVEMENT OF BOTH ELECTRICAL POTENTIAL AND TRANSMEMBRANE pH GRADIENT. S. Naito* and T. Ueda. Mental Health Research Inst., Depts. of Pharmacology and Psychiatry, University of Michigan, Ann Arbor, MI 48109.

There is increasing evidence that glutamate plays a neurotransmitter role in the central nervous system. We have recently demonstrated that glutamate is specifically taken up into certain highly purified brain synaptic vesicles in an ATP-dependent, but sodium-independent manner (Naito, S. and Ueda, T., J. Biol. Chem. 258:696-699, 1983). Here we report that the glutamate uptake is driven by an electrochemical proton gradient generated by a proton translocating ATPase in the vesicle membrane. Thus, proton pump ATPase inhibitors such as DCCD and trimethyltin caused a substantial reduction in the glutamate uptake. The glutamate uptake was also inhibited by FCCP and thiocyanate, agents known to attenuate membrane potential generated by Mg-ATPase, and by proton gradient dissipators such as nigericin (in the presence of potassium ions) and ammonia. Kinetic experiments have indicated that these inhibitors reduce Vmax without significantly changing Km for glutamate. The ATP-dependent uptake of glutamate was observed in a sucrose medium containing impermeable anions (e.g., maleate). The addition of 2 mM Cl^-, a permeable anion, caused a marked stimulation of the uptake; Vmax was increased 4-fold, whereas Km for glutamate was hardly affected. In the presence of excess chloride ions, the uptake was reduced in a concentration-dependent manner. These observations support the notion that the maximal driving force for glutamate uptake into synaptic vesicles is provided by the combination of an optimal membrane potential and pH gradient.

165.8 INHIBITION OF ACTIVE UPTAKE OF ACETYLCHOLINE INTO TORPEDO ELECTRIC ORGAN SYNAPTIC VESICLES BY β-BUNGAROTOXIN. D.C. Anderson* and S.M. Parsons. DEPT. OF CHEMISTRY, UNIV. OF CALIF., SANTA BARBARA, CA 93106.

The presynaptic toxin β-bungarotoxin (β-BuTX) specifically inhibits acetylcholine (ACh) release at peripheral nerve terminals with a time lag that is shortened by nerve terminal activity, suggesting the possible importance of a vesicle-associated target in the toxicity. We have found that β-BuTX potently blocks active transport of ACh into purely cholinergic synaptic vesicles from Torpedo californica electric organ. The inhibition is Ca^{2+}-dependent, with an apparent K_m for Ca^{2+} of 0.4 mM. The potency of inhibition increases with preincubation of β-BuTX and Ca^{2+} with vesicles. The apparent K_i of β-BuTX decreases from 60 nM with no preincubation to 8 nM with 8 hour preincubation. The inhibition of active uptake of ACh by β-BuTX is correlated with activation of the vesicular Ca^{2+}/Mg^{2+} ATPase, which we have shown is coupled to active transport of ACh via a proton gradient (Anderson, D.C. et al., Biochemistry 21, 3037, 1982). ATPase activation by β-BuTX has an apparent K_m for Ca^{2+} identical to that for inhibition of active transport of ACh, and shows a time-dependent increase in toxin effectiveness similar to that seen with active transport of ACh. β-BuTX maximally activates the vesicle ATPase at 3×10^{-8}M while blocking 91% of active ACh transport at 6×10^{-8}M. This is consistent with β-BuTX acting to block ACh uptake by uncoupling the ATPase from active transport of ACh in a fashion similar to protonophoric uncouplers such as nigericin. The maximal activations of the ATPase by β-BuTX·Ca^{2+} and nigericin are not additive, and neither activates triton X-100 solubilized ATPase activity. In the presence of Ca^{2+}, phospholipase A_2's (PLA_2's) from cobra venom, porcine pancreas, Vipera russelli and bee venom block active uptake of ACh with apparent K_i's of 3 nM after 1 hour preincubation. Cobra venom PLA_2 activates the vesicular ATPase with a concentration-dependence similar to that of β-BuTX·Ca^{2+}. The maximal activation of the ATPase by cobra venom PLA_2 is not additive with activation by nigericin or β-BuTX·Ca^{2+}. Thus the PLA_2 activity of β-BuTX appears to be important for its uncoupling of the vesicular ATPase and the resulting blockade of active ACh loading by synaptic vesicles.

165.9 IS COPPER-MEDIATED OXIDATION OF THIOLS A GENERALIZED MECHANISM FOR THE RELEASE OF NEUROPEPTIDES? By G.E. Rice and A. Barnea, Depts. Ob-Gyn. and Physiol., Green Ctr. Reprod. Biol., Univ.of Texas Health Sci. Ctr., Dallas, Tx. 75235.

Previously, we demonstrated that copper, in a chelated form, stimulates the release of luteinizing hormone releasing hormone (LHRH) from isolated hypothalamic granules. Since copper occurs in a chelated form and in high concentrations in the hypothalamus, copper may also affect the release of other peptides from hypothalamic storage granules. To assess the generality of the copper-stimulated release process, we have determined the effects of copper on the release of α-melanocyte stimulating hormone (α-MSH); another peptide which is localized in a population of neurons distinct from LHRH. We addressed two specific questions: (i) What is the active form of copper that interacts with the α-MSH granule (ionic or chelated)?; and (ii) Is copper-stimulated α-MSH release a result of the oxidation of thiol groups? Granules were isolated from hypothalami of adult male rats and were then incubated at 37°C for 3-5 min in a buffered medium. α-MSH contained in granules was separated from α-MSH released into the medium by gel filtration chromatography and quantified by RIA. When granules were incubated with various copper chelates, CuATP-stimulated α-MSH release by 54 ± 6% (mean ± S.E.), Cu tartarate by 56 ± 4%, CuBSA by 32 ± 5% and Cu histidine by 29 ± 2%. Neither CuEDTA nor $CuCl_2$ stimulated α-MSH release. Therefore, copper, in the form of CuATP, was used in subsequent experiments. CuATP-stimulated α-MSH release from granules incubated under N_2 was 43% of that from granules incubated under air. Furthermore, the reducing agent dithiothreitol (DTT) inhibited CuATP-stimulated α-MSH release ($p < 0.01$), whereas oxidized DTT did not do so. Pretreatment of granules with the thiol-blocking reagents iodoacetic acid or 5, 5-dithio-bis-(2-nitro-benzoic acid) inhibited CuATP-stimulated α-MSH release by 52 ± 3 and 38 ± 4%, respectively. We conclude that chelated copper, rather than ionic copper, is the active form of the metal and the action of copper involves the oxidation of thiols. These data are similar to those observed for the copper-stimulated release of LHRH. Hence, the effects of copper on the permeability of granule membranes may be a generalized phenomenon which underlies a susceptibility of storage granules to the redox status of the cellular millieu. Such a process may modulate the responsiveness of the granules to other stimuli that initiate exocytosis.

165.10 THE UPTAKE AND RELEASE OF ^{3}H-GABA IN RAT CEREBELLAR CULTURES. Lisa R. Scribner and Richard W. Burry, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio, 43210.

The inhibitory neurotransmitter gamma aminobutryic acid (GABA) has been shown to be released in a potassium-stimulated, calcium-dependent manner (Pearce, B.R., et al., Brain Research, 206:485, 1981). In order to study this K^+-stimulated release, the neurons need to be first loaded with a tritiated neurotransmitter. Lasher (Brain Research, 69:235, 1974) found that the uptake of ^{3}H-GABA into cerebellar neurons was Na^+ dependent and age related. With this information, the present study was designed to examine what levels of potassium were needed to cause release from cultured cerebellar neurons. Also noted in the study was the age dependence necessary for the K^+-stimulated release of ^{3}H-GABA.

Dispersed rat cerebellar cultures at 7 days in vitro were loaded with 4.24 uM ^{3}H-GABA, washed in solutions containing different concentrations of K^+, and processed according to standard autoradiographic procedure. A 3mM K^+ wash solution left a substantial density of silver grains over the GABA-ergic neurons. This suggests that 3mM K^+ concentration is not adequate for release of the ^{3}H-GABA. However autoradiograms of 7 day in vitro cultures treated with a wash solution containing 50mM K^+ revealed sparsely labeled GABA-ergic neurons. Thus, a wash solution containing 50mM K^+ stimulated the release of the tritiated neurotransmitter.

Next the release of ^{3}H-GABA was investigated with a series of scintillation experiments. Cerebellar cultures were loaded with 4.24 uM ^{3}H-GABA and washed with 3mM K^+ or 50mM K^+ solutions. These solutions were collected and counted. Low levels of ^{3}H-GABA were detected in cultures 7 days in vitro in both the 3mM K^+ and 50mM K^+ wash solutions. By 14 days in vitro, the amount of ^{3}H-GABA released was significantly higher in the 50mM K^+ wash solutions than in the 3mM K^+ wash solution.

These data indicate that the cultures were mature enough by 7 days in vitro to take up the ^{3}H-GABA. Upon addition of different K^+ concentrations, various levels of ^{3}H-GABA were detected in the autoradiograms and liquid scintillation counts. The scintillation data suggest that the release of ^{3}H-GABA is dependent on the age of the neurons, and that only more mature neurons were able to release ^{3}H-GABA in a stimulation dependent manner. Supported by NIH Grant to R.W.B. NS-15894 and funds from the Spinal Cord Injury Research Center at The Ohio State University, NIH Grant NS-10165.

165.PO LIGHT MICROSCOPIC OBSERVATIONS OF GRANULE RELEASE FROM MAST CELLS. C. Edwards, M. Curran* and M. Brodwick*. Dept. Biol. Sciences, SUNY Albany, Albany, N.Y. 12222 and Dept. Physiol., Univ. Texas Med. Br., Galveston, TX 77550.

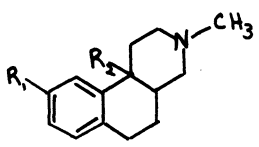
Mast cells release granules containing histamine, heparin and other compounds in response to various secretogogues. This degranulation is thought to occur by exocytosis. In the mutant mouse, beige, the granules are enlarged; the average diameter is about 1.7 μ. These mice resemble the Chediak-Higashi syndrome in humans. In peritoneal mast cells, with video enhanced Nomarski differential-interference microscopy, the granules appear to be denser than the rest of the cell. Perfusion with the ionophore A-23187 and elevated Ca^{2+} causes the emptying of the granules, and this is accompanied by swelling. There is no evidence for flattening of the vesicles. The empty vesicles stain with ruthenium red, a marker for heparin sulfate.

Supported by NIH grants NS07681 and NS13778.

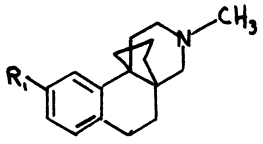
CHARACTERIZATION OF NONCHOLINERGIC RECEPTORS

166.1 STRUCTURE-ACTIVITY STUDIES OF LIGANDS INTERACTING WITH RAT CORTEX PHENCYCLIDINE RECEPTOR. Laurane E. Geary, Dennis M. Zimmerman*, J. David Leander and Vin Kalra*. Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The ability of compounds to displace ^{3}H-phencyclidine (PCP) from rat brain membrane receptors was compared with their ability to produce PCP-type catalepsy in pigeons (Chen, G., 1965. Arch. Intern. Pharmacodyn. 157: 193-201). There was a high degree of correlation between the two tests using derivatives of the phenylcyclohexylamines, dioxolanes, and benzomorphan series previously reported to produce PCP-like effects.



I. Benz[f]isoquinolines



II. Bridged benz[f]isoquinolines

Two series, benz[f]isoquinolines (I) and bridged benz[f]-isoquinolines (II), were found to have PCP-like activities. Using these two tests, the structure-activity relationships at the PCP receptor of these series were studied. It was found that replacement of the methyl-substituted nitrogen with an allyl or methylcyclopropyl group significantly increased receptor affinity and potency in the pigeon catalepsy test. Substitution of a propyl group for methyl at R_2 significantly reduced activity. In addition, a hydroxyl-substituted phenyl was found to be of critical importance for maximum PCP-like activity.

These studies suggest that compounds from several different classes can interact strongly with the PCP receptor and that the receptor binding and catalepsy assays described can be utilized to develop compounds with high affinity and selectivity for this receptor.

166.2 Specific Binding of [^{3}H]-Amantadine to Dog Striatal Membranes

R. Michael Allen, M.D., VAMC and UTHSCD, Dallas, Texas

I have reported previously that amantadine can displace [^{3}H]spiperone and ADTN from striatal total particulate and crude P2 preps (Allen, Neurosci., abstr. 1981) and more recently appears to effect a change in the affinity state of the D_2 receptor (Allen, Neurosci., Abstr. 1982). In this experiment, binding studies were carried out using custom synthesized [^{3}H] amantadine (ICN, inc. 25 ci/mM) to determine if a binding site for amantadine existed in the striatum. The binding assay was carried out at 37° in a Tris salt buffer system, pH 7.34, in a 2-5ml total incubation volume with a tissue concentration of 2.5 mg/tube. The samples were incubated for 30 min., filtered using Whatman GFB filters pretreated with a 0.4% bovine serum albumin solution (found previously to markedly reduce nonspecific filter binding), and washed 3x with 5ml ice cold Tris HCl pH 7.1. Nonspecific binding was determined with 10^{-5} spiperone, stereospecific binding with 10^{-5} (±) Butaclamol. The binding was maximally defined by spiperone with a K_D of 750 pM, Bmax 180 fmol/mg prot. Butaclamol stereospecifically yielded a K_D of 1.25nM and Bmax of 157 fmol/mg prot. Correlation for scatchard and Hill Plot analyses was >.96 for each. Inhibition experiments were carried out with (±) Butaclamol (IC_{50} (+) But 3nM (-) But 150nM) Apomorphine (IC_{50} 1uM); (±) Sulpiride (IC_{50}>10uM), cold amantadine (IC_{50} 1nM) which was quite variable and is probably due to ternary complex formation; ketanserin (IC_{50}>10uM), and rimantadine, a non DA active analogue of amantadine (no displacement). These results indicate that specific amantadine binding is found in the striatum, is probably to a site related to the D_2 receptor (possibly the D_2Lo) and not to the proposed D_4 site of (Sokoloff et al; in Molecular Pharmacology of Neurotransmitter Receptors 163-173, 1983) due to its resistance to sulpiride displacement. Further conclusions and methodological details will be presented.

Supported by Veterans Administration research funds, and a grant from E.I. Dupont Co.

166.3 CHARACTERIZATION OF [^{3}H]PHENCYCLIDINE (PCP) BINDING TO RAT HIPPOCAMPAL SECTIONS USING AUTORADIOGRAPHY. M. R. Kozlowski and L. A. Lebel*, Department of Metabolic Diseases and General Pharmacology, Pfizer Inc., Groton, CT 06340.

Autoradiographic measurement of neurotransmitter receptor binding in brain sections offers many advantages over binding assays employing brain homogenates. These advantages include simultaneous visualization of binding in several brain regions, ease of measurement of binding in small brain areas and elimination of filtration artifacts. This technique has been used to study the binding in brain of several ligands including ^{3}H-PCP (Quirion et al., PNAS 78, 5881, 1981). However, ^{3}H-PCP binding was quantified by scintillation counting rather than autoradiography. The present study reports the characteristics of ^{3}H-PCP binding to rat brain sections measured autoradiographically.

^{3}H-PCP bound in a saturable fashion to rat brain sections and was displaced by excess unlabeled PCP. The areas of densest binding in the forebrain included the hippocampus, neocortex and neostriatum. Scatchard analysis of ^{3}H-PCP binding to hippocampus yielded a K_D of 279.5 ± 35 nM and a B_{max} of 3057 ± 406 fmol/mg prot., in agreement with the results of homogenate assays (Vincent et al, PNAS 76, 4678, 1979). Similar K_D's were observed for ^{3}H-PCP binding to neocortex, neostriatum and olfactory bulb, suggesting that each of these regions contains the same type of ^{3}H-PCP binding site. SKF 10,047 and 1-(1-phenylcyclohexyl)-3-methylpiperidine (PCMP) stereoselectively inhibited ^{3}H-PCP binding to hippocampal sections with the (+)-isomers being mnore potent than their respective (-)- isomers (Table 1). In addition, the relative potencies of SKF 10,047, PCMP, ketamine and dexoxadrol in inhibiting PCP binding agreed well with their abilities to produce PCP-like behavioral effects (Browne et al., in press).

In summary, the present study demonstrates the usefulness of autoradiographic analysis of ligand binding to brain sections not only for obtaining a global picture of specific binding but also for measuring its characteristics.

Table I

Compound	K_I (nM)	Relative Potency
PCP	413	1.00
(+)SKF 10,047	310	1.44
Dexoxadrol	683	0.65
(+)PCMP	912	0.49
(-)SKF 10,047	2,100	0.21
Ketamine	4,169	0.11
(-)PCMP	10,400	0.04

166.4 β-ALANINE BINDING IN THE PRESENCE AND ABSENCE OF SODIUM. L.M. Orensanz, M.C. Azuara* and I. Fernández*. Dpto. de Investigación, Centro Especial Ramón y Cajal; and Dpto. de Bioquímica, Colegio Universitario Arcos de Jalón, Madrid, Spain.

β-Alanine binds to synaptosomal-mitochondrial preparations in a "specific" manner. Previous studies have indicated that this binding may be representative of interaction with synaptic receptors for this amino acid. Although much of the experimental work leading to this hypothesis was performed in a bicarbonate-buffered, sodium-free medium, it has been suggested that β-alanine binding to synaptic receptors may be studied in the presence or absence of sodium, using very low concentrations of β-alanine, and strychnine to define the synaptic receptor component. This communication reports results of experiments designed to investigate the possibility of studying β-alanine binding in several sodium-free and sodium-containing media. Effects of antagonists and uptake inhibitors on binding as well as pharmacological and saturation characteristics of binding in the absence of sodium were also studied.

Experiments were conducted in rat tissue containing brain stem plus spinal cord. Specific binding was higher in sodium-containing than in sodium-free media and was absent when extensively frozen/thawed membranes were used. Binding in the presence of sodium was inhibited by strychnine and the uptake inhibitor arecaidine, whereas without sodium, it was only sensitive to strychnine. Using either Tris or bicarbonate-buffered media, binding was equally sensitive to cold β-alanine and strychnine and equally insensitive to arecaidine, demonstrating the existence of strychnine sensitive and insensitive β-alanine sites. Saturation experiments in the bicarbonate-buffered medium showed a similar affinity of β-alanine for the two types of sites.

Present results show the need to avoid sodium when studying β-alanine binding to its putative synaptic receptors. Binding is better detected in a bicarbonate-buffered medium than in Tris media, although their pharmacological specificity is identical.

166.5 AMINE RECEPTORS ON EMBRYONIC SENSORY NEURONS. K. Dunlap and D.R. Canfield*. Department of Physiology, Tufts University School of Medicine, Boston, MA 02111.

Nor-epinephrine (NE) has been shown to decrease the conductance of voltage-dependent Ca channels in the soma membrane of embryonic chick sensory neurons in vitro (1,2). This action of NE results in a decrease in the Ca-dependent soma action potential duration (APD). Dopamine (DA) can mimic this effect of NE on APD.

NE and DA act over a 1000-fold concentration range, with ED_{50}'s of 1.4 μM and 0.3 μM, respectively. Maximal concentration is 10 μM for both; however, DA is somewhat less potent, producing a maximal 29% decrease in APD as compared to 43% for NE. Several lines of evidence suggest that these two agonists act via the same receptor. Saturating doses of the two, when applied together, do not produce any larger response than that produced by either applied alone. In addition, NE added to the bath blocks the response of the neurons to DA with an ID_{50} identical to the ED_{50} measured for NE's agonist activity (i.e., the dose-response curves are identical for NE, whether it acts as an agonist or as a blocker of DA effects).

Inhibition of NE and DA responses by antagonists also suggest that these two amines act via the same receptor. Characteristic of an α_2 adrenergic receptor, the transmitter-induced decreases in APD are blocked by yohimbine (α_2) and phentolamine (α) but not prazosin (α_1) or propranolol (β). The ID_{50}'s for yohimbine and phentolamine are 5 nM and 160 nM, respectively. In contrast, the decrease in APD is not mimicked by clonidine or xylazine, both selective α_2 agonists. In fact, in higher concentration clonidine blocks the NE-induced decrease in APD (ID_{50} of 5 μM). This receptor also has properties of a dopaminergic receptor in that the transmitter-induced decrease in APD is blocked by haloperidol (ID_{50} = 10 nM).

These results show that the amine receptor which mediates the decrease in APD cannot be definitively classified as either adrenergic or dopaminergic, as it exhibits a pharmacology characteristic, in part, of both receptor types. (Supported by PHS Grant NS 16483).

1. K. Dunlap & G.D. Fischbach (1978) Nature 276: 837-839.
2. K. Dunlap & G.D. Fischbach (1981) J. Physiol. 317: 519-535.

166.6 THE PRESENCE OF HIGH AND LOW AFFINITY BINDING SITES FOR ^{3}H-IMIPRAMINE IN RAT CEREBRAL CORTEX. P.G. Conway and D.J. Brunswick*, Depts. of Psychiatry and Pharmacology, University of Pennsylvania and Veterans Administration, Phila., PA 19104.

It has been demonstrated previously that the tricyclic antidepressant drug imipramine binds to a high affinity binding site in rat brain tissue in vitro. Our preliminary findings suggested that there may also be a low affinity binding component of ^{3}H-imipramine. Thus this study was performed to further evaluate the binding characterisics of ^{3}H-imipramine in rat cerebral cortex. Saturation experiments were performed according to the method of Raisman et al. (Eur. J. Pharmacol. 61:373-380, 1980) by incubating fresh rat cerebral cortex membranes with a wide range of ^{3}H-imipramine concentrations (0.5nM to 50nM) for 60 minutes at 0°C. Specific binding was defined as that inhibited in the presence of 10uM desipramine and represented 70-80% of total binding at 2nM ^{3}H-imipramine. Scatchard analysis of the data revealed a biphasic, concave curve indicating that imipramine does not bind to a single population of non-interacting binding sites in the rat cortex. Additional evidence for non-homogeneous binding of ^{3}H-imipramine to rat cerebral cortex was obtained by studying the dissociation rates of imipramine. After equilibrating ^{3}H-imipramine (2nM) with cerebral cortex membranes for 60 minutes at 0°C, 10uM desipramine was added and the incubation was allowed to continue for various lengths of time (40 sec to 60 min). When the ^{3}H-imipramine specifically bound (defined using 10uM desipramine) was plotted against the time of dissociation a biphasic curve was exhibited with apparent half lives of dissociation of 2.5±.42 (mean±S.E.M.) and 18.5±2.46 min (n=3). Further support for non-homogeneous binding of ^{3}H-imipramine to rat cortex was obtained from competition curves derived by incubating cerebral cortical membranes with ^{3}H-imipramine (2nM) and various concentrations of unlabelled imipramine (.01nM to 100uM). These curves were shallow and Hill analysis gave coefficients of 0.60±.04 with IC-50 values of 11±1.8nM (n=3). Thus analysis of saturation, dissociation and competition curves indicate that ^{3}H-imipramine binds to low (micromolar) as well as high (nanomolar) binding sites in rat cortex when specific binding is defined with 10uM desipramine. These results are similar to those of Reith and coworkers (J. Neurochem. 40:389-395, 1983), who demonstrated both high and low affinity binding sites for ^{3}H-imipramine in mouse cerebral cortex. (Supported by Research Funds from the Veterans Administration and NIMH Grants MH 14654 and MH 36761.)

166.7 PROTEOLYTIC GENERATION AND PURIFICATION OF BENZODIAZEPINE BINDING SITE FRAGMENTS. K. L. Klotz*, J. W. Thomas[1], J. H. Neale and J. F. Tallman[2]. Department of Biology, Georgetown University, Washington, D.C. 20057; Biological Psychiatry[1], NIMH, Bethesda, MD 20205 and Department of Pharmacology[2], Yale University, New Haven, CT 06510.

The high affinity, membrane bound benzodiazepine (BZ) receptor site appears to be a component of a GABA receptor-ionophore complex. We have used proteolytic enzymes to generate water soluble fragments of the BZ receptor which we have purified subsequently without the use of detergents. The BZ binding site was photolabeled with ^{3}H-flunitrazepam and subsequently digested using trypsin, chymotrypsin or a combination of these enzymes. The effects of various enzyme concentrations and digestion times upon proteolysis were determined. The size of the tryptic fragments was estimated using SDS polyacrylamide gel electrophoresis. Trypsin digestion converts the 50K dalton receptor subunit into a 40K dalton fragment and then a 25K dalton fragment which remains membrane bound. Following enzymatic digestion of membranes, an approximately 5K dalton radiolabeled fragment is released into the aqueous supernatant.

Receptor digestion using combined trypsin and chymotrypsin for 20 minutes at 37° C released approximately 40% of the photolabeled BZ binding sites from rat brain membranes. The receptor fragment generated by enzymatic digestion was purified by Amicon YM10 filtration, Sephadex G50 gel filtration and reverse phase octadecyl silicone HPLC. Radiolabeled peaks of material which emerged from the HPLC were further purified by varying the chromatographic conditions. Microgram quantities of photolabeled BZ receptor fragments containing the BZ binding site have been partially purified using this method.

166.8 GLUTAMATE RECEPTOR: BIOCHEMICAL IDENTIFICATION IN HOUSEFLY MUSCLE. M. T. Filbin*, A. T. Eldefrawi and M. E. Eldefrawi*. Dept. of Pharmacology and Exp. Ther., University of Maryland School of Medicine, Baltimore, Maryland 21201.

Thoraces of house flies (Musca domestica L.) were homogenized (10% w/v) in Van Harreveld's buffer, pH 7.4. The following antiproteases were included at all stages of the preparation: PMSF, pepstatin, trypsin inhibitor, aprotinin and iodoacetamide. The homogenate was filtered through cheesecloth and centrifuged at 50,000 x g for 30 min. After resuspension of the pellet and centrifugation at 1,000 x g for 10 min, the supernatant was recentrifuged at 50,000 x g for 30 min. The pellet was washed and resuspended (1 g original tissue/ml) in 50 mM Tris-HCl, pH 7.1, and was used within 1 h of preparation. The membrane preparation (75 µl, 100-800 µg prot.) was incubated for 2 h at 4°C with [^{3}H]-L-glutamate (100 nM; 1 µCi) with and without 0.5 mM unlabelled L-glutamate to account for the nonspecific binding. The preparation was centrifuged at 12,500 x g for 7 min, after which the pellet was washed, and its radioactivity counted. Specific [^{3}H]L-glutamate binding was directly proportionate to tissue concentration up to 300 µg of protein per sample and saturated at concentrations of L-glutamate greater than 1.2 µM. Scatchard analysis of the binding data revealed consistently one binding site of K_D 0.57 ± 0.09 µM (mean of 5 expt.). The B_{max}, however, was found to vary from 10-40 pmol/mg prot., the higher density of binding sites being apparent in those preparations in which the flies were greater than 6 days old. Preincubation of the membranes for 5 min at 70°C eliminated specific [^{3}H]L-glutamate binding. Furthermore, specific binding was also abolished by preincubation of the membranes for 1 h at 21°C with trypsin, chymotrypsin or protease. Binding was also stereoselective as a greater sensitivity to the L- than to the D-isomer of glutamate was observed. Of the drugs tested only glutamate-like ligands were effective in inhibiting specific [^{3}H]L-glutamate binding. The order of potency was as follows: L-glutamate>L-aspartate>> D-glutamate=L-glutamate diethyl ester>N-methyl-D-L-aspartate>> kainate. A crude extract of the venom from the bee wolf Philanthus triangulum inhibited specific binding. The following drugs at 1 mM had an insignificant effect: ibotenate, proctolin, d-tubocurarine, γ-aminobutyric acid and glycine. It is suggested that [^{3}H]L-glutamate is binding to an L-glutamate receptor in this fly thoracic muscle membrane preparation. (Supported in part by NIH grant ES02594 and Army Research Office grant DAAG 29-81-K-0161.)

166.9 ANTIBODIES AGAINST THE BOVINE BRAIN GLUTAMATE BINDING PROTEIN. S. Roy and E.K. Michaelis, Dept. of Human Development and Ctr. for Biomedical Research, University of Kansas, Lawrence, KS 66045.

A small molecular weight glycoprotein that has high affinity binding sites for L-glutamic acid has been purified from rat brain synaptic membranes and from bovine brain crude membrane fraction. Based on the selectivity of the protein binding sites for various L-glutamate analogs and on protein's lack of glutamate metabolizing enzymatic activity, it was proposed that this binding protein may be the recognition macromolecule of the L-glutamate receptor complex (Michaelis et al., Mol. Cell. Biochem. 38, 163-179, 1981). We report here on our studies of raising antibodies in rabbits against the purified bovine brain glutamate binding protein (GBP).

Underivatized GBP as well as GBP derivatized with dinitrobenzene evoked a specific immune response in immunized rabbits. The antibody titer was measured by the solid phase enzyme-linked immunosorbent assay (ELISA). Our results show that antibodies against bovine GBP extensively reacted with rat brain GBP indicating thereby that both proteins are immunologically homologous. A bacterial glutamate-aspartate binding protein, however, did not show any cross-reaction with the anti GBP antibodies. The specificities of the antibodies were also investigated by measuring their cross-reactivity with glutamate metabolizing enzymes. Glutamate dehydrogenase, glutamine synthetase and γ-glutamyl transpeptidase showed little or no cross-reactivity with anti GBP antibodies. A crude bacterial glutamate decarboxylase preparation gave small to moderate cross-reaction with the anti GBP antibodies. The sensitivity of the ELISA assay and the specificity of the antibodies were such that GBP levels as low as 3-10 ng could be detected. Thus it should be possible to use these anti GBP antibodies to quantitate and localize this binding protein in the brain and other tissues and to explore the role of this binding protein in the function of the physiologic glutamate receptors. (Supported by grants AA 04732 from NIAAA, DAAG 29-79-C-0156 and DAAG 29-83-K-0065 from U.S. Army Res. Office, and Biomedical Research grant 4073-706, the University of Kansas).

166.10 STRUCTURAL REQUIREMENTS OF THE SODIUM-INDEPENDENT BINDING SITE OF 4-AMINOBUTANOIC ACID (GABA). S.L. Early and E.K. Michaelis Dept. of HDFL, Univ. of Kansas, Lawrence, KS 66045.

Studies aimed at defining the structural requirements of the sodium-independent binding site of 4-aminobutanoic acid (GABA) have depended mainly on the modification of the 3-carbon backbone and the carboxylic acid. Only recently has any attempt been made to study the effect of modifying the amino group of GABA to study the effect of modifying the amino group of GABA on the binding of [^{3}H]-GABA to brain tissue. We report here on the effects of some N-alkylated, N-acylated, and non-acidic derivatives of GABA on the sodium-independent binding of [^{3}H]-GABA to bovine cerebellar synaptic plasma membranes.

Under the assay conditions used in this study, GABA had a K_D of 14.5 nM and a B_{max} of 2800 pmol/mg protein as determined by Scatchard analysis. The Scatchard plot over the concentration range 7 to 112 nM appears linear. A curvilinear plot at higher concentrations of [^{3}H]-GABA was observed.

Eighteen analogues with modified carboxylic acid groups, most of them analogues of bicuculline, were tested. Only 5 inhibited binding by more than 50% at 1 mM. AETS (2-aminoethyl-2-aminoethanethiosulfonate) and kojic amine (2-aminoethyl-5-hydroxy-4-H-pyran-4-one) inhibited binding with IC_{50} values of 51.2 uM and 4 uM (as reported by others), respectively. Our results suggest that bicuculline's inhibitory potency is due to its rigid conformation and hydrophobicity, which allows bicuculline to interact with the GABA binding site without a free carboxylic acid group.

The most potent N-monoalkylated derivative of GABA was N-benzyl GABA with an IC_{50} of 37.6 uM. Tritylation of GABA gave a very lipophilic compound with an IC_{50} of 233 uM. It was observed that N-trityl-GABA inhibition was not time dependent. However, the compound does decompose in Tris sulfate buffer, presumably to GABA and a triphenylmethane derivative.

All attempts to modify the 4-amino group of GABA via an amide/peptide bond failed to produce a compound with any significant ability to inhibit GABA binding. Initially, we observed potent inhibition by the derivatives. However, we found that the activities of these compounds could be attributed to the presence of 0.036%-0.236% GABA. Schiff bases formed from GABA and a ketone or aldehyde were also potent inhibitors of [^{3}H]-GABA binding. However, the source of inhibition was not the parent compound, but rather the GABA released by the hydrolysis of the Schiff bases in solution. From these data, the free amine appears to be necessary for binding to the [^{3}H]-GABA binding site. Additionally, care needs to be exercised in data interpretation when working with compounds synthesized from GABA. Supported by Biomed. Grant 4073.

166.11 ADENOSINE RECEPTOR SITES IN PURIFIED ATRIAL SARCOLEMMAL MEMBRANES. M.L. Michaelis, T.E. Kitos*, and T. Mooney*, Ctr. Biomedical Research, Univ. of Kansas, Lawrence, Kansas 66045.

Adenosine (Ado) has multiple regulatory actions in many organ systems. It has marked depressant actions in the CNS and potent negative inotropic effects in the heart. Ado receptors may be coupled to adenylate cyclases which in some cells are inhibited and in others stimulated by the ligand's interaction with its receptor sites. The properties of Ado receptors in CNS have been studied through analysis of the binding interactions between Ado analog and Ado recognition sites in particulate fractions. The studies reported here involve the use of radioligand binding assays to delineate the basic characteristics of Ado binding sites in a preparation of highly purified sarcolemmal membranes from bovine atria.

Binding of the stable Ado analog [^{3}H] 2-chloroadenosine (2-ClAdo) to atrial myocyte membranes was measured in 20 mM potassium phosphate buffer, pH 7.4, at 4°C for 120 min, and incubations were terminated either by rapid filtration or by centrifugation at 4°C. Specific [^{3}H] 2-ClAdo binding was determined by subtraction of the radioactivity present in samples incubated in the presence of 100 µM L-phenylisopropyladenosine (L-PIA), a potent adenosine agonist. Scatchard analysis of the data obtained over a ligand concentration range from 0.5-750 nM indicated the presence of multiple binding sites, and the best least squares fit of the data to the Scatchard equation required the assumption of 3 classes of binding sites with interactions among the sites. The estimated B_{max} and K_D values for the 3 sets of sites were: 40 fmol/mg protein and 4 nM; 260 fmol/mg protein and 63 nM; and 5,500 fmol/mg protein and 667 nM. The displacement of bound [^{3}H] 2-ClAdo by several N^6-substituted Ado agonists also confirmed the presence of at least 2 classes of binding sites. The relative order of potencies for various agonists in displacing [^{3}H] 2-ClAdo was: 2-ClAdo > N^6-cyclohexyladenosine > L-PIA > N^6-methyladenosine > D-PIA. The IC_{50} for two xanthines believed to be receptor antagonists were: isobutylmethylxanthine, 0.6 µM, and theophylline, 2.1 µM. Adenosine uptake inhibitors such as papaverine and desmethyldiazepam required concentrations greater than 100 µM to produce 50% inhibition. These studies confirm the presence of specific Ado binding sites in atrial sarcolemmal membranes, and the preliminary pharmacological characterization suggests that these sites may be of the A_1 type which are generally associated with inhibition of adenylate cyclase activity. (Supported by the Amer. Heart Assoc., Ks. Affiliate, NS 16364, and BRSG RR5606.)

166.12 GLUCOCORTICOIDS ENHANCE PGE_1-STIMULATED CYCLIC AMP FORMATION IN CULTURED MURINE NEUROBLASTOMA CELLS (CLONE N1E-115). Carlos Forray* and Elliott Richelson. (SPON: R. Weinshilboum) Depts. of Psychiatry and Pharmacology, Mayo Clinic & Fdn., Rochester, MN 55905.

Adrenal glucocorticoids have been shown to regulate adrenergic receptor-mediated adenylate cyclase stimulation in brain tissue and in glial cells in culture. This finding has led to the hypothesis that glucocorticoids play an important role in endocrine-mediated receptor sensitivity changes. We determined whether these effects could be generalized to other receptors coupled to adenylate cyclase. To do these studies, we used murine neuroblastoma clone N1E-115 cells possess prostaglandin E_1 (PGE_1) receptors coupled to adenylate cyclase, since several neuroblastoma clones had been shown to be sensitive to induction of enzymes by glucocorticoids.

Corticosterone (Cort) or dexamethasone (Dex) added to the incubation medium of N1E-115 cells, induced a 40% increase in PGE_1-stimulated AMP (cAMP) formation. This increase was due to an increase in the maximum response without an apparent change in the ED_{50}. The time course of the facilitation of the PGE_1 response had a latency of 18 to 24 hrs and remained constant for at least 72 hrs following Dex or Cort incubation. The dose response curve for the steroids gave an ED_{50} of 2.6 µM for Cort and of 0.08 µM for Dex, suggesting that these hormones were active within the range of physiological concentrations. Other steroids such as progesterone, β-estradiol and testosterone had no significant effect on the PGE_1 response or in altering the glucocorticoid effects. Inhibitors of RNA or protein synthesis actinomycin-D (1 µg/ml) or cycloheximide (30 µM), respectively, blocked the effect of glucocorticoids.

These results suggest that glucocorticoids alter receptor regulation in neural cells through a genomic mechanism of action and that these effects may be functioning for other receptors coupled to adenylate cyclase. Furthermore, this study suggests that cells in culture may be a suitable model for studying these hormonal effects at the molecular level. (Supported by Mayo Foundation and USPHS Grant MH27692.)

166.13 DISCRIMINATION OF MULTIPLE S-2 BINDING SITES BY METHYSERGIDE IN CORTICAL TISSUES OF MOUSE AND HUMAN BRAIN. DG Morgan*, J Marcusson*, and CE Finch. Andrus Gerontology Center and Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Serotonin binding sites in the CNS have been classified as S-1 sites (labeled by ^{3}H-serotonin) and S-2 sites (cortical sites labeled by ^{3}H-spiperone) (Peroutka and Snyder, Fed. Proc. 42:213). We report here the resolution of two previously unidentified S-2 binding components which we designate S-2A and S-2B.

Membrane preparations of whole mouse cortex or human frontal cortex were incubated with ^{3}H-spiperone in 10 mM MOPS/TRIS buffer (pH 7.2) and 4 mM $CaCl_2$ with or without competing drugs, for 30 minutes at 25° and separated from free ligand by filtration.

Ketanserin, a selective S-2 antagonist, produced biphasic displacement curves. The initial phase (10^{-9} - 10^{-7} M ketanserin) displaced approximately 40% of the total binding. A second phase occured at ketanserin concentrations in excess of 10^{-6} M ketanserin, however, similar displacement could be observed with extensively boiled tissue preparations. Hence, this second phase was assumed to represent displaceable nonspecific binding. A concentration of 0.5 µM ketanserin was subsequently used to define S-2 specific ^{3}H-spiperone binding.

Methysergide, a more general serotonin antagonist, also produced biphasic displacement curves. However, unlike ketanserin, the initial phase (10^{-9} - 10^{-7} M) displaced only 20% of the total binding, and no displacement from boiled membranes was obtained with methysergide. When methysergide competitions were conducted in the presence of a saturating concentration of ketanserin (0.5 µM), no additional displacement by methysergide was observed. Thus the two phases of methysergide competition involve the same binding sites as the S-2 specific phase of ketanserin displacement. We refer to the fraction of ^{3}H-spiperone binding with high affinity for methysergide as S-2A binding sites, and that fraction with a lower affinity as the S-2B subtype.

Saturation analyses using 0.3 µM methysergide to discriminate S-2A from S-2B indicated equal densities of S-2A and S-2B binding sites in human frontal cortex (130 fmoles/mg protein) and whole mouse cortex (50 fmoles/mg protein). The affinity of ^{3}H-spiperone for S-2A sites was twice that for S-2B sites (K_d = 0.67 and 1.45 nM respectively)

The biphasic displacement of ^{3}H-spiperone by methysergide was unaffected by guanine nucleotides, protease inhibitors, or the ionic constituents of the assay and wash buffers. Other experiments reported at this meeting indicate age related reductions in S-2A binding density with no change in S-2B recognition sites (Marcusson et al). (Glenn Fndn & NIA AG-03272)

167.1 CO-LOCALIZATION OF VASOPRESSIN, NEUROPHYSIN AND NORADRENALIN IMMUNOREACTIVITY IN SUBPOPULATIONS OF RAT LOCUS COERULEUS AND SUBCOERULEUS. A.R.Caffé*, F.W.van Leeuwen* and H.W.M.Steinbusch+, Netherlands Institute for Brain Research, 28, IJdijk, 1095 KJ Amsterdam; +Free University, Medical Faculty, Dept. of Pharmacol., Van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands.

It was recently shown that vasopressin (VP) and neurophysin (NF) immunoreactive cell bodies are present not only in the supraoptic, paraventricular and suprachiasmatic nuclei, but also in a variety of perikarya within the bed nucleus of the stria terminalis (BST), medial amygdaloid nucleus (AME), dorsomedial hypothalamus (DMH) and locus coeruleus (LC). In the BST, AME and DMH, parvocellular VP and NF elements are present while medium-sized, multipolar cells (width 15-20 µ, length 24-30 µ) are prominent in the LC and subcoeruleus (Van Leeuwen and Caffé, 1983; Caffé and Van Leeuwen, 1983).

In order to reveal whether or not noradrenalin (NA) and VP co-exist within perikarya of the LC, 10 µ thick cryostat sections were collected. The rats had been treated with colchicine 24-48 h prior to fixation with 4% paraformaldehyde and 0.5% glutaraldehyde. Consecutive sections were incubated according to the unlabeled antibody enzyme method, using antibodies raised against VP, NA and NF.

NA immuno-reactive cell bodies could be demonstrated throughout the entire LC and subcoerulear area. A considerable number of NA-immunoreactive cells within the LC was also immunoreactive for VP or NF, suggesting co-localization of these compounds.

With regard to the biological relevance of such co-existence, it is of interest that vasopressin appears to facilitate memory consolidation processes, via modulation of noradrenergic transmission in limbic midbrain terminals of the dorsal noradrenergic bundle (Kovács et al., 1979). The mechanism of this interaction is still unknown.

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Kovács, G.L., Bohus, B., Versteeg, D.H.G., Neurosci. *4*,1529, 1979.
Van Leeuwen, F.W., Caffé, A.R., Cell Tiss. Res. *228*, 525, 1983.

167.2 LEU-ENKEPHALIN, DYNORPHIN AND BOMBESIN CONTENTS OF PURIFIED NORADRENERGIC VESICLES. R. L. Klein[1], R. Day[2]* and S. Lemaire[2]. Depts. of Pharmacology, U. Miss. Med. Ctr.[1], Jackson, MS 39216 and U. Sherbrooke[2], Quebec, Canada J1H 5N4.

Antibodies specific to Leu-enkephalin(L-ENK), Dynorphin 1-13 (DYN), Bombesin(BOM), Vasoactive intestinal polypeptide(VIP), neurotensin(NT) and Substance P(Sub-P) were used to analyse by RIA a highly purified fraction of large dense cored noradrenergic vesicles(LDVs) which can be prepared from the bovine splenic nerve. Preliminary data indicate that VIP, NT and Sub-P were not above detection limits in the purest LDV fractions, but traces of VIP occurred in the whole nerve homogenate.

LDV fractions contained L-ENK at 1.3-1.7 nmol/mg protein, representing a 69-fold purification from the 10,500xg for 15 min supernatant of the nerve homogenate in which > 85% L-ENK was particulate. The data are uncorrected for peptide losses during processing or for non-specifically adsorbed protein in the vesicle fraction or for vesicle purity.

LDV fractions contained DYN at 0.19-0.25 nmol/mg protein representing an average 66-fold purification and > 87% DYN was particulate in the nerve homogenate.

LDV fractions contained BOM at 0.83-0.95 nmol/mg protein representing an average 77-fold purification and > 95% BOM was particulate in the nerve homogenate.

A preliminary ratio of L-ENK:DYN:BOM in the purest LDV fraction is ~ 7:1:4. By radioreceptor and HPLC assays, approximately equal amounts of L-ENK and M-ENK have been shown to co-exist with noradrenaline(NA) in the LDVs (Wilson et al., 1980) and have been calculated at 0.4-0.8 nmol ENKs/mg protein(corrected) which is equivalent to 58-100 molecules ENKs/LDV (Klein et al., 1982). After trypsin treatment, cryptic ENK activity can be unmasked to double the value by radioreceptor assay. The present RIA data confirm about a two-fold higher content of L-ENK compared to the radioreceptor assay and that DYN accounts for a portion of the 40-60% LDV opioids of > 600 but < 5000 Daltons in size. Thus, at least two synthesis pathways for opioids can be inferred to be present in the splenic nerve, estimated to consist of ~ 98% sympathetic C-fibers.

Supported by the American Heart Association 82-604 to RLK and the Medical Research Council of Canada to SL.

167.3 DIFFERENTIAL RELEASE OF SOMATOSTATIN-14 AND SOMATOSTATIN-28(1-12) FROM RAT HYPOTHALAMUS. C. Bakhit, L. Koda, R. Benoit*, J.H. Morrison and F.E. Bloom. A.V. Davis Center, The Salk Institute, La Jolla, CA 92037 and *Laboratories of Neuroendocrinology, The Salk Institute.

Recent investigations aimed at identifying the pro-somatostatin precursor have resulted in the characterization of two somatostatin-related peptides: somatostatin-28 (SS28), which contains the original tetradecapeptide somatostatin-14, and the dodecapeptide somatostatin-28(1-12) which corresponds to the NH_2-terminal portion of SS28.

In an effort to investigate how the somatostatin-related peptides are stored and released we have examined the in vitro release of SS14 and SS28(1-12)-like immunoreactivity (LI) from rat hypothalamic slices following in vivo or in vitro treatment with cysteamine, a drug previously shown to have specific depleting effects of SS14 but which does not influence the tissue stores of SS28 or SS28(1-12)-LI (Bakhit et al, Regulatory Peptides, in press, 1983). Cysteamine (300 mg/kg, s.c. 4 hrs) administration to rats resulted in a marked depletion of hypothalamic SS14 and a profound decrease in the potassium evoked in vitro release of SS-14 without a significant change in the content or release of SS28(1-12)-LI. Further, cysteamine (1mM) administered in vitro enhances the spontaneous release and markedly potentiates the potassium evoked release of SS14. In vitro exposure to cysteamine, however, does not alter either the spontaneous or potassium evoked release of SS28(1-12)-LI. Selective immunohistochemical visualization of hypothalamic neuronal cell bodies and fibers following cysteamine administration shows a marked disappearance of the SS14 immunoreactive fibers and cell bodies with no apparent change in the SS28(1-12) immunoreactive fibers or cell bodies. These data suggest, that in rat hypothalamus, SS14 and SS28(1-12) are either stored and released separately from the same terminals or are released from different neuronal systems. This work supported by USPHS HL 25457, AA 07273, AA 03504, and MRC (Canada) fellowship to R.B.

167.4 ACETYLCHOLINESTERASE AND SOMATOSTATIN-LIKE-IMMUNOREACTIVITY COEXIST IN A SUBPOPULATION OF NEURONS IN CULTURES FROM RAT CEREBRUM. J. R. DELFS, C. H. ZHU* AND M. A. DICHTER. DIVISION OF NEUROSCIENCE, CHILDREN'S HOSPITAL, BOSTON, MA 02115.

In brains of persons with senile dementia of the Alzheimer type, there are low levels of both choline acetyltransferase and of somatostatin (Davies et al. Nature 288:279-280, 1980). These abnormalities suggest that somatostatinergic and cholinergic systems may interrelate in the brain. In cultures of rat cerebrum, somatostatin-like-immunoreactivity (SOM-LI) can be localized by histochemistry to neurons (Delfs et al. Nature 283:676-677, 1980). Other studies have shown that neurons in these cultures stain for acetylcholinesterase (AChE) (Dichter and Mesulam, J.Histochem. Cytochem.29:306-308, 1981). In the present study, we asked whether SOM-LI and AChE might coexist in individual neurons.

For cultures, 15 to 16 day fetal rat cerebri were dissected, mechanically and enzymatically dissociated, and grown in a modified MEM with 5% rat serum. At 5 to 6 weeks cultures were treated with 100 nM colchicine for 24 hours, then fixed, and stained, first for AChE by the acetylthiocholine-copper sulfate method of Koelle, and then by the indirect immunofluorescent method of Coons with a rabbit anti-SOM antibody (furnished by Seymour Reichlin).

Out of 391 neurons identified prospectively to contain AChE, 59 (15%) were also seen to stain for SOM-LI. Out of 140 neurons identified prospectively as staining for SOM-LI, 31 (22%) were also seen to stain for AChE. Of the neurons staining for both SOM-LI and AChE, 13% were monopolar, 18% were bipolar or bitufted, 17% were stellate, and 13% resembled pyramidal neurons.

When an identical double staining procedure was carried out for cholecystokinin-octapeptide-LI (CCK-8-LI) and AChE, simultaneous staining for both substances in the same neuron was never observed.

These results demonstrate that in cultures of rat cerebrum there exists a subpopulation of neurons in which SOM-LI and AChE coexist. Whether the AChE activity in these neurons signifies cholinergic or a cholinoceptive neurons must await further studies.

(This work was supported by NS15362, NS00608, and Core Grant HD06276.)

167.5 COEXISTENCE OF SUBSTANCE P, CORTICOTROPIN RELEASING FACTOR AND ACETYLCHOLINESTERASE IN NEURONS OF THE NUCLEUS TEGMENTI DORSALIS LATERALIS, J. A. Olschowka, D. I. Diz* and D. M. Jacobowitz, Lab. of Clinical Science, NIMH and NIGMS, Bethesda, MD 20205.

The two neuropeptides, substance P (SP) and corticotropin releasing factor (CRF), have both been described as having a wide distribution throughout the rat central nervous system. The patterns of terminal arborization and location of immunoreactive cell bodies are similar for these two peptides in a number of regions, e.g. medial frontal cortex (MFC), septum, thalamus, nucleus tegmenti dorsalis lateralis (ntdl), etc. The cell bodies of the ntdl are particularly interesting for they have been shown to contain acetylcholinesterase (AChE) and project to the MFC, septum and thalamus. The purpose of the present study is to 1) determine if SP, CRF and AChE coexist within neurons of the ntdl and 2) determine to what areas they may project.

To determine the coexistence of SP, CRF and AChE, male rats were given colchicine (100 µg, intracisternal), perfused 2 days later and processed for the indirect immunohistochemical procedure. Cryostat sections of the ntdl were stained first for CRF, photographed and then the antiserum was removed by elution with acidified $KMnO_4$ (Tramu, G. et al., J. Histochem. Cytochem. 26: 322, 1978). The sections were then restained for SP and photographed. Alternatively, sections were stained for AChE and then SP.

The projections of ntdl neurons were determined by injecting rats with either True Blue (2.5%, 42-300 nl) or Fast Blue (5%, 83-300 nl) into the MFC, anterior cingulate cortex, parietal cortex, septum or dorsal thalamus. After 4-7 days, the rats were given colchicine (100 µg, intracisternal), perfused 2 days later and processed for either SP or CRF immunofluorescence or AChE histochemistry.

Following the staining of the same ntdl section for both SP and CRF, there appeared to be complete coexistence of the two peptides. AChE histochemistry revealed a larger number of neurons within the ntdl, a small proportion of which also contained SP. Following the injection of True Blue or Fast Blue into the MFC, septum or thalamus, moderate numbers of cells within the ipsilateral ntdl were labeled, as well as a few neurons contralaterally. A small proportion (20-50%) of these dye-labeled neurons were observed to also contain AChE, SP or CRF. However, not all AChE, SP or CRF cells contained the fluorescent dye. Dye injections into the anterior cingulate or parietal cortices, areas with no SP or CRF innervation, failed to label ntdl neurons. These results suggest that SP, CRF and AChE coexist within a subpopulation of ntdl neurons and that these cells project to a number of forebrain regions.

167.6 SUBSTANCE P IN THE ASCENDING CHOLINERGIC RETICULAR SYSTEM. S.R. Vincent, K. Satoh and H.C. Fibiger. Depts. of Psychiatry and Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The existence of an ascending cholinergic reticular system has been postulated by Shute and Lewis to arise from cells in the midbrain and pontine tegmentum. These cholinergic neurons have recently been identified immunohistochemically using antibodies to choline acetyltransferase. We have found that pharmacohistochemical protocol for acetylcholinesterase utilizing diisopropylfluorophosphate (DFP) can also be used to visualize selectively these cells. In the rat, the neurons in this cell group begin in the midbrain reticular formation just caudal to the substantia nigra and extend dorsocaudally in the lateral portion of the mesencephalic and pontine reticular formation. In the rostral pons they extend medially in the dorsolateral tegmentum and enter the periventricular gray, forming a dense cluster in the laterodorsal tegmental nucleus of Castaldi, just medial to the rostral pole of the locus coeruleus. We have discovered that all of the cholinergic neurons in this particular cell group can also be specifically stained by a novel enzyme histochemical technique based on NADPH-diaphorase activity. This method stains the cell bodies, dendrites and fibers of these cholinergic neurons making possible detailed morphological studies of this cell group. Also, NADPH-diaphorase histochemistry can be readily combined with immunohistochemistry. A population of substance P neurons has been found in the midbrain and pontine tegmentum, in particular within the laterodorsal tegmental nucleus. Some of these substance P cells appear to project to forebrain areas such as the septum and prefrontal cortex. By combining the indirect immunofluorescence technique for substance P with NADPH-diaphorase histochemistry or acetylcholinesterase pharmacohistochemistry, we have found that about one third of the cholinergic neurons in these tegmental areas display substance P immunoreactivity. Thus substance P and acetylcholine coexist in a population of neurons in the midbrain and pontine tegmentum that project to the forebrain.

Supported by the Medical Research Council of Canada.

167.PO VIP, CCK, AND MET-ENKEPHALIN IN PIA ARACHNOID AND CEREBRAL ARTERIES AFTER UNILATERAL LESIONS OF CAT TRIGEMINAL GANGLIA. M.A. Moskowitz, T.V. Norregaard*, L.-Y. Liu-Chen, R. Weatherwax*, S. Michener*, V.L.W. Go*. Neurology and Neurosurgery Services, Mass Gen Hosp, Harvard Medical School, Boston, MA 02114; Gastroenterology Dept, Mayo Clinic, Rochester, MN 55905.

Trigeminovascular projections to cat cerebral arteries comprising the circle of Willis have been identified by axonal transport studies using horseradish peroxidase histochemistry (Mayberg et al, Science '81). The neurotransmitter, substance P has been found in some of these perivascular fibers as determined by immunohistochemical and RIA data following unilateral trigeminal ganglionectomy (see Abst.: Liu-Chen, et al; Norregaard, et al). The neuropeptides, vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK), and met-enkephalin (m-ENK) have also been localized in primary sensory neurons where they may function as putative neurotransmitters or neuromodulators.

In this report, we examined the pia arachnoid and its accompanying arteries for the presence of iVIP, iCCK and im-ENK in cat by RIA; when found, we then determined by lesioning studies, whether or not iVIP was contained within sensory neurons projecting from trigeminal ganglia. Levels of VIP were approximately 35 and 1 fm/mg protein in cat cerebral arteries and trigeminal ganglia, respectively; immunoreactivity exhibited parallel displacement over a 100-fold dilution. The authenticity of VIP was determined by gel filtration chromatography (Sephadex G-50 resin) and co-elution of VIP-like activity with synthetic peptide. Trigeminal lesions were performed surgically in 13 cats using an extradural approach and microsurgical methods. Three to six weeks later, animals were sacrificed, pia arachnoid harvested from each hemisphere, and assays performed on extracts of tissue homogenates. IVIP levels in cat vessels decreased by approximately 35% on the side of the lesion. Decreases of similar magnitude were measured for im-ENK but not iCCK. Levels of this peptide increased significantly in pia and arachnoid ipsilateral to the lesion. These preliminary findings indicate that iVIP and im-ENK arise in part from trigeminal nerve fibers which surround and invest arteries of the circle of Willis. ICCK, shown recently to coexist with iSP in primary sensory neurons, did not appear to be contained within perivascular trigeminal fibers, at least as reflected by increases in levels measured after unilateral trigeminal lesions. Other sources of iVIP, im-ENK and iCCK in addition to the trigeminal ganglia remain to be determined.

168.1 CHARACTERIZATION OF A PUTATIVE NICOTINIC ACETYLCHOLINE RECEPTOR FROM RAT BRAIN. G. Kemp*, L. Stone*, B. Morley and M. McNamee, Neurosciences Program, Univ. of Alabama in Birmingham, Boy's Town Institute, Omaha, Neb., and the Dept. of Biochem. and Biophysics, University of California, Davis, California.

^{125}I-α-Bungarotoxin (^{125}I-BGT) is known to bind with high affinity and almost absolute specificity to nicotinic acetylcholine receptors (AChRs) isolated from the mammalian neuromuscular junction and from the electric organs of a variety of elasmobranches and teleosts. ^{125}I-BGT also binds to a closely related protein found in mammalian autonomic ganglia and CNS tissue. It has been suggested that the identity of this protein is that of the ganglionic nicotinic AChR long known to be responsible for primary synaptic connections in the autonomic nervous system.

Using a protocol developed to purify AChR from mammalian skeletal muscle, this protein has been purified to apparent homogeneity. Purification steps include Naja naja siamensis α-toxin (cobratoxin) biospecific adsorption, DEAE-Sephadex ion exchange chromatography, and concanavalin A-Sepharose 4B-adsorption. The purified product binds approximately 3 pMoles of ^{125}I-BGT per μg of protein. Denaturing polyacrylamide gel electrophoresis reveals polypeptide chains 47,000, 50,000, 57,000, and 62,000 daltons.

The affinity ligand (^{3}H)-maleimidobenzyl trimethylammonium iodide (^{3}H-MBTA) has been used to establish that the ligand binding site in peripheral AChR's is associated with the 39-44 K dalton α-chain. ^{3}H-MBTA completely abolishes ^{125}I-BGT binding to brain AChR, and labels the 50K chain.

It is well documented that peripheral AChRs bind BGT at sites on each of two α-chains. When bound to a cobratoxin-Sepharose 4B biospecific adsorption matrix, it can be shown that only one of these sites participates in the interaction with the affinity resin, leaving the second site free to interact stoichiometrically with ^{125}I-BGT. In contrast, brain AChR adsorbed to the same affinity resin is unable to bind ^{125}I-BGT. This result suggests that brain AChR has only one α-toxin binding site per molecule. If an additional ligand site or sites are present on brain AChR, as is the case for peripheral AChRs, then these sites are not blocked by BGT. Activation by nicotinic agonists acting at these proposed but undocumented sites may be responsible for the known inability of α-toxins to block agonist mediated conductance in neuronal tissues.

(Supported in part by NIH grant 14262.)

168.2 IN VIVO REGULATION OF ^{3}H-ACETYLCHOLINE BINDING SITES IN BRAIN BY NICOTINIC CHOLINERGIC DRUGS. Rochelle D Schwartz and Kenneth J. Kellar. Department of Pharmacology Georgetown University Schools of Medicine and Dentistry, Washington, DC

Nicotinic cholinergic receptors in rat brain have been studied using [^{3}H]-acetylcholine ([^{3}H]ACh) of high specific activity (Schwartz et al., Mol. Pharmacol. 22, 56-62, 1982). In the presence of a cholinesterase inhibitor to prevent hydrolysis and atropine to block muscarinic cholinergic receptors, [^{3}H]ACh binds rapidly, reversibly and with high affinity to rat brain membranes associated with the synaptosomal fraction. The [^{3}H]ACh binding sites are unevenly distributed throughout the brain; the highest levels of binding are found in the thalamus, cortex and striatum. Nicotinic cholinergic agonists such as (-)nicotine, cytisine and carbachol are at least 10-1000 times more potent (K_I = 1-13 nM) than nicotinic cholinergic antagonists in displacing [^{3}H]ACh binding.

Recently we have shown that [^{3}H]ACh binding sites in rat brain are down-regulated after chronic treatment of rats with the cholinesterase inhibitor diisopropyl fluorophosphate (Schwartz and Kellar, Science 220, 214-216, 1983). In addition, [^{3}H]ACh binding sites were shown to up-regulate after repeated nicotine administration. In the present study, we compared the effects of repeated administration of cotinine, the major metabolite of nicotine, and of the nicotinic agonist, cytisine with those of nicotine on [^{3}H]ACh binding sites in several areas of rat brain. All drugs were injected (6.3 umol/kg) SC twice daily for 10 days. Repeated nicotine administration resulted in a 38%, 18% and 25% increase in [^{3}H]ACh binding in the cortex, thalamus and striatum, respectively. However, nicotine decreased [^{3}H]ACh binding in the hypothalamus by 47%. Cotinine had no effect in the cortex or thalamus, but decreased [^{3}H]ACh binding 25% and 29% in the striatum and hypothalamus, respectively. Cytisine had similar effects to nicotine in the cortex (25% increase), thalamus (18% increase) and hypothalamus (53% decrease). However, repeated administration of cytisine decreased [^{3}H]ACh binding in the striatum by 25%. While nicotine is most likely not acting via its metabolite, cotinine, it is known to have both agonist and antagonist properties. (The same is true for cytisine.) Up-regulation could be explained by an antagonistic action of nicotine and cytisine at [^{3}H]ACh recognition sites. In addition, the different effects of these two drugs in different brain regions suggest the existence of sub-populations of [^{3}H]ACh binding sites in different areas of the brain.

168.3 CHRONIC REGULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS OF CULTURED CELLS BY AGONIST OCCUPANCY. D.E. Robinson* and R. McGee, Jr., Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

In many receptor systems chronic changes in receptor occupancy by an agonist induce compensatory changes in the cell in an attempt to maintain homeostasis. However, it is unknown if such factors also regulate the neuronal nicotinic acetylcholine (ACh) receptor. This question has been studied using a model system, the clonal pheochromocytoma cell line, PC12, which possesses functional "neuronal" nicotinic acetylcholine receptors. An ion flux assay using $^{86}Rb^+$ as the tracer ion was used to monitor the cellular response to receptor stimulation by agonists. Monolayer cultures of the cells were pretreated with ouabain to inhibit Na^+,K^+-ATPase, and then stimulated for 20 sec with the agonist in the presence of tracer amounts of $^{86}Rb^+$ in a Na^+-depleted salt solution. Initial experiments have examined the changes in receptor function that occur upon chronic treatment with the non-hydrolyzable agonist, carbamylcholine (carbachol). Following chronic treatments carbachol was removed from the cultures for 30 min, sufficient time to allow complete resensitization of the receptors. A time-dependent decrease in the maximal response to carbachol was observed after treatment with carbachol for 1 to 10 days. The extent of decreased responsiveness of the receptors was dependent on the concentration of carbachol between 50 uM and 1 mM, concentrations very similar to those which activate the receptors. Upon removal of the carbachol from the culture medium, receptor function began to return towards control values within 24 hr and was completely recovered by 4 days. This down-regulation of receptor activity occurred without any changes in the EC_{50} for receptor activation, suggesting that the receptors which remained functional were unaltered. Mecamylamine, a nicotinic ganglionic antagonist which potently blocked acute receptor stimulation by carbachol, antagonized the carbachol-induced functional down-regulation of the receptors. In contrast, d-tubocurarine, a neuromuscular antagonist which is thought to act as a channel blocker, did not antagonize the chronic effects of carbachol. The simplest interpretation of these results is that chronic treatment with an agonist results in a change in the number of functional nicotinic acetylcholine receptors in the membranes of these cells. Since a channel blocker did not inhibit down-regulation, the results also suggest that receptor occupancy alone directly triggers down-regulation.

168.4 MONOCLONAL ANTIBODIES TO TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR THAT CROSS-REACT WITH SPECIFIC SUBSETS OF MAMMALIAN PERIPHERAL NEURONS AND SMOOTH MUSCLE. Edward Hawrot, Janet Holliday*, Barry Schweitzer*, and Linda L.Y. Chun*. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. of Neurology, Mass. General Hospital, Boston, MA 02114.

Two mouse monoclonal antibodies (mAb) generated against Torpedo electric organ nicotinic acetylcholine receptor (AChR) but with considerable cross-species recognition of skeletal muscle AChR have been further characterized. One antibody (27.43.37) immunoprecipitates both murine muscle AChR and Torpedo AChR labeled with ^{125}I-α-bungarotoxin (^{125}I-BuTX). It binds primarily to the α-subunit of Torpedo AChR on immunoblots with some minor cross-reactivity to the δ-subunit. Recognition of the α-subunit is not reduced by prior treatment of AChR with endoglycosidase-H. Immunofluorescence studies with frozen sections indicate that mAb 27.43.37 binds to mouse and rat skeletal muscle endplates, to the longitudinal and circular smooth muscle layers of guinea pig ileum, to rat superior cervical ganglion (SCG) neurons, and to subsets of rat sensory neurons.

A second mAb (23.12.18) also immunoprecipitates ^{125}I-BuTX-labeled AChR from both mouse muscle and Torpedo. Its subunit specificity is unknown since antigen recognition is lost upon SDS-denaturation of AChR. In frozen sections, mAb 23.12.18 binds mouse and rat skeletal muscle endplates, rat SCG neurons, subsets of rat sensory neurons, rat myenteric plexus neurons, and rat sciatic nerve. The rat pheochromocytoma cell line, PC12, is also stained by mAb 23.12.18.

In order to characterize the nature of the recognized antigens, PC12 cells and SCG explants obtained from neonatal rats were metabolically labeled with ^{35}S-methionine. In addition, membrane fractions prepared from guinea pig ileal smooth muscle were labeled with ^{125}I using lactoperoxidase. Detergent extracts of the labeled proteins were immunoprecipitated with the mAbs covalently bound to Sepharose beads. Analysis of the immunoprecipitates by SDS-gel electrophoresis has led to the identification of 3 or 4 prominently labeled bands in the molecular weight range of 45 to 70 kd. The interrelationship between the labeled proteins being immunoprecipitated from the various tissue sources is under investigation as is the further characterization of the properties of the neuronal and smooth muscle antigens.

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168.5 ULTRASTRUCTURAL LOCALIZATION OF 43K PROTEIN IN TORPEDO POSTSYNAPTIC MEMBRANES WITH MONOCLONAL ANTIBODIES. Stanley C. Froehner, Barnaby E. Wray*, and Robert Sealock*. Department of Biochemistry, Dartmouth Medical School, Hanover, N. H. 03756 and *Department of Physiology and The Neurobiology Program, University of North Carolina, Chapel Hill, N. C. 27514.

In addition to the subunits of the acetylcholine receptor, other proteins that may play important roles in synaptic function are present in highly-purified postsynaptic membranes from Torpedo electric organ. The major non-receptor component of these membranes (43K protein) has an apparent mol. wt. of 43,000 and displays the properties of a peripheral membrane protein. We have prepared monoclonal antibodies (mabs) to this protein and have used them to investigate the localization of this component at the electrocyte synapse. Receptor-rich membranes were extracted with 10mM lithium diiodosalicylate and the solubilized proteins (predominantly 43K protein) were used to immunize mice. Spleen cells were fused with NS-1 myeloma cells and hybridomas secreting active antibodies were cloned. The specificity of the mabs was determined by an immunoblot technique. Five hybridomas secrete mabs that react with a 43K protein of Torpedo membranes. Immunoblots of membrane proteins separated by two-dimensional gel electrophoresis show that the reactive 43K components have isoelectric points of 7.5-8.0. The anti-43K mabs do not recognize either the acetylcholine receptor or Torpedo muscle actin. Ultrastructural studies on Torpedo electric organ using these anti-43K mabs indicate that the 43K protein is restricted to the cytoplasmic surface of the postsynaptic membrane. Furthermore, the distribution of the 43K protein and the receptor appear to be very similar and possibly coextensive. This work was supported by a grant from the Muscular Dystrophy Association (S.C.F.) and by N.I.H. grants NS-14871 (S.C.F.) and NS-15293 (R.S.).

168.6 COMPARISON OF LIGHT AND HEAVY, EMBRYONIC AND ADULT FORMS OF THE TORPEDO ACETYLCHOLINE RECEPTOR USING MONOCLONAL ANTIBODIES. B. Holton, S.J. Tzartos and J.-P. Changeux. Neurobiologie Moléculaire, Institut Pasteur, 75724 Paris-Cedex 15, FRANCE.

The acetylcholine receptor (AChR) from adult Torpedo marmorata electric organ exists in a Light form ($\alpha_2\beta\gamma\delta$) of apparent molecular weight 250,000. The association of two Light forms via an intermolecular δ-δ disulfide bridge results in the AChR Heavy form, which predominates in vivo. With the aim of distinguishing between such states, we screened a library of 38 monoclonal antibodies for the ability to immunoprecipitate the ^{125}I-α-bungarotoxin-labeled Heavy or Light forms. None of the nine anti α-subunit mAbs tested distinguished between the two forms. On the other hand, the 29 anti β, γ and δ subunit mAbs precipitated more Heavy than Light form.

The Heavy and Light forms also exist in embryonic electric organ. We tested the same collection of mAbs against extracts of embryonic tissue. Most of the anti β, γ and δ subunit antibodies precipitated more adult than embryonic AChR, but the anti α-subunit mAbs made no distinction. Parallel measurements of the Heavy to Light form ratios in extracts from embryonic and adult electric organs revealed only 35% Heavy form in the embryo against 78% in the adult. Addition of the alkylating agent, N-ethyl maleimide (NEM) to electric organ preparations increased the percentage of Heavy forms in embryonic but not in adult extracts. When ^{125}-I toxin-labelled adult AChR was mixed with embryonic electric organs and present throughout the membrane preparation, the Heavy to Light receptor ratio did not change. In addition, a carefull study of embryonic and adult Light forms and of embryonic and adult Heavy forms did not reveal any difference in their reactivity towards the 38 mAbs tested. Thus, we conclude that our mAbs can not distinguish Torpedo embryonic and adult AChR by intrinsic structural differences but by the Light/Heavy form ratio. The ability of the anti-β, γ and δ subunit mAbs to differentiate between the two forms may serve as a tool for their localization.

168.7 ACUTE ELEVATION OF cAMP DOES NOT EFFECT THE FUNCTIONAL PROPERTIES OF A NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR. R. McGee, Jr. and B. Liepe* (SPON: F. G. Standaert), Dept. of Pharmacology, Georgetown Univ. Sch. of Med., Washington, D.C. 20007

Nicotinic acetylcholine (ACh) receptors from Torpedo electric organs (models of muscle receptors) have been shown to be phosphorylated by processes dependent on cAMP or Ca^{++}, as well as processes independent of these modulators. However, it is unknown whether or not these phosphorylations influence receptor function. For neuronal nicotinic ACh receptors, it is unknown whether similar phosphorylations occur, and, if so, whether they alter receptor function. Although the neuronal receptors are not readily amenable to direct analysis of their state of phosphorylation, their functional properties can be investigated under conditions known to alter cellular rates of phosphorylation. We have begun these studies by looking for cAMP-dependent alterations in receptor functions. Intact pheochromocytoma cells (clone PC12) were exposed to concentrations of forskolin (10^{-8} to 10^{-5} M) which produced 2-100 fold increases in cellular cAMP and increased cAMP-dependent protein phosphorylation. The responsiveness of the neuronal nicotinic ACh receptors then was assessed as agonist-induced uptake of $^{86}Rb^+$ into the cells. The uptake assay was conducted in a Na^+-depleted salt solution containing ouabain to inhibit Na^+,K^+-ATPase. At 37°C $^{86}Rb^+$ uptake induced by activation of the receptors with carbamylcholine was linear for at least 20 sec. Under all conditions of elevated cAMP (2-100 fold increases, 1-40 min elevation of cAMP prior to stimulation) no changes in receptor-mediated uptake of $^{86}Rb^+$ were observed. Neither the maximal response elicited by saturating agonist concentrations nor the agonist concentration/receptor response relationship was altered by elevation of cAMP. The rapid desensitization of the receptors also was not changed by elevation of cAMP; the entire time course for agonist-induced uptake of $^{86}Rb^+$ was the same in the presence and absence of forskolin. Finally, even the distribution between desensitized and excitable receptors achieved upon prolonged exposure to the agonist was unchanged by elevation of cAMP. Thus, within the time frame of 1 to 40 min the functioning of a neuronal nicotinic ACh receptor is unaltered by conditions known to enhance cAMP-dependent protein phosphorylation. It remains to be determined if alterations in cAMP may play a role in long-term adaptive responses. Supported by NIH grant NS 16777.

168.8 PHOSPHORYLATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR BY Ca^{2+}/PHOSPHOLIPID-DEPENDENT PROTEIN KINASE, AND COMPARISON WITH ITS PHOSPHORYLATION BY cAMP-DEPENDENT PROTEIN KINASE. R. L. Huganir, K. A. Albert* and P. Greengard. Dept. Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

We have found that the purified nicotinic acetylcholine receptor from Torpedo californica is phosphorylated by a Ca^{2+}/phospholipid-dependent protein kinase purified from bovine brain. We showed previously that the receptor is phosphorylated by the catalytic subunit of cAMP-dependent protein kinase purified from bovine heart. The Ca^{2+}/phospholipid-dependent protein kinase phosphorylates only the δ subunit of the receptor, whereas the catalytic subunit of the cAMP-dependent protein kinase phosphorylates the γ and δ subunits. The stoichiometry of phosphorylation under the conditions used was 0.3 mol phosphate/mol of δ subunit for the Ca^{2+}/phospholipid-dependent protein kinase and 1.0 mol phosphate/mol of γ or δ subunit for the cAMP-dependent protein kinase. Phosphorylation of the receptor by the cAMP-dependent protein kinase appears to be of physiological significance (Huganir and Greengard, PNAS, 80, 1130-1134, 1983). We are currently attempting to determine the physiological significance of the phosphorylation of the receptor by the Ca^{2+}/phospholipid-dependent protein kinase, by comparing the relative rates and specificities of the phosphorylation of the receptor by the two kinases.

We have also studied the phosphorylation of the nicotinic acetylcholine receptor by endogenous protein kinases in subcellular fractions from Torpedo electric organ. Endogenous membrane-bound and cytosolic cAMP-dependent protein kinase phosphorylates the γ and δ subunits of the receptor. We are currently attempting to demonstrate endogenous Ca^{2+}/phospholipid-dependent phosphorylation of the δ subunit of the receptor. The possible effects of the phosphorylation of the acetylcholine receptor by the two kinases on the function of the receptor and its associated channel are also being investigated. (Supported by USPHS Grants MH-17387 and NS-08440 (P.G.). R.L.H. is the recipient of a Muscular Dystrophy Association Fellowship.)

168.9 KAPPA-BUNGAROTOXIN: AN UNUSUAL SNAKE NEUROTOXIN WHICH BLOCKS NICOTINIC TRANSMISSION IN MAMMALIAN AND AVIAN AUTONOMIC GANGLIA. V. A. Chiappinelli and S. E. Dryer*. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Kappa-Bungarotoxin (KBgT) is a minor α-neurotoxin present in the venom of the snake Bungarus multicinctus. The major α-neurotoxin contained in the venom is the well-known α-bungarotoxin (ABgT). KBgT has been purified to apparent homogeneity via a series of cation-exchange columns (1).

Electrophysiology. Both extracellular and intracellular recording from isolated ganglia have been used to characterize the physiological effects of KBgT. In the chick ciliary ganglion KBgT blocks nicotinic transmission at a dose of 75 nM. In the rat superior cervical ganglion a blockade is observed only at considerably higher doses (800 nM). In both ganglia, nicotinic transmission recovers after several hours of washing out the toxin.

Biochemistry. KBgT is a highly basic polypeptide (pI=9.1). It appears to be purified in a dimerized state, with a molecular weight for the dimer of approximately 12,500 daltons as determined by gel filtration on Sephacryl 200 or by sedimentation equilibrium centrifugation. The dimer is physiologically active. The dimer is broken down by treatment with the detergent sodium dodecyl sulfate, yielding a weight for the monomer of 6,500 daltons as determined by gel electrophoresis or by gel filtration in the presence of sodium dodecyl sulfate. The amino acid composition of KBgT indicates that it is not a cleavage product of ABgT.

Binding Experiments. While most α-neurotoxins (e.g. ABgT) bind with high affinity (K_d=1 nM) to a nicotinic site in both the chick ciliary ganglion and the rat superior cervical ganglion, α-neurotoxins frequently have no effect on nicotinic transmission through these ganglia at concentrations which saturate this site (2,3). Using ^{125}I-labeled KBgT, a search for sites other than those seen by ABgT was initiated. In the chick ciliary ganglion, such a site was identified, which may be associated with the physiological nicotinic receptor in the ganglion. In contrast, when binding was studied in the chick optic lobe, where ABgT blocks nicotinic transmission (4), ^{125}I-KBgT did not identify any additional nicotinic binding sites.

These results indicate that an unusual snake α-neurotoxin, KBgT, may be a useful probe for the physiological nicotinic receptor in several neuronal systems. (Supported by NIH Grant NS 17574.)

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3. Chiappinelli, Cohen and Zigmond (1981) Brain Res. 211, 107.
4. Oswald and Freeman (1981) Neuroscience 6, 1.

168.10 PARTIAL PURIFICATION AND CHARACTERIZATION OF AN ENDOGENOUS FACTOR WHICH INTERACTS WITH THE NICOTINIC-LIKE α-BUNGAROTOXIN RECEPTOR. M. Quik, Department of Pharmacology, McGill University, Quebec, Canada, H3G 1Y6.

Although the neuronal α-bungarotoxin (α-BGT) binding site has the properties of a nicotinic acetylcholine receptor, its nature remains controversial because α-BGT does not block the physiological functions mediated by nicotinic receptors. This could suggest that the α-BGT site, although regulated by nicotinic cholinergic drugs, is the target for an as yet unidentified endogenous ligand, which plays a role in other aspects of receptor regulation. Evidence that a factor which competes with α-BGT for its binding site, exists in brain supernatant has previously been obtained (Brain Res. 245:57, 1982). Experiments to partially purify and characterize this factor are now presented.

A 45,000 xg supernatant was prepared from rat or bovine brain. This supernatant was heated and subsequently centrifuged at 100,000 xg. The factor, which competed for α-BGT binding, was active after this procedure and present in both the supernatant and sedimented material. Attempts to purify the 100,000 xg supernatant by gel chromatography or other conventional purification techniques resulted in minimal recovery of an apparent low molecular weight material. These observations suggested that the factor in supernatant is in a soluble, possibly low molecular weight form, which is readily adsorbed. On the other hand, a purification of the endogenous material, which competed for α-BGT binding, could be achieved after an acetonitrile/trifluoroacetic acid extraction of the 100,000 xg pellet. A 50% inhibition of α-BGT binding occurred using 50 µg/ml of this extract; this represents an approximate one hundred fold purification. The effect of the factor on α-BGT binding was approximately linear with protein and was not altered by prior exposure of the extract to peptidase, neuraminidase, phospholipase or lipase activity. No activity was recovered after passage of the 100,000 xg acetonitrile/trifluoroacetic acid extract through a gel filtration column, although the activity associated with the original 100,000 xg pellet could be eluted from the column in the void volume.

These results suggest that the endogenous ligand which competes for the α-BGT binding site is a fairly stable factor. It appears to exist in a soluble form of possibly low molecular weight which is readily absorbed, and also in a higher molecular weight or aggregated state.

This research is supported by the MRC of Canada.

168.11 LECTIN SENSITIVITY OF NICOTINIC ACETYLCHOLINE RECEPTORS IN CULTURED CHICK CILIARY GANGLION NEURONS. A. Messing and N. K. Gonatas. Div. of Neuropathology, Univ. of Penn. Sch. of Med., Philadelphia, PA 19104.

The effects of various lectins and toxins on neuronal nicotinic acetylcholine receptor function have been studied in primary cultures of chick ciliary ganglion neurons.

Dissociated monolayer cultures from 8-day chick embryos were prepared as in Messing (Brain Res., 232:479, 1982) and were used for experiments between days 5 through 9 in vitro. Neuronal response to acetylcholine receptor activation was measured as influx of radioactive ions by a modification of the method of Huang, Catterall and Ehrenstein (J. Gen. Physiol., 71:397, 1978). Ion influx was measured at 4°C in a high potassium-low sodium medium designed to stabilize membrane potential near zero, with acetylcholine as the agonist and cesium-137 as the tracer ion. Non-specific influx was assessed in parallel cultures by incubation in medium lacking acetylcholine.

Exposure to 1 mM acetylcholine for 30 seconds (within the linear range of the time course) produced a 5-10-fold stimulation of cesium-137 influx. Acetylcholine-stimulated influx was inhibited more than 95% by 10 µM d-tubocurarine, but was insensitive to both 1 µM tetrodotoxin and 1 µM α-bungarotoxin.

Cultures were exposed to 50 µg/ml of concanavalin A, wheat germ agglutinin, lentil lectin, cholera toxin or 10 µg/ml tetanus toxin for one hour at either 4°C or 37°C, then assayed for acetylcholine-stimulated cesium-137 influx at 4°C. Concanavalin A inhibited agonist-induced ion flux by 80% at both temperatures; the other lectins and toxins were without effect. Succinyl-concanavalin A was ineffective at concentrations up to 125 µg/ml, and could not protect against the concanavalin A inhibition. However, inhibition by concanavalin A was eliminated by prior incubation of the lectin with 0.2 M α-methyl-D-mannoside and subsequent co-incubation with the sugar. These results suggest a specific interaction between concanavalin A and neuronal nicotinic acetylcholine receptors. (Supported by U.S.P.H.S. grants NS-05572-19 and NS-07064-03).

168.12 BENZOMORPHAN INTERACTIONS WITH ACETYLCHOLINE RECEPTOR-ION CHANNEL COMPLEXES FROM TORPEDO CALIFORNICA. Coleman T. King, Jr.* and Robert S. Aronstam. Department of Pharmacology, Medical College of Georgia, Augusta, GA 30912.

Benzomorphan interactions with acetylcholine receptor complexes from Torpedo californica electric organ were investigated using radiolabelled probes for receptor and ion channel binding sites, and compared to their selectivity for various opiate receptor subtypes.

Alpha-bungarotoxin binding to the ACh receptor was not decreased by more than 10% by any of the benzomorphans at a concentration of 100 uM. All of the benzomorphans competitvely inhibited phencyclidine ([^{3}H]PCP) and perhydrohistrionicotoxin ([^{3}H]HTX) to ion channel sites.The benzomorphans can be divided into 2 groups on the basis of their affinity for the ion channel: Phenazocine, N-allylnormetazocine, pentazocine and cyclazocine inhibited ion channel binding with high affinity (IC50's = 1 to 5 uM), while ketacyclazocine and ethylketocyclazocine were substantially less active. With each drug, benzomorphan affinity was increased when 1 uM carbarnylcholine was included in the incubation medium. Thus, ion channel affinity is not strictly limited to benzomorphans which stimulate sigma opiate receptors, and is not a certain indication of psychotomimetic potency.

Compound	Opiate Activity	IC50, uM [^{3}H]PCP	[^{3}H]PCP/CARB	[^{3}H]HTX/CARB
Penazocine	µ	0.9	0.4	1.3
Ketocyclazocine	κ	200	126	56
Ethylketocyclazocine	κ	115	36	20
Cyclazocine	κ,σ	1.8	1.5	5
Pentazocine	κ,σ	1.8	0.6	4
N-allylnormetazocine	σ	4.5	1.8	5

(Supported by DA-03303).

168.13 THE SPECIFICITY OF A MYASTHENIC SERUM FOR DEVELOPMENTALLY DIFFERENT FORMS OF THE ACETYLCHOLINE RECEPTOR. Marie-Paule Roisin*, Yong Gu* and Zach W. Hall, Div. Neurobiol. Dept. Physiol., Univ. Calif., San Francisco, CA 94143

During development of the neuromuscular junction, the acetylcholine receptor (AChR) undergoes changes in its functional and biochemical properties that occur in several stages before and after birth. We have previously shown (Weinberg and Hall, 1979) that many patients with myasthenia gravis have antibodies in their sera that distinguish two developmental forms of the AChR. We have found one serum that shows remarkable specificity when tested with AChR from rat or mouse muscle. Antibodies in this serum recognize only an α-bungarotoxin (α-BuTx) binding site on AChRs partially purified from denervated or embryonic muscles; they do not bind to other sites on these receptors nor do they bind to receptors purified from adult muscle (Gorin et al, 1982).

We have used this serum to determine when during development the immunological reactivity of the AChR at endplates changes. Immunocytochemical experiments on frozen sections of rat muscle show that AChRs at endplates in neonatal animals bind the antibodies, but that during the second and third postnatal weeks, the AChRs at endplates cease to be recognized by the antibodies. This immunological transition occurs during the same developmental period in which the mean channel open time of the AChRs changes and in which postsynaptic folds appear.

We have characterized the biochemical specificity of the anti-AChR antibodies in this serum in two ways. First, we have examined the effect of the antibody on toxin-binding to intact myotubes of the C_2 mouse muscle cell line. The AChRs in C_2 myotubes have two binding sites for α-BuTx that can be distinguished by their different affinities for d-tubocurarine (dTC). Our experiments show that the antibodies specifically block binding of toxin to the high-affinity dTC site, and do not affect equilibrium binding to the low-affinity dTC site. Thus a maximum of 50% inhibition of toxin-binding is obtained.

We have also found that the antibodies block 50% of toxin-binding to solubilized AChR from Torpedo electric organ. The effect of the antibodies on the Torpedo receptor and on the AChR from denervated rat leg muscle can be completely inhibited by N-acetylglucosamine, but is unaffected by N-acetylgalactosamine, sialic acid, mannose, galactose or glucose. Our results thus suggest that during the postnatal maturation of endplates in rat muscle, the AChR undergoes a change in glycosylation near one of the toxin-binding sites. Supported by grants from the NIH and MDA.

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2) Gorin, P. et al. (1982) Soc. Neurosci. Abstr. 8, 336.

168.14 MECHANISM OF PHENCYCLIDINE BINDING TO THE TORPEDO CALIFORNICA ACETYLCHOLINE RECEPTOR. R. E. Oswald, M. J. Bamberger* and J. T. McLaughlin*. Department of Pharmacology, Cornell University, Ithaca, NY 14853.

Phencyclidine (PCP) binds to the acetylcholine receptor (AChR) and inhibits ion flux at a site distinct from the acetylcholine binding site. This high affinity site seems to be present in one copy per 250 000 dalton acetylcholine receptor monomer (Heidmann, T., Oswald, R. E. & Changeux, J. P., Biochemistry, in press). The nature of this site remains unresolved; however, the voltage-dependence of inhibition of ion flux suggests that these agents may sterically block the ion channel (Albuquerque et al., PNAS, 77:1224, 1980). The kinetics of [^{3}H]PCP association are 10^3- to 10^4-fold more rapid when PCP and carbamylcholine (carb) are added simultaneously to the AChR (presence of a large proportion of open channels) than when PCP is added to AChR which has been previously equilibrated with carb (mainly desensitized AChR) or AChR in the absence of carb (mainly "resting" AChR). The mechanism of PCP binding to the AChR previously equilibrated with carb was investigated with thermodynamic, viscosity and kinetic experiments. The thermodynamic data suggested that both association and dissociation rates in the presence and absence of carb were highly dependent upon temperature with transition state enthalpies on the order of 25 kcal/mole and Q_{10}'s (20° to 30°) of 4 to 6. Viscosity had no effect on association rates but increased dissociation rates. The interpretation of the thermodynamic and viscosity studies was that the binding is not limited by diffusion but rather by an energy barrier associated with the interaction between the AChR and PCP. The pseudofirst order association rate constant was measured as a function of AChR and PCP concentration. In the presence of carb, the association rate was linearly dependent on AChR concentration and virtually insensitive to PCP concentration. In the absence of carb, the association rate seemed to be a hyperbolic function of both PCP and AChR concentration. The minimal model capable of explaining the data is a mechanism by which PCP binds to two states of the AChR, one state having a high affinity (nanomolar K_D) and constituting a low percentage of AChR and the other with a lower affinity (micromolar K_D) and constituting a higher percentage. Evidence that the high affinity site corresponds to an open channel conformation of the AChR will be discussed.

This work was supported by grants from the Muscular Dystrophy Association and the NIH (1R23NS18660-01A1NEUB).

168.15 A SIMPLE DEVICE FOR PERFORMING LARGE NUMBERS OF CENTRIFUGAL GEL FILTRATION RECEPTOR BINDING ASSAYS SIMULTANEOUSLY. J. A. Lewis and S. A. McLafferty*. Dept. of Biological Sciences, Univ. of Missouri-Columbia, Columbia, MO 65211.

Ligand binding to a detergent-solubilized receptor can be assayed by centrifuging ligand-receptor solutions through mini-columns of G-25 Sephadex to separate receptor-bound ligand from free ligand. A limitation of this standard technique is that during the time required to load a series of columns, bound ligand may begin dissociating from receptor on the first-loaded columns while additional columns are being loaded. We find that Becton-Dickinson 26 gauge syringe needles clipped or bent to remove the beveled point can be used as loading caps in the tops of assay columns (1 cc. B-D tuberculin syringe barrels) to hold each assay aliquot above its column until the start of centrifugation loads all columns simultaneously by forcing liquid through the needle openings onto the top of each column. Up to 72 assays can be loaded, equilibrated, and run simultaneously in one set of centrifuge adapters. With extra sets of adapters, one person can easily perform several hundred binding assays a day. The height to which Sephadex columns are packed and the speed of initial centrifugation turn out to be important variables in achieving precise reproducibility. We demonstrate the usefulness and reliability of our modified technique in assaying the Triton X-100 solubilized levamisole receptor of the nematode Caenorhabditis elegans by [^{3}H]-meta-aminolevamisole binding. The receptor assayed is one for which a number of receptor-deficient mutants have been obtained by selection for levamisole resistance.

169.1 EFFECT OF SHORT-TERM DESENSITIZATION ON MUSCARINIC RECEPTOR BINDING IN INTACT MURINE NEUROBLASTOMA CELLS (CLONE N1E-115). Michael McKinney*, Scott Stenstrom* and Elliott Richelson (SPON: J. W. McLaren). Depts. of Psychiatry and Pharmacology, Mayo Foundation, Rochester, MN 55905.

Muscarinic agonist binding sites in intact N1E-115 cells were studied with the equilibrium binding of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) and carbachol. The data were analyzed with an interative non-linear computer fitting program employing models for multiple independent agonist binding sub-types.

Intact naive N1E-115 cells at 15°C exhibited three agonist sites with dissociation constants for carbachol of 2.7 nM, 1.35 μM, and 56 μM, with relative proportions of 29%, 24% and 47%, respectively. These sites correlate with the "super-high", "high", and "low" agonist sites in the literature. The super-high site appeared to be absent when the binding was performed with homogenates of N1E-115 cells.

Confluent N1E-115 cells were desensitized with 1 mM carbachol for 20 minutes at 37°C. After three washes with cold medium, the binding of [^{3}H]QNB and its displacement by carbachol was performed at 15°C to prevent resensitization. Neither the total receptor number nor the receptor's affinity for [^{3}H]QNB was changed by short-term desensitization. Conversely, the curve for agonist displacement of [^{3}H]QNB was markedly shifted to the right. Computer analysis revealed that the major change occurred in the binding affinities of all three agonist binding sites with the new dissociation constants as follows: super-high=141 nM, high=109 μM, low=855 μM.

The data suggest that muscarinic receptor binding in homogenates differs from that in intact cells in that the proportions of agonist sites may change. However, in either homogenates or intact cells there were two sites with dissociation constants near the ED_{50}'s for inhibition of PGE_1 mediated cAMP formation and stimulation of cGMP formation, respectively. The decrease in agonist binding affinity of the muscarinic receptor that occurred during prolonged treatment with agonists may be part of the mechanism whereby cells become desensitized.

Supported by the Mayo Foundation and USPHS grants MH 27692 and AM 07147.

169.2 COMPARISON OF ^{3}H-PIRENZEPINE AND ^{3}H-QNB BINDING TO MUSCARINIC RECEPTORS IN RAT BRAIN. Gary R. Luthin* and Barry B. Wolfe (Spon: M. Wax) Dept. Pharmacol., Univ. Pennsylvania Sch. Med., Philadelphia, PA 19104

Pirenzepine (Pz) is an antagonist at muscarinic cholinergic receptors and inhibits the effects of muscarinic receptor agonists (Gil and Wolfe, Neurosci. Abs. 1983). Pz, as well as atropine and scopolamine, inhibit the binding of ^{3}H-antagonists to muscarinic receptors. Atropine inhibition curves of ^{3}H-QNB binding have Hill coefficients of 1, suggesting that atropine and ^{3}H-QNB interact with one site, or with multiple sites having equal affinities. Pz also inhibits (^{3}H)-QNB binding, but Hill coefficients of competition curves are less than one in some tissues. This is consistent with the presence of either receptor subtypes or different affinity states of muscarinic receptors. Watson et al. (Life Sci. 31:2019, 1982) recently have demonstrated binding of ^{3}H-Pz to rat brain membranes. In this study, we have characterized ^{3}H-Pz binding in rat brain and compared the binding profile of ^{3}H-Pz to that of ^{3}H-QNB. Initial experiments were performed in rat cerebral cortex. At 32°C, the binding of ^{3}H-Pz was rapid, with a k_1 of 8.75 x $10^6 M^{-1} min^{-1}$, reversible, with a k_{-1} of 0.128 min^{-1}, and of high affinity and saturable. Scatchard plots of equilibrium ^{3}H-Pz binding data were linear over the range 0.3-75 nM ^{3}H-Pz. The Kd calculated from the saturation data was 15.2 nM, which agrees with the value of 14.6 nM calculated from the ratio k_{-1}/k_1. The pharmacological profile of ^{3}H-Pz binding was identical to that of (^{3}H)-QNB binding. The rank order of potencies of muscarinic drugs to compete with both ligands was QNB > atropine~scopolamine > pirenzepine > oxotremorine > carbachol. All antagonists inhibited ^{3}H-Pz and ^{3}H-QNB binding with Hill coefficients of 1 (except Pz vs ^{3}H-QNB). The Hill coefficients for agonists were all less than one. The Hill coefficients for agonists were increased in the presence of 5 mM magnesium plus 100 μM GTP for both ligands. Saturation experiments with both ligands revealed the concentration of (^{3}H)-Pz binding sites was approximately half of the concentration of (^{3}H)-QNB binding sites in cortex, striatum, and hippocampus. However, in pons-medulla, the ratio of ^{3}H-Pz:^{3}H-QNB binding sites was 0.2, and in cerebellum, no ^{3}H-Pz binding sites were detectable. We postulate that Pz interacts with both a high- and low-Kd recognition site in brain, and that the high-Kd site is not detectable in direct binding assays using ^{3}H-Pz. In cortex, Pz interacted with ^{3}H-QNB binding sites in a complex fashion best fit by a two-site model with Kd values of 8 nM and 160 nM, and with a ratio of low Kd:high Kd binding sites of 65:35. In cerebellum, Pz interacted with (^{3}H)-QNB sites in a simple fashion described by the law of mass action with a Kd of 212 nM. From these data, it appears that Pz may selectively interact with a subpopulation of muscarinic recognition sites in brain. It remains to be shown whether this selective interaction of Pz is due to the presence of multiple receptor subtypes or different affinity states of the receptors. (Supported by GM 31155).

169.3 DIFFERENT PHARMACOLOGICAL PROFILES FOR TWO MUSCARINIC-MEDIATED RESPONSES. Daniel W. Gil* and Barry B. Wolfe. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Several hormones modulate phosphatidylinositol (PI) turnover, associated with cytosolic calcium mobilization, and adenylate cyclase activity through separate receptor subtypes. This arrangement has been best demonstrated for catecholamines, with α_1-adrenergic receptors mediating an increase in PI turnover and calcium fluxes and α_2-adrenergic receptors mediating an inhibition of adenylate cyclase activity (Fain, J.N. and Garcia-Sainz, J.A., Life Sci. 26:1183, 1980). Muscarinic cholinergic receptors also modulate these biochemical responses and analogy with α receptors suggests that muscarinic receptors may exist as two subtypes, each modulating a distinct biochemical response. We have therefore investigated the pharmacological profiles of muscarinic receptor-stimulated PI turnover and muscarinic receptor-inhibited adenylate cyclase activity.

The cleavage of the phosphorylated inositol head group from the glycerol backbone is the receptor-linked step in PI turnover, so the accumulation of inositol 1-phosphate (I 1-PO_4) is a measure of receptor-mediated PI turnover. 10 mM LiCl maximally inhibited the enzyme, inositol 1-phosphate phosphatase, and I 1-PO_4 accumulation remained linear for over 80 min. We have measured the 60-min muscarinic-stimulated accumulation of ^{3}H-I 1-PO_4 in rat parotid slices preincubated with ^{3}H-inositol. Adenylate cyclase activity was studied in rat ventricle homogenates by measuring the production of ^{32}P-cAMP during a 500-sec incubation with ^{32}P-ATP. Adenylate cyclase activity was increased with either 7.5 μM isoproterenol or 100 μM forskolin and its activity remained linear for at least 12 min.

The potencies of several muscarinic drugs in the two assay systems have been determined. The potency of the agonist, oxotremorine, was similar in both assay systems. Using Schild analysis, however, the potencies of some muscarinic antagonists differed. Pirenzepine was a more potent (16x) blocker of muscarinic-stimulated PI breakdown than muscarinic-mediated inhibition of adenylate cyclase activity. Atropine was also a more potent (5x) inhibitor of the muscarinic-mediated PI effect than the muscarinic-mediated adenylate cyclase effect. The antagonist potencies were not altered by using different muscarinic agonists, different temperatures, or by stimulating adenylate cyclase with forskolin rather than isoproterenol. The ganglionic stimulant, McN A343, was a weak partial agonist for I 1-PO_4 accumulation, but a full agonist for adenylate cyclase inhibition. While the reason that the muscarinic antagonists appear to be selective is not yet clear, the differences in potencies are consistent with the existence of separate muscarinic receptor subtypes modulating PI turnover and adenylate cyclase activity. (Supported by NIH GM-31155 and NSF SPE-82-64157).

169.4 'EFFECT OF ZINC ON ^{3}H-OXOTREMORINE DISPLACEMENT BY MUSCARINIC AGONISTS AND ANTAGONISTS. C.P.Smith and F.P.Huger Dept. of Biochemistry, Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876

Muscarinic receptor heterogeneity has been explained by the presence of three different agonist binding sites (SH, H and L) which bind agonists at widely different affinities but bind antagonists at equal affinities. The ability of guanine nucleotides, ions and sulfhydryl reagents to alter the relative proportions of agonist binding sites indicate that these proportions are probably not due to the existence of molecularly heterogeneous non-interconvertible sites. The finding that Zn^{+2} enhances the ability of muscarinic agonists to displace ^{3}H-antagonists from muscarinic receptors prompted us to investigate the effect of 1mM $ZnSO_4$ on ^{3}H-oxotremorine binding in rat forebrain membranes. Bound ^{3}H-oxotremorine was captured by centrifugation after a 15-minute incubation at 30°C, then washed, dried, solubilized and measured by liquid scintillation. Nonspecific binding, measured in 2 μM atropine sulfate or 2 μM oxotremorine sesquifumerate, did not differ, either in the presence or absence of 1 mM $ZnSO_4$. The results indicate that homogenization and incubation in 1 mM $ZnSO_4$ can effectively enhance ^{3}H-oxotremorine binding, apparently through an increase in binding sites. A saturation experiment showed a 10-fold increase in the number of binding sites due to the presence of 1 mM $ZnSO_4$. At low ^{3}H-ligand concentrations (3-7nM), zinc-treated rat forebrain membranes showed nearly a three-fold increase in bound ^{3}H-oxotremorine as compared to control membranes. Under these conditions, agonist affinities were not affected by zinc-treatment, but muscarinic antagonists showed a 10-fold decrease in ability to displace bound ^{3}H-oxotremorine in 1 mM $ZnSO_4$. The selective, high-affinity antagonist pirenzepine and McN-A-343, a unique agonist, were not distinguished from classical agonists (oxotremorine, carbachol) or antagonists (atropine, scopolamine), but pilocarpine, a partial agonist of low efficacy, had an intermediate decrease in ability to displace ^{3}H-oxotremorine in the presence of 1 mM $ZnSO_4$.

169.5 CHARACTERIZATION OF THE EFFECT OF ZINC ON THE DISPLACEMENT OF ^{3}H-QNB BY MUSCARINIC AGONISTS AND ANTAGONISTS. F.P.Huger and C.P.Smith. Dept. of Biochemistry, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876

The effects of certain heavy metals and sulfhydryl agents on muscarinic cholinergic receptor binding have been reported by several laboratories. We have shown that incubation of membranes from rat forebrain in the presence of 1mM Zn^{2+} shifts the agonist displacement curves of ^{3}H-QNB to the left, with little or no effect on antagonist displacement curves. Further studies were done to optimize and characterize the effect of zinc on muscarinic receptor binding.

Experiments were performed with membranes isolated from discrete brain regions (frontal cortex, striatum, hippocampus and cerebellum) showed that the effect of zinc on muscarinic agonist affinity for QNB-labeled sites was consistent in all regions tested. The effect of zinc was concentration-dependent up to 1 mM. At a concentration of 10 mM Zn^{2+}, there was no specific binding of ^{3}H-QNB. This loss of specific binding was associated with a loss of membrane protein. The possibility of an anion effect was ruled out by comparing treatments with $ZnSO_4$, $ZnCL_2$, $Zn(NO_3)_2$ and $Zn(C_2H_3O_2)_2$. All of these salts shifted the concentration-response curves for carbachol, with no effect on atropine. Evaluation of several other heavy metals revealed that Cd^{2+}(.01 mM) and Cu^{2+}(.01 mM) also shifted the agonist displacement curve, while Co^{2+} (1 mM) and Hg^{2+} (.001 mM) had no effect. Like zinc, none of these metals affected the displacement curves of atropine. Reincubation of zinc-treated membranes with dithiothreitol reversed the zinc effect on agonist affinity. These data support the hypothesis that zinc increases muscarinic agonist affinity by binding to critical sulfhydryl groups which control the conformation of the receptor.

169.6 DISTRIBUTION AND FUNCTION OF M1 AND M2 MUSCARINE RECEPTORS IN DIFFERENT TISSUES. L.T. Potter and J. Luber-Narod. University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

Measurement of the prevalence of M1 and M2 receptors is facilitated by agents which uncouple high-affinity agonist-receptor complexes, and by conditions which maximize the different binding properties of the two remaining populations of uncoupled receptors. These goals are achieved in 20 mM Tris-1 mM $MnCl_2$ plus 0.1 mM GppNHp at 37°C, or in 50 mM sodium phosphate-1 mM EDTA plus 1 mM NEM at 25°C. The latter is much less expensive. Membranes were prepared in 50 mM phosphate-10 mM EDTA plus 0.15 M sodium perchlorate to extract non-membrane proteins and facilitate pipetting. Unselected membranes were assayed with a 20-fold excess of 1 nM (-)^{3}H-quinuclidinyl benzilate, by competition with carbachol and/or pirenzepine.

From all mammals so far studied (human, rat, rabbit, dog, monkey, cat, guinea pig, sheep) M1 and M2 receptors show the same affinities and very similar prevalences. There are two clear patterns. 75-90% of the sites in the forebrain (cortex, striatum, hippocampus etc.), ganglia and various glands (including intestinal lining) are of the M1 type, which shows ~ 100-fold lower affinity for carbachol and ~ 15-fold higher affinity for pirenzepine than M2. The conducting system of the heart has many of these sites, which may lie on cholinergic cell bodies. M1 receptors appear to regulate neural firing (M-currents) and glandular secretion. In contrast, 80-90% of the sites in the brainstem and lower midbrain (medulla, pons, cerebellum, superior colliculus), forebrain cholinergic nuclei, smooth muscle and heart are of the M2 type, which is sensitive to GppNHp or NEM. Activation of these receptors attenuates activation of adenylate cyclase. Both receptors are used postsynaptically. M2 sites predominate in cholinergic neurons. Drugs can be selected for the regulation of specific tissues.

Supported by the National Parkinson Foundation.

169.7 CHANGES IN M1 AND M2 MUSCARINE RECEPTORS IN ALZHEIMER'S DISEASE AND AGING, AND WITH LESIONS OF CHOLINERGIC NEURONS IN ANIMALS. D.C. Mash and L.T. Potter. University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

Our autoradiographs of muscarine receptors in rat and human brains demonstrate that regions rich in cholinergic cell bodies (magnocellular neurons in the basal forebrain of the rat, nucleus basalis of Meynert in humans, and the medial septum/diagonal band of Broca) are heavily labelled with ^{3}H-quinuclidinyl benzilate in the presence of enough pirenzepine to prevent binding to M1 receptors. Carbachol prevents this labelling at concentrations which occlude M2 receptors. Although most cortical receptors are M1 in type (labelled in carbachol but not pirenzepine), M2 receptors are apparent in laminae having the highest levels of choline acetyltransferase. In the hippocampus there is a striking correspondence between the localization of M2 receptors, the distribution of the septal-hippocampal projection, and acetylcholinesterase. Evidence that M2 receptors are transported in cholinergic nerves further favors the idea that M2 receptors are neurochemical markers for localizing cholinergic tracts. Acetylcholine is known to inhibit its own release, while receptor antagonists increase release. Our working hypothesis, therefore, is that M2 receptors function in cholinergic terminals to inhibit transmitter release, and that a centrally-acting M2-selective antagonist may prove useful for increasing acetylcholine release in people with diminished numbers of cholinergic terminals.

We are seeking evidence for a decrease in M2 receptors in the cerebral cortex with Alzheimer's disease, with aging in humans, and in rats with kainic acid lesions in the ventral globus pallidus. So far, test tube data have yielded a consistent picture: there is a moderate (~ 20%) loss of total QNB sites in the frontal cortex, with a smaller loss in M2 sites. These changes are being correlated with changes in choline acetyltransferase. The localization of changes in receptors is being studied by autoradiography. If enough terminals remain in all layers of the cortex, there will be reason to focus further on drugs which work via these terminals. In any case, drugs which block M1 receptors (e.g., scopolamine, trihexyphenidyl, antihistamines, and prochlorperazine) should be used with great care in demented patients, including those with Parkinson's disease.

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169.8 SELECTIVITY OF DRUGS FOR M1 AND M2 MUSCARINE RECEPTORS. J. Luber-Narod and L.T. Potter. University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

We have examined ~ 30 commonly-used clinical and laboratory drugs which block or activate muscarine receptors, for their M1/M2 selectivity, by conducting competition assays against (-)^{3}H-quinuclidinyl benzilate. Two sets of conditions were used. In 50 mM sodium phosphate-1 mM EDTA at 37°C, and in physiological media (Krebs) with added GppNHp or NEM, ligands bind to only two populations of uncoupled receptors, analyses are easy, and quantitative data are obtained. The results are particularly applicable to laboratory work. In physiological media without uncoupling agents, analyses are complicated by the presence of high- and low-affinity agonist-receptor complexes; however the results are relevant for the selection of drugs in medicine, and for distinguishing physiological effects. Potency and selectivity series were obtained in both conditions, with complex results.

Scopolamine is an example of a highly potent antagonist which is M1-selective only in EDTA. Oxotremorine is a highly potent agonist with little selectivity. McN-A-343 is a moderately potent agonist which is M1-selective only in physiological media. Carbachol is highly M2-selective in general. Dicyclomine (Bentyl) is ~ 100-fold more potent and slightly more M1-selective than the prototype antagonist, pirenzepine, (which is available only in Europe). It would appear to have advantages for treating ulcers over less selective drugs like propantheline (Pro-Banthine). Trihexyphenidyl (Artane) is one of the most M1-selective anti-Parkinson drugs. The nicotine receptor antagonists, gallamine and pancuronium, are also weakly M2-selective antagonists; alpha-neurotoxins are inactive. New selective drugs are clearly needed and possible.

Supported by the National Parkinson Foundation.

169.9 N-ETHYL MALEIMIDE DISTINGUISHES M1 AND M2 MUSCARINE RECEPTORS. D.D. Flynn and L.T. Potter. University of Miami School of Medicine, P.O. Box 016189, Miami, Florida 33101.

The binding of (-)^{3}H-quinuclidinyl benzilate to unselected membranes from the hippocampus and brainstem of the rat was examined in the presence and absence of NEM. NEM has two clear effects. Low concentrations (1 μM at 37°, 10 μM at 25°C) maximally diminished the high affinity of brainstem receptors (which are ~ 90% M2) for carbachol, moving competition curves to the right and almost to isotherms. The only effect on hippocampal receptors (which are ~ 75% M1) was to uncouple high-affinity carbachol binding to M2 sites. Thus low concentrations of NEM diminish selectivity between M1 and M2. More NEM progressively increased the affinity of both receptors, especially M2, such that in 1 mM NEM at 25°C the carbachol affinity of M2 became ~ 100 times that of M1, greatly facilitating distinction between the uncoupled receptors. This effect was slightly facilitated by freezing membranes before assays. NEM had no effect on competition curves for the M1-selective antagonist, pirenzepine. The mechanism whereby NEM uncouples agonist-M2 complexes was apparent from dissociation rate experiments: NEM increased the rate of loss of ^{3}H-oxotremorine-M from M2 more than 20-fold, with little or no effect on M1. GppNHp has a similar effect. NEM probably uncouples high affinity agonist-M2 complexes by interacting with a guanine nucleotide binding protein. Presumably it increases affinities at high concentrations by direct actions on both receptors.

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169.10 BIOCHEMICAL CHARACTERIZATION OF CHOLINERGIC MUSCARINIC RECEPTORS IN MEMBRANE FRACTIONS FROM BOVINE AIR-WAY SMOOTH MUSCLE. A. Ponte Sucre*, I.L. de Becemberg* and M. Alfonzo*. (SPON: T. Koch). Seccion de Biomembranas, Instituto de Medicina Experimental, Universidad Central de Venezuela, Apartado 50587 Sabana Grande, Caracas, Venezuela.

The air-way caliber in many species including humans is regulated by the parasympathetic nervous system via the vagus nerve. This physiological regulation is effected by the contraction of the air way smooth muscle, which is blocked by atropine indicating thus that muscarinic receptors are involved.

Most of the data regarding muscarinic receptors in air-way smooth muscle are concerned with their pharmacological characterization. In this work we have used [^{3}H]-QNB binding to study some biochemical properties of muscarinic receptors associated to highly purified membrane fractions obtained by subcellular fractionation of bovine air-way smooth muscle. [^{3}H]-QNB binding shows high affinity, specificity and saturability, with an estimated Kd of 1.6nM and a Hill number (HN) of about 1 in the absence of experimental ions. Incubation of membrane fractions in the presence of monovalent cations (0.154M) increased the specific binding about 20% when KCl was used and 40% when either NaCl or LiCl was used. In addition, when KCl was used, Kd=1.8nM and HN=1.39±0.18 (slope±SD); when NaCl was used Kd=2.66nM and HN=1.84±0.12; and when LiCl was used Kd=2.66nM and HN=2.00±0.13. Incubation of membrane fractions with Tris/PO_4 or Hepes/Imidazol, instead of Tris /HCl, did not modify the effect of NaCl.

Our results suggest that an increase in the number of receptors and/or the cooperativity among them can be produced by the incubation of membrane fractions in the presence of monovalent cations. Furthermore, the specific binding and the HN increases as the atomic radius of the cation used decreases (K Na Li). This suggest that the functional properties of the muscarinic receptor are influenced by its environment, i.e., that there are specific interactions which depend on the ion's radius and are thus subjected to physical restrictions. At NaCl concentrations similar to those present in the extracellular fluid the specific binding to membrane fractions shows optimal values and the HN increases from near 1 to 1.84, thus indicating an increased cooperativity among the receptors. Indeed Ghosh (1981) has pointed out that in those systems where positive cooperativity exists and depends on the presence of a cofactor, such cooperativity shows optimal values at the physiological concentrations of the cofactor.

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Ghosh (J. Theoret Biol, vol.93, pag. 395, 1981)

169.11 PROPERTIES OF A SOLUBILIZED GUANYL NUCLEOTIDE SENSITIVE MUSCARINIC ACETYLCHOLINE RECEPTOR. M.W. Gainer and N.M. Nathanson. Dept of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Extensive studies on membrane homogenates have shown that muscarinic receptors consist of at least two classes of sites which have similar high affinities for antagonists but differ in their affinities for agonists. Previous attempts to solubilize muscarinic receptors have yielded preparations which retain specific high affinity binding of antagonists but bind agonists to only a single class of low affinity sites and thus have lost the heterogeneity of agonist binding characteristic of the membrane bound receptor.

In this study, a combination of 10 mM CHAPS and 1 M NaCl solubilized 15-40% of the specific receptor binding sites from bovine brain membranes. This solubilized receptor preparation binds the muscarinic antagonist, [^{3}H] QNB, with high affinity to a single class of sites (K_D=301 pM). Moreover, this solubilized preparation exhibits heterogeneity in the binding of the agonist, carbachol, as well as guanyl nucleotide regulation of agonist binding, identical to that of the membrane bound receptor. Therefore, CHAPS can solubilize muscarinic receptors from bovine brain membranes which retain the agonist and antagonist binding properties characteristic of the membrane bound receptor.

The solubilized receptor preparation can be adsorbed to wheat germ lectin sepharose and specifically eluted with N-acetylglucosamine with a several-fold increase in specific activity, suggesting that lectin chromotography may be a useful step in the purification of the receptor.

Sucrose gradient sedimentation analysis of the solubilized receptor showed that the receptor sedimented in a single peak with a sedimentation coefficient of 5S.

Sepharose 4B chromotography of the solubilized receptor preparation resulted in an elution profile containing two peaks of activity. Studies are being undertaken to determine if the receptor in these two peaks exhibit similar biochemical properties.

169.12 LIGAND-BINDING CHARACTERIZATION OF INSECT ACETYLCHOLINE RECEPTORS. Sarah C.R. Lummis* and David B. Sattele*. (SPON: A. Taylor). ARC Unit of Insect Neurophysiology and Pharmacology, Dept. of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, U.K.

Radiolabelled ligand-binding studies have been used to investigate acetylcholine receptors in the central nervous system of the cockroach Periplaneta americana. In studies using the nicotinic cholinergic receptor ligand, α-bungarotoxin and the muscarinic cholinergic receptor ligand, quinuclidinyl benzilate, particulate preparations of cockroach nerve cords were shown to contain distinct saturable components of specific binding. L-[benzilic-4, 4'-^{3}H]-quinuclidinyl benzilate bound to a single class of non-cooperative binding sites. Scatchard analysis yielded a K_d of 8nM and a B_{max} of 138pmol/mg. The dissociation constant calculated from the association and dissociation rate constants was 1.9nM. The pharmacological profile of the binding component was that expected for a muscarinic acetylcholine receptor: dexetimide, atropine, scopolamine and unlabelled quinuclidinyl benzilate were the most potent inhibitors of binding, whilst α-bungarotoxin and nicotine were ineffective. The N-[propionyl-^{3}H]-propionylated α-bungarotoxin binding component was saturable (B_{max} = 910pmol/mg) and of high affinity (K_d = 4.4nM). Rate constants for association and dissociation yielded a dissociation constant of 0.6nM. Specific α-bungarotoxin binding was displaced most effectively by the nicotinic ligands d-tubocurarine (K_i=8.5 x 10^{-7}M), nicotine (K_i=3.4 x 10^{-6}M) and unlabelled α-bungarotoxin (K_i=7.7 x 10^{-10}M). The muscarinic ligands quinuclidinyl benzilate (K_i=1.4 x 10^{-4}M) and atropine (K_i=2.0 x 10^{-5}M) were much less effective. Decamethonium (K_i=1.3 x 10^{-4}M) was a weak inhibitor. The cell body membrane of the fast coxal depressor motoneurone (D_f) of the cockroach metathoracic ganglion was particularly sensitive to nicotinic cholinergic ligands (David J.A. & Sattele D.B., J. Exp. Biol., 1983, in press): α-Bungarotoxin was much more effective (I_{50} = 6.4 x 10^{-8}M) in blocking the depolarization resulting from ionophoretic application of acetylcholine, than either quinuclidinyl benzilate (I_{50}=1.0 x 10^{-4}M) or decamethonium (I_{50} = 2.8 x 10^{-3}M). It is concluded that the membrane component characterized in α-bungarotoxin binding studies is a constituent of a cell body acetylcholine receptor of an identified motoneurone.

170.1 **CHARACTERIZATION OF ENKEPHALIN-LIKE PEPTIDES IN BOVINE SPLANCHNIC NERVE,** **T.D. Hexum. Dept. Pharmacology, Univ. Neb. Med. Ctr., Omaha, NE 68105**

Chromaffin cells from the adrenal medulla contain opiate as well as cholinergic receptors. Acetylcholine-induced catecholamine secretion can be reduced by opiate agonists such as etorphine. The source of the endogenous opiate material that can interact with these receptors may be either the adreno-medullary chromaffin granules or the splanchnic nerve both of which contain enkephalin-like peptide (ELP) material (Schultzberg et al., 1978 Neurosci. 3, 1169-1186). The ELP content of adrenal chromaffin granules has been well-characterized and found to exist primarily as polymeric forms of met^5- and leu^5-enkephalin (high MW) with lesser amounts of the pentapeptides and related compounds (low MW). The nature of the ELP content of splanchnic nerves has not been determined.

Splanchnic nerves from pigs were desheathed, minced and homogenized in 1 N acetic acid containing 0.02 N HCl and 0.1 % 2-mercaptoethanol. The homogenate was subjected to a chloroform:methanol extraction procedure (1:2). ^{3}H-Met^5-enkephalin added to the extraction mixture was recovered in the methanol layer (93% recovery). The ELP content of pig splanchnic nerve, determined radioimmunochemically using a C-terminal directed antiserum to met^5-enkephalin, was 1.4 pmoles ELP/mg protein. Trypsinization followed by treatment with carboxypeptidase B increased the immunoreactivity to 5.2 pmoles ELP/mg protein indicating the presence of precursor (high MW) material. Chromatography of the material extracted in the methanol layer on BioGel P-2 showed the presence of low and high MW ELP in approximately equal proportions. A peak of immunoreactive material co-eluted with met^5-enkephalin. This pattern is similar to that reported for other nervous tissue such as the striatum but unlike that observed in adrenal chromaffin granules.

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170.2 OPIOID PEPTIDES IN RAT PITUITARY ANTERIOR LOBE.
C.J. Molineaux, J.G. Rosenberger and B.M. Cox. Department of Pharmacology, Uniformed Services University, Bethesda, MD 20814.

The presence in pituitary corticotrophs of pro-opiomelanocortin and the opioid peptide β-endorphin derived from it, is well established. However, peptides derived from the two other known opioid peptide gene products, prodynorphin and proenkephalin, are also present in anterior lobe (AL), suggesting a possible local role for endogenous opioids in pituitary function in addition to their probable function in pituitary regulation at the level of the hypothalamus.

We have measured by RIA the levels of several dynorphin-(Dyn-) and enkephalin-like peptides in rat AL. The level of ir-Dyn A-(1-13) in AL was 2.32 pmol/gland, an amount comparable to that found in the neurointermediate lobe. The total ir-α-neo-endorphin (α-nEND) concentration was lower (0.42 pmol/gland. However, gel filtration analysis indicated that there was very little authentic Dyn A-(1-17) and α-nEND present. The level of ir-Dyn A-(1-8), another small peptide derived from prodynorphin, was also very low (0.08 pmol/gland). Most of the Dyn A and α-nEND immunoreactivity in AL extracts eluted with apparent M_r in the range 6-10 kdaltons. The absolute amounts of these peptides cannot be determined because the RIA cross-reactivities of these putative precursor peptides are unknown.

ir-Leucine-enkephalin (LE) is also present in AL, at a level of 1.18 pmol/gland. LE might be generated from either prodynorphin or proenkephalin. We have therefore measured the concentration of the C-terminal fragment of proenkephalin, [Met^5]-enkephalin-Arg-Phe (ME-RF), with an antiserum which has negligible cross-reactivity with the other opioid peptides known to be present in AL, but which apparently also recognizes enkephalin precursors. The ir-ME-RF level in AL was 5.52 pmol/gland. Gel filtration analysis suggests that ir-ME-RF in AL is also present largely in a high molecular weight form. Thus, it appears that the low levels in AL of the small molecular weight forms of opioids derived from prodynorphin and proenkephalin do not result from a deficiency of precursor peptides.

In initial experiments examining dynamic changes in opioid peptide levels in AL, levels of each of the peptide immunoreactivities were measured at intervals throughout a 24 hr period. A significant circadian variation was observed in the level of ir-ME-RF, with the level at 0100 hr being approximately twice that found at 1300 hr. Other peptide immunoreactivities remained relatively stable.

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170.3 EXAMINATION AND CHARACTERIZATION BY COLUMN COUPLED RADIORECEPTOR ASSAYS OF ENDORPHINS RELEASED FROM THE SPINAL CORD OF THE CAT DURING CONTROL AND HIGH INTENSITY STIMULATION OF THE SCIATIC NERVE. T.L. Yaksh*, F. Nyberg*, G. Harty*, L. Terenius* and K. Jhamandas* (SPON: P.J. Dyck). Dept. of Neurosurg. Res., Mayo Clinic, Rochester, MN; Depts. of Pharmacol., Uppsala Univ., Uppsala, Sweden; Queens Univ., Kingston, Ontario, Canada.

We have previously shown that met-enkephalin like immunoreactivity is released from the spinal cord by high intensity stimulation of the sciatic nerves (J. Neurophysiol. 46:1056,1981). We have extended these experiments to determine if other materials acting at opiate binding sites are released. Cats are anesthetized (chloralose-urethane), prepared for spinal superfusion with a concentric polyethylene catheter inserted through the cisternal membrane. Artificial CSF with albumin (120 μg/ml) and bacitracin (30 μg/ml) was infused at 100 μl/min through the inner catheter (PE-10) which extended to the sacral cord (35 cm). The outflow was collected through the outer catheter (PE-90) which extended to the mid-thoracic cord (15 cm). Samples were lyophilized and reconstituted in 0.2 M acetic acid, and passed through an Amicon PM-10 filter prior to passing over a Sephadex G-10. Further separation was achieved using column electrophoresis in agarose suspension. Opiate displacement activity was examined using brain synaptosomes and ^{3}H-dihydromorphine (^{3}H-DHM) as described elsewhere (Life Sci. 16:1759, 1975). Separation of activity on the G-10 column revealed two fractions (I and II) which appear before and after the salt fractions and account for over 70% of ^{3}H-DHM displacing activity. Sciatic nerve stimulation resulted in a 30 and 5 fold increase in FI and FII, respectively. Electrophoretic separation of FI at alkaline and acid pH revealed 2 major pools which co-migrated with α-neoendorphin and dynorphin 1-13. FII activity was isographic with enkephalin hexapeptides. Radioimmunoassays revealed the presence of dynorphin. Though relatively little activity co-migrated with met-enkephalin radioimmunoassays revealed low levels comparable to that previously reported in spinal superfusates. In all cases, the activity peaks identified on the agarose columns was elevated by sciatic nerve stimulation. These observations confirm the stimulated release of non-enkephalin activity which co-migrates with extended enkephalin and dynorphin fragments. Whether these several fractions represent neurotransmitters or metabolic by-products is not known, but as they derive from metabolically distinct precursors, their increase in the extracellular fluid by sciatic nerve stimulation suggests that they may serve as markers for activity in different populations of endorphin releasing neurons. Can we now determine what is the effective physiological drive for the several populations of neurons from which these markers are derived? (NS16541/TLY; DA01502, B83-04X-03766-12C/LT)

170.4 CHARACTERIZATION OF IN VITRO β-ENDORPHIN PROCESSING IN THE BRAIN AND GUT: IMPLICATIONS. Thomas P. Davis, Alison J. Culling* and Hans Schoemaker*. Dept. of Pharmacology, University of Arizona College of Medicine, Tucson, Arizona 85724.

In vitro, central, proteolytic processing of β-endorphin (βE; βE 1-31) by membrane-bound enzymes results in the formation of among others αE (βE 1-16), γE (βE 1-17) and their des-tyrosine-analogs. Clinical studies suggest that these endorphins may function in brain homeostases and a variety of behavioral adaptive processes due to their amphetamine (αE) and neuroleptic (γE)-like activities.

Recent evidence from our laboratory indicates that βE is also processed in the isolated small intestine while significantly increasing motility. Analysis of the venous effluent identified, among others, several α- and γ-type endorphins. Upon testing these peptide fragments, it was noted that αE, γE and their des-tyrosine-analogs significantly increased motility at doses of 1 μg/ml. These responses were characterized by increases in phasic contractions of constant amplitude and frequency.

These observations led to the hypothesis that a balance exists between α-, β- and γ-type endorphins in specific tissues that is necessary for "normal" physiological activity. To address this hypothesis, a new, improved HPLC procedure, capable of separating 28 different βE related fragments in a single chromatographic analysis, was developed. In vitro βE metabolism by membrane-bound enzymes from post-mortem brain and regions of the small intestine were studied. βE (20μM) was incubated in PBS buffer at 37°C with twice-washed membrane preparations. After 30-120 min time-course incubations, samples were boiled for 15 min and centrifuged for 60 min at 15,000xg. The supernatant was assayed for βE related peptide fragments by HPLC. The peptides were separated on a ODS-5μ column using a series of curvelinear gradients of acetonitrile against 0.1M NaH_2PO_4 buffer (pH 2.1) with detection at 210nm. The most striking difference was between the mucosa and nerve/muscle regions of the small intestine. The mucosa metabolized βE very rapidly with a $T_{\frac{1}{2}}$ of 22 min, whereas βE in the muscle had a half-life of 82 min. The mucosa also exhibited a very random rate of fragment formation which differed qualitatively and quantitatively from the nerve/muscle region and the brain. The brain was very similar to the nerve/muscle region of the small intestine in that it metabolized βE slowly ($T_{\frac{1}{2}}$=141 min vs 82 min) and yielded a very similar pattern and ratio of α- and γ-type endorphins. Therefore, these data suggest that βE processing in the nerve/muscle region may be occurring at the nerve and that a specific balance of α- and γ-type endorphins does exist in different tissues. The question of whether this balance in the gut is altered in pathological states, as we have shown centrally in schizophrenia, remains to be established.(Supp. by PMA Grant)

170.5 THE ACTION OF ENDOPEPTIDASE OF BRAIN MEMBRANES WITH DYNORPHIN-RELATED PEPTIDES. M. Knight, L. Steardo,* C.A. Tamminga, T.N. Chase. NINCDS ETB, Bethesda, Maryland 20205.

An enkephalin-generating endopeptidase (EGE) purified from rat brain membranes has been previously shown to form enkephalin from peptides containing the amino-terminal enkephalin sequence. Although the tryptic fragment of Beta endorphin, Beta-LPH61-69 was initially used as the assay for this peptidase, EGE also produces enkephalin from enkephalin Arg^6 and generates enkephalin and Leu-enkephalin from precursors in striatal extracts of rat. Thus, this endopeptidase may function in brain as the final processing enzyme in enkephalin biosynthesis from proenkephalin precursors.

The possibility exists that EGE may metabolize the dynorphin-alpha-neoendorphin precursor and fragment peptides. Since the known specificity of the enzyme is to cleave a medium chain peptide sequence after a Met or Leu amino acid, Leu-enkephalin could be cleaved from alpha-neoendorphin and dynorphin, or dynorphin 1-8 could be generated directly by proteolysis of the Ile^8 peptide bond in dynorphin 1-17, 1-13, or 1-11. To answer this question, dynorphin peptides at 10^{-5}M were reacted with 6 ug/ml EGE enzyme at 37°; at progressively longer reaction times the products were analyzed by HPLC and radioimmunoassay for the production of Leu-enkephalin and dynorphin 1-8. There was no formation of Leu-enkephalin or dynorphin 1-8 from dynorphin 1-13 and 1-17 at times up to 90 min. Thus, the enzyme apparently lacks affinity for these peptides. However, when the EGE was reacted with dynorphin 1-8 and with alpha-neoendorphin octapeptide amide, a rapid production of Leu-enkephalin was noted. Thus the EGE enzyme may have an affinity for these smaller peptides and produce Leu enkephalin. These in vitro results suggest a role for EGE in generating Leu-enkephalin from the dynorphin-alpha neoendorphin system. Also, the enzyme could function as the inactivating enzyme for dynorphin 1-8. Indeed, the presence of EGE in either synthetic granules or on the nerve ending cell membrane may define the neurochemistry of a neuron as a functional enkephalin cell.

170.6 PHENCYCLIDINE-INDUCED INHIBITION OF STRIATAL ACETYLCHOLINE RELEASE: COMPARISONS WITH MU, KAPPA, AND SIGMA OPIATE AGONISTS. S.M. Leventer* and K.M. Johnson. Dept. of Pharmacol. and Toxicol. Univ. Tex. Med. Br., Galveston, TX 77550.

Phencyclidine (PCP) has been shown to share a number of characteristics with certain psychotomimetic opioids. In the rat, cyclazocine and PCP produced a similar behavioral pattern, including backwards walking and side-to-side head movements. In the chronic spinal dog, both SKF10047 and PCP increased pulse rate, dilated pupils, and decreased the flexor reflex. Finally, in rats trained to discriminate PCP from saline, SKF10047 and cyclazocine elicited PCP-appropriate responding.

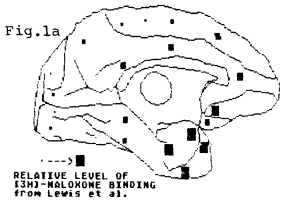
Previous studies in our laboratory have shown that PCP produced a concentration-dependent inhibition of K^+-stimulated acetylcholine (ACh) release from rat striatal slices. In order to further characterize the similarities between PCP and the opiates, the present study compared the effects of PCP to those of morphine, ethylketocyclazocine (EKC) and SKF10047, putative agonists at mu, kappa and sigma binding sites respectively.

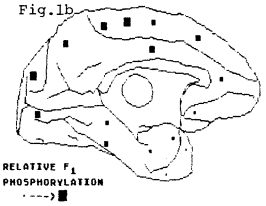
EKC and the (+)-isomer of SKF10047, like PCP, inhibited K^+-stimulated ACh release in a concentration-dependent manner. This effect was antagonized by 0.1 μM naloxone and 0.1 μM haloperidol. Since it is probable that naloxone is not an antagonist at the sigma/PCP receptor, but is an antagonist at the mu and kappa receptor, these data indicate that the effect of PCP and SKF10047 may result from interaction with mu and/or kappa receptors. Morphine, however, had no effect on ACh release in this preparation, suggesting that the inhibition of ACh release observed in this study may be mediated via kappa opiate receptors. The reversal of this effect by haloperidol suggests that EKC and SKF10047, like PCP, may inhibit ACh release indirectly by stimulating striatal dopamine release. This work was supported by ADAMHA grant #DA 02073.

170.7 PHOSPHORYLATION STATE OF 45-49 kD PROTEINS IN MONKEY CEREBRAL CORTEX: CORRESPONDENCE WITH OPIOID RECEPTOR GRADIENTS. R. Nelson*, D. Friedman, J. B. O'Neill, M. Lewis, M. Mishkin, and A. Routtenberg. Cresap Neuroscience Laboratory, Northwestern University, Evanston, IL 60201, and Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

Stereospecific 3H-naloxone binding sites in rhesus monkey cerebral cortex, which represent one or more classes of opioid receptors, increase in density along cortical pathways which sequentially process modality-specific sensory information (Fig.1a; Lewis, et al., Science, 1981). Since certain opioid peptides affect protein phosphorylation (Bar et al., Eur.J.Pharm., 1980), we examined endogenous phosphorylation of electrophoretically separated proteins from cerebral cortex of 3 rhesus monkeys. We now report: (a) an inverse correlation (Fig. 1b) across 22 cortical regions between the density of 3H-naloxone binding sites and the in vitro phosphate incorporation of Band F1, a 45 kD protein ($r=-0.609, df=20, p<.01$); (b) the presence of a 47 kD protein with similar properties to the 45 kD band (Ca++-stimulated, cAMP-stimulated phosphorylation) found only in cortical regions of highest 3H-naloxone binding; (c) a positive correlation between the phosphorylation of a Ca++-stimulated, cAMP-independent 49 kD protein and 3H-naloxone binding levels ($r=+0.564, df=20, p<.01$). These findings suggest a local control of protein phosphorylation in monkey cerebral cortex corresponding to opioid receptor levels.

A 48 kD brain phosphoprotein termed B-50 proteolytically cleaves following dialysis to a 46 kD phosphoprotein, B-60 (Zwiers et al., FEBS Letters, 1981). B-50 phosphorylation is also sensitive to dynorphin (Zwiers et al., Life Sci., 1981). The 45-49 kD phosphoproteins observed in monkey cortex may correspond to B-50 and B-60, suggesting that these 2 proteins may occur physiologically and that endogenous opioids may determine which form predominates. (Supported by MH25281 and MH263-MD-229713 to A.R.)





170.8 ISOLATION AND CHARACTERIZATION OF METORPHAMIDE, A NOVEL AMIDATED OPIOID OCTAPEPTIDE FROM BOVINE BRAIN. E. Weber, F. Esch*, P. Bohlen*, J. D. Barchas and C. J. Evans. Nancy Pritzker Lab., Dept. Psychiatry, Stanford Univ. Med. Sch., Stanford, CA 94305, and Labs. for Neuroendocrinology, Salk Institute for Biological Studies, La Jolla, CA 92037

Recent studies have begun to yield information on the post-translational proteolytic processing of opioid peptide precursors in brain. Most work has been done on pro-dynorphin whose processing appears to be unusual in that at least two peptides, dynorphin A(1-8) and dynorphin B, are generated from this precursor by proteolytic cleavages at single arginine residues rather than at classical double basic amino acid residues thought to be primarily involved in peptide precursor processing (1,2).

In order to examine whether pro-enkephalin, the second major opioid peptide precursor, also gives rise to opioids that are generated by a single arginine cleavage, we have begun to examine brain tissue from several species for the presence of such cleavage products. Here we show that extracts from bovine caudate contain an amidated opioid octapeptide with the following following structure: Tyr-Gly-Gly-Phe-Met-Arg-Arg-Val-NH_2. The peptide has been named metorphamide. Metorphamide is contained within pro-enkephalin and is apparently generated by a single arginine cleavage followed by transformation of a carboxyterminal glycine into an amide group. Metorphamide was detected by a specific RIA and purified to homogeneity by gel-filtration chromatography and reverse phase HPLC. The purified peptide was chemically characterized by amino acid composition analysis and automated Edman degradation in the gas/liquid phase peptide sequenator.

1. Weber, E., Evans, C.J., Barchas, J.D. Nature 299, 77, 1982.
2. Cone, R., Weber, E., Barchas, J.D., Goldstein, A. J. Neurosci., in press.

170.9 HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES OF ENKEPHALIN-LIKE PEPTIDES IN HIPPOCAMPUS, RETINA, AND COCHLEA. Douglas W. Hoffman LNO, Bldg 36, Rm. 5D08 National Institutes of Health, Bethesda, MD 20205

Previous studies from this lab have reported the presence of enkephalin-like peptides in the guinea pig cochlea, retina and hippocampus, using immunofluorescent, chromatographic, and radioimmunoassay techniques. The high-performance liquid chromatographic (HPLC) studies, utilizing an isocratic elution of trifluoroacetic acid (TFA) in acetonitrile from a reversed phase column, demonstrated the presence of three enkephalin-like fractions in these tissues, as determined by radioimmunoassay (RIA) of the HPLC fractions. Two of these fractions can be identified by retention time in HPLC as met- and leu-enkephalin, respectively. The third immunoreactive HPLC fraction elutes earlier than met- or leu-enkephalin standards from the reversed-phase column, and, like the fractions identified as met- and leu-enkephalin, co-elutes from a Biogel P-2 column with synthetic enkephalin standards, and is active in inhibiting the binding of labeled opiate ligands to rat brain membranes. It appears likely that this earlier eluting fraction contains a chemically modified or extended leu-enkephalin-like molecule, possibly a dynorphin fragment, although neither dynorphin (1-8) or alpha-neo-endorphin (1-8) have the retention time of this fraction.

Gradient elution of tissue sonicates, utilizing a shallow gradient from 20% to 30% acetonitrile in 0.05% TFA, indicates that the earlier eluting fraction is composed of two or more immunoreactive and bioactive peptides, as determined by RIA and radioreceptor assays of HPLC gradient fractions. Further studies are directed toward purification and identification of these chromatographically separated enkephalin-like peptides.

170.10 ISOLATION AND AMINO ACID SEQUENCE OF A PRO-DYNORPHIN C-TERMINAL FRAGMENT FROM BOVINE CAUDATE NUCLEUS. C. J. Evans, F. Esch*, P. Bohlen*, J. D. Barchas and E. Weber. Nancy Pritzker Lab., Dept. Psychiatry, Stanford Univ. Med. Sch., Stanford, CA 94305, and Labs. for Neuroendocrinology, Salk Institute for Biological Studies, La Jolla, CA 92037.

The complete primary structure of the common precursor to neo-endorphins and dynorphins was recently determined. The precursor contains the amino acid sequences of α-neo-endorphin, dynorphin A and dynorphin B (rimorphin). Dynorphin B is located near the carboxyterminus of the precursor.

The release of dynorphin B involves cleavage at a single arginine residue. This processing site is 16 residues from the C-terminus of the precursor. One would predict that cleavage at this site results not only in the release of dynorphin B but also a fragment constituting the C-terminus of the precursor (pro-dynorphin C-fragment). We have raised antibodies to a synthetic decapentapeptide fragment representing the C-terminus of pro-dynorphin as predicted from the porcine mRNA nucleotide sequence. Using radioimmunoassay as detection system, we isolated and characterized natural pro-dynorphin-C-fragment immunoreactive material from bovine caudate nucleus. Amino acid composition analysis showed that the peptide consisted of 15 amino acid residues. Microsequencing in the gas/liquid phase sequenator demonstrated that bovine pro-dynorphin-C-fragment differed by one amino acid from the porcine peptide as predicted from the mRNA sequence (serine was substituted for the asparagine residue at position 6). The N-terminal amino acid was serine (an arginine as predicted from the mRNA sequence was missing from the N-terminus) showing that the processing enzyme(s) cleave(s) on the carboxyl side of the arginine residue. Dynorphin-B was also isolated from bovine caudate nucleus. Amino acid composition analysis confirmed its structure (i.e., no arginine was present at the C-terminus of dynorphin B). Since the arginine residue at the cleavage site is not present on either dynorphin-B or pro-dynorphin-C-fragment, it must be eliminated during the processing. The cleavage at this single arginine residue is therefore analogous to those at double basic residues and is consistent with a trypsin-like cleavage followed by carboxypeptidase-B-like activity. The pro-dynorphin C-fragment that we have isolated may be a good marker peptide for the presence of pro-dynorphin in neuronal tissues.

170.11 POMC IN RHESUS ANTERIOR PITUITARY + PLASMA: EVIDENCE OF N-ACETYLATED β-ENDORPHIN AND α-MSH C. Cahill*, S. Watson, M. Knobloch* and H. Akil (SPON: G. Goldstein). Mental Health Res. Insti., Univ. of Michigan, Ann Arbor, Michigan 48109

Pro-opiomelanocortin (POMC) related peptides have been extensively studied in rat tissue and plasma. Yet, in rhesus monkey pituitary and plasma they have not been well characterized. We have characterized POMC related peptides by immunocytochemistry, multiple radioimmunoassays (RIA's), molecular sieving and HPLC.

Immunocytochemical staining of the pituitary demonstrated N-acetyl-β-endorphin and α-MSH in some of the anterior lobe (AL) corticotrophs. The AL was carefully separated from the thin band of the intermediate lobe (IL) seen in monkey. The dissection was conservative to avoid contamination from IL. RIA's of extracted AL has shown N-acetyl-β-endorphin-like and α-MSH-like immunoreactivity. Molecular sieving chromatography of the extracted AL has shown that the β-endorphin-sized peak is larger than the β-LPH peak with a ratio of 1.7:1 unlike the situation in the rat where the β-endorphin/β-LPH ratio is 0.5:1. Further, a significant proportion of the β-endorphin-sized peak is N-acetylated (33%), whereas less than 8% acetylation is seen in rat β-endorphin-sized material derived from the AL. Overall, less than 3% of the total β-endorphin-like immunoreactivity in the rat AL is N-acetylated whereas, over 20% of the immunoreactivity is N-acetylated in monkey AL. This pattern along with the presence of α-MSH-like immunoreactivity suggests that post-translational events more similar to the rodent intermediate lobe, and is mirrored by the plasma profiles. Molecular sieving chromatography of extracted plasma demonstrated a pattern similar to that seen in the AL. N-acetyl-β-endorphin forms and α-MSH peaks are clearly identifiable with 40% of the β-endorphin-sized material being N-acetylated. After pretreatment with intraventricular colchicine, N-acetyl-β-endorphin and α-MSH-like immunoreactivity disappears from plasma. These data suggest that rhesus monkey corticotrophs process POMC differently than rat with a pattern falling between AL and IL patterns of the rodent.

This work was supported by NIDA Center grant DA00154 to HA and SJW.

170.12 ISOLATION OF MULTIPLE-SIZED IMMUNOREACTIVE FORMS OF DYNORPHIN-A IN THE SUBSTANTIA NIGRA. R.M. Dores, H. Akil and S.J. Watson. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109

Immunocytochemical studies indicate that pro-dynorphin, the common precursor for and α-neo-endorphin, dynorphin A and dynorphin B, has a distribution distinct from either the enkephalin or β-endorphin opioid networks in the central nervous system of the rat (Khachaturian et al., Peptides, 3:941-54, 1982). Two anatomically distinct terminal field regions in the pro-dynorphin system are the posterior lobe of the pituitary, a neuroendorcine region and the substantia nigra, the major dopaminergic cell group in the CNS.

In order to assess whether dynorphin A is differentially processed in these regions acid/acetone extracts of the posterior pituitary and substantia nigra were separately fractionated by gel filtration chromatography on a Sephadex G-50 column equilibrated in 10% formic acid and analyzed by radioimmunoassay. Two dynorphin A antisera were used: antiserum #54 is specific for the COOH-terminal region of DYN A(1-17) and has less than 0.1% crossreactivity for DYN A(1-8); antiserum #78 is specific for the COOH-terminal region of DYN A(1-8) and has approximately 1% crossreactivity for DYN A(1-17) and less than 0.1% crossreactivity for [Leu]enkephalin.

Three peaks of dynorphin A-related material were detected in the extracts of both regions: a 4K peak of DYN A(1-17)-related material, a peak of DYN A(1-17)-sized material, and a peak of DYN A(1-8)-sized material. In the substantia nigra these peaks represented respectively 1%, 3%, and 96% of the total immunoreactivity, while in the posterior pituitary these peaks represented 11%, 28%, and 61% of the total immunoreactivity. These results argue that the dynorphin A region of pro-dynorphin is differentially processed in these regions to yield DYN A(1-8)-sized material as a major end product in the substantia nigra, and a mixture of DYN A(1-17)- and DYN A(1-8)-sized material as major end products in the posterior pituitary. Experiments are in progress to analyze the forms of dynorphin A present in the cell bodies associated with these distinct terminal regions.

This research was supported by NIDA Grant DA02265 and NIDA Center grant DA00154 to HA and SJW.

170.13 β-ENDORPHIN IMMUNOREACTIVITY IN THE RAT PITUITARY: POSTNATAL DEVELOPMENT. N.E. Alessi, H. Khachaturian, S.J. Watson and H. Akil. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

Previous studies of β-endorphin ontogeny have focused mainly on developmental changes in content of different brain regions (Bayon, A., et al., Brain Res., 179:93-101, 1979). Little attention has been given to the ontogeny of β-endorphin in the pituitary gland. Specifically, post-translational alterations of the β-endorphin molecule which may occur at different developmental stages, such as changes in the ratio of multiple forms or in acetylation of β-endorphin, have not been studied. Our current studies of the postnatal development of β-endorphin in the anterior (AL) and intermediate (IL) lobes of the rat pituitary have begun to address these issues.

Postnatal (P1, P7, P14, P21 days) and adult male Sprague-Dawley rats were sacrificed, and the pituitaries were immediately separated into AL and IL (also containing neural lobe). The region of the AL in contact with the IL was discarded. Tissue was extracted in acid-acetone with iodoacetamide and phenylmethylsulfonyl fluoride as inhibitors. Radioimmunoassays were performed utilizing antibodies which recognize β-endorphin immunoreactivity (i.r.) and N-acetylated-β-endorphin i.r. The preliminary results are summarized in the following table. Values of β-endorphin i.r. are expressed in pmol/lobe (mean ± SEM).

	P1	P7	P14	P21	Adult
AL	1.8 ± 0.0 (n=5)	6.1 ± 1.7 (n=5)	10.4 ± 0.4 (n=6)	16.2 ± 4.5 (n=6)	164 ± 40 (n=5)
IL	2.3 ± 0.4 (n=5)	5.6 ± 0.6 (n=6)	6.5 ± 0.8 (n=5)	12.4 ± 5.8 (n=4)	1,010 ± 308 (n=4)

From P1 to P21, the total β-endorphin i.r. increased 9 fold in AL and 5 fold in IL. From P21 to adult, β-endorphin i.r. increased more than 50 fold in IL and 10 fold in the AL. Therefore, the quantity of β-endorphin i.r. increased more than 250 fold from P1 to adulthood in the IL and more than 90 fold in the AL. On the other hand, acetylated β-endorphin i.r. increased only 30 fold in the IL and 10 fold in the AL.

Ongoing work at this time involves the characterization of β-endorphin i.r. and N-acetylated β-endorphin i.r. following the molecular sieving of pooled AL and IL extracts at selected postnatal dates. These studies will provide information on the ontogeny of multiple forms of β-endorphin and its post-translational processing during development.

This work was supported by NIDA grant DA02265 to HA and SJW.

170.14 SOME NEW INSIGHTS IN THE REGULATION OF BETA-ENDORPHIN BIOSYNTHESIS AND RELEASE. H. Akil, H-L. Lin and Y. Ueda. Mental Health Research Institute, Univ. of Michigan, Ann Arbor, MI 48109.

While numerous studies have examined the effects of various manipulations on brain beta-endorphin (βE) levels, few have uncovered substantial changes in content. Clearly, we need to develop strategies for more dynamic measures and their validation in given models. In our laboratory we have attempted this by studying βE systems in pituitary and in brain and the effects of drugs or of acute and chronic stress. The biochemical tools for more dynamic measure include: 1) pulse-chase experiments, involving labeling peptides and their purification on immunoaffinity columns. 2) Measurement of the pre- prohormone/prohormone ratio, using specific signal peptide, βE and ACTH antibody affinity columns. 3) Studies of multiple forms of E (N-acetylated and C-terminus cleaved) using HPLC /RIA.

Our studies suggest two new insights into the system: 1) activation of the system (with stress or drugs) which causes release of βE leads to increases not only in the biosynthesis of the precursor, but in the processing of it, resulting in shorter half lives of the precursors and intermediates. This may be due to some induction of the enzymes involved in cleavage and in post-translation modification. 2) There is some suggestion that the most modified, highly processed material is preferentially released, at least in the case of the neurointermediate lobe.

Whether these observations apply to all brain areas under other behavioral or pharmacological models remains to be established.

This work was supported by NIDA grant DA02265 to HA.

170.15 BETA-ENDORPHIN IN DEPRESSION. J. Matthews*, S.J. Watson, J. Greden* and H. Akil (SPON: J.A. Taren). Dept. of Psychiatry, University of Michigan, Ann Arbor, Michigan 48109.

For several years it has been clear that at least fifty percent of endogenously depressed (ED) patients exhibit abnormal regulation of their hypothalamo-pituitary-adrenal axis (HPA). The dexamethasone suppression test (DST) has been useful in demonstrating this abnormal regulation by showing that ED patients tend to suppress plasma cortisol less postdexamethasone challenge as compared to either normal or psychiatric control subjects. In addition, circadian rhythm studies show that ED patients secrete more cortisol over a 24 hour period than controls. These cortisol measures reflect adrenal activity and thus implicate abnormal adrenal function. We however have been studying changes in plasma POMC peptides in ED patients in order to see whether these pituitary products exhibit analogous regulatory defects thus suggesting that either pituitary or brain is the source of the regulatory defect.

Patients were catheterized and four plasma samples were collected around the 4 p.m. time period on the baseline day as well on the day following 1 mg oral dexamethasone (given at 11 p.m.). Plasma was assayed for β-END/ β-LPH and cortisol across 35 patients.

We have found that dexamethasone can suppress β-END/β-LPH in plasma of normal subjects and psychiatric controls at 16 hours postadministration. The ED patients as a group suppressed β- END/ β-LPH less than control groups. Further, those ED patients which escaped suppression of β-END/β-LPH were not necessarily the same as those whose cortisol escaped suppression. The general conclusion is that plasma POMC peptide measures postdexamethasone point to an abnormality in either pituitary or brain regulation in ED patients and such measures may be a sensative marker for diagnosis. Furthermore, a combination of β-END or cortisol abnormalities tends to be seen in a reasonably large percent of ED patients.

Studies in progress involve circadian rhythm studies of β-END /β-LPH and ACTH, ACTH plasma responses to the DST, and CRF challenge tests.

This work was supported by NIMH grant MH36168 to SJW.

170.16 RELEASE OF ENDOGENOUS DOPAMINE (DA) FROM RAT STRIATUM IN VITRO: EFFECTS OF L-GLUTAMIC ACID (L-GLU) AND A DELTA OPIOID AGONIST. Marc Marien* and Khem Jhamandas* (SPON: J.V. Milligan). Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Previous investigations of opioid effects on striatal DA release in vitro have focused on the release of exogenous or newly-synthesized radiolabelled transmitter. We have observed that morphine depresses the L-GLU-evoked release of preloaded ^{3}H-DA from superfused slices of the rat striatum (Marien et al., Can. J. Physiol. Pharmacol. 61, 43, 1983). The aim of the present study was to determine if opioids modify the release of endogenous striatal DA in vitro. These experiments examined the effects of D-Ala^2-D-Leu^5-enkephalin (DADL), a delta opioid receptor agonist, on the spontaneous and the L-GLU-stimulated release of endogenous DA in rat striatal slice superfusates. Slices (300 μm thickness, 50 mg weight) were placed in 1.0 mL chambers and superfused for 70 min with a modified Kreb's-Henseleit medium (0.5 mL/min). The DA in 10-min superfusate samples was isolated by alumina adsorption and cation-exchange chromatography, and quantitated by high performance liquid chromatography with electrochemical detection (HPLC-ED). The limit of sensitivity of this method was 40-50 pg DA per mL superfusate. The baseline rate of DA release from the slices was 330 pg DA/g tissue/min; this basal release rate was not affected by a 30-min exposure to DADL (5 nM). Nomifensine (10 μM), a blocker of DA reuptake, increased the basal DA release rate 10-fold. In the presence of nomifensine, L-GLU (1 mM, 5-min exposure) increased the basal release of DA by 530%; in these experiments, DADL (50 nM, 50-min exposure) had no effect on the baseline (unstimulated) release of DA, but significantly reduced the L-GLU-evoked release to 420%. The post-superfusion tissue levels of DA and the DA metabolite dihydroxyphenylacetic acid (DOPAC) were not significantly changed by DADL.

This study demonstrates that the release of endogenous striatal DA in vitro, under the experimental conditions employed, is measurable by HPLC-ED; the release of DA in this system is stimulated by L-GLU and this action is modulated by a delta receptor agonist.

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170.17 Co-release of enkephalins and precursors with catecholamines from the perfused cat adrenal in situ. J. Rossier, M. Chaminade, A.S. Foutz, G. Patey and P.E. Chabrier+, C.N.R.S. Physiologie Nerveuse, 91190 Gif-sur-Yvette, and +Institut Beaufour, Les Ulis, France.

Several studies have indicated that enkephalin immunoreactive products or opiate-like products are coreleased with catecholamines from the adrenal medulla. In the present study we have carefully characterized the nature of the released enkephalins (E). Met-enkephalin (ME), Leu-enkephalin (LE), the octapeptide (OE) and the heptapeptide (HE) are released from perfused adrenals in a molar ratio similar to that found in the biosynthetic precursor, proenkephalin. The left adrenal of anesthetized cats was perfused with Krebs solution. Catecholamines (CA), ME, LE, OE, HE and large E containing peptides (ECP) were assayed in the perfusate by various specific assays. Electrical stimulation (St) of the left splanchnic nerve (N) at 15 Hz induced an immediate release (10 to 20 times over baseline values) of these various molecules. During St, 2.17 nmole of CA (about 1.2 % of the gland content) are released per min with 0.87 pmole of ME (1.3 %), 2.41 pmole of ECP (1.%) and 0.34 pmole of HE (1.5 %). Perfusion of the gland with Ach (0.1mM) or K^+ (50mM) induced an even greater release, about 70 times over baseline for all the molecules. During K^+ St the release per min was 10.41 nmole (5.6 %) CA, 3.45 pmole (5.%) ME, 19.4 pmole (7.6 %) ECP and 1.02 pmole (4.9 %) HE. As for CA, a rapid fatigue of the secretion of various E was noticed during a 10 min St by N, Ach or K^+. During the first 2.5 min period output was always 2 to 3 times higher than the output during the fourth 2.5 min period. During the four 2.5 min periods of the N St the molar ratios of CA/ME (2420), CA/HE (9500) and CA/ECP (1025) were quite stable and close to the ratios found in extracts of the gland (2690, 7930 and 720 respectively). We examined the ratios of ECP/ME during N and K^+ St. This ratio was 2 fold greater during K^+ than during N St. When a second 10 min K^+ St was repeated the ECP/ME increased 4 fold as compared to N St. When perfusates obtained during N or K^+ stimulation were analysed on gel filtration, their elution profiles were strikingly different. With N St the main peak of ECP has Mr 4000, with K^+ St the main peak had Mr between 20,000 and 5000. These results may indicate a heterogeneity in the population of chromaffin granules. They may be classified into mature granules in which proenkephalin is fully processed into ME, LE, OE and HE, and in immature granules containing partially processed precursors. Physiological stimulation by the splanchnic nerve will induce the release of mature granules. When intense K^+ St are repeated immature granules containing the partially processed precursors are also released.
Abbreviations: N St, Nerve stimulation; ECP, enkephalin containing peptides; HE,heptapeptide ;OE,Octapeptide; ME,Met-enkephalin.

170.18 LC-EC OF NEUROPEPTIDES. L.H. Fleming* and N.C. Reynolds, Jr., Dept. of Neurol. (Milwaukee Clinical Campus), Univ. of WI Med. School and Sect. of Neurol.-Dept. of Med., Mt. Sinai Med. Cntr., Milwaukee, WI 53201.

The existence of many closely-related neuropeptides has complicated research efforts to clearly establish their functional significance in normal and disease states (Panerai, A.E. et al., In: Regulatory Peptides: From Molecular Biology to Function, ed. by E. Costa and M. Trabucchi, p. 139. Raven Press, New York, 1982). Highly specific and sensitive techniques are required to quantify neuropeptides present in physiological fluids and tissues at low concentrations. Radioimmunoassay (RIA) is frequently used to quantify methionine (ME) and leucine enkephalin (LE) (Przewlocki, R. et al., Br. Res. 174: 357, 1979) but has been limited for some neuropeptides by the lack of highly specific antibodies. Because of its selectivity, high performance liquid chromatography (LC) has been used to separate neuropeptides in biological samples. Chromatographic fractions are quantitated by RIA for each peptide of interest because the concentration of neuropeptides (pico-to-femto-molar) is below the limits of detection by far UV spectroscopy (Loeber, J.G. and J. Verhoef, Meth. Enzmol. 73: 261, 1981). This procedure is time consuming, expensive and introduces additional experimental variables.

LC with electrochemical detection (LC-EC) has been used to identify and quantify catechol- and indol-amines from small samples of brain, blood, urine and CSF (Kissinger, P.T. et al., Life Sci. 28: 455, 1981). The observation of the electroactivity of ME and LE (Meek, J.L. et al., Neuropharmac. 16: 151, 1977) prompted us to explore the possible usefulness of electrochemical detection for neuropeptide standards. In an effort to optimize the separation of ME and LE from other closely-related peptides (i.e. Dynorphin 1-6, β-Neo-endorphin, etc.), several different reverse phase columns and isocratic mobile phases were used. ME and LE were chromatographed on 5μ Ultrasphere-Octyl, 10μ Aquapore 300, 10μ μBondapak and 5μ Biophase ODS columns using various dilutions of phosphate buffer, pH 2.3 containing acetonitrile. Hydrodynamic voltammograms showed that in this mobile phase, ME and LE are oxidized by a glassy carbon working electrode at relatively high potentials, > + .90v relative to Ag/AgCl reference electrode. Dynorphin 1-6, α-Neo-endorphin 1-10, β-Neo-endorphin etc. are also oxidized at these potentials permitting their simultaneous measurement. LC-EC offers an efficient and inexpensive alternative to the use of RIA for studies of neuropeptides in selected brain regions.

170.19 IN VIVO ENKEPHALIN RELEASE FROM THE GLOBUS PALLIDUS OF THE RAT: DIURNAL RHYTHM AND RELATIONSHIP WITH GABA RELEASING SYSTEMS. A. Bayon, B. Anton* and D. Diaz-Pontones*. Instituto de Investigaciones Biomedicas. Universidad Nacional Autonoma de Mexico, 04510 Mexico D. F. Mexico.

We have previously shown that endogenous immunoreactive enkephalin is released in vivo from the globus pallidus and that this release is tonically inhibited by endogenous GABA (Bayon et al; Neurosci. letts., 24:65, 1981, Bayon et al; Soc. Neurosci. Abstr. Vol. 7, p. 800, 1981). During those investigations it was incidentally observed that enkephalin release was higher in the evening than in the morning hours. Given the link observed between GABA and enkephalin systems in the pallidum, we decided to study and compare the diurnal variation in the in vivo release of these messengers. Methodological details of the push-pull-cannula perfusion technique used in these experiments have been reported elsewhere (Bayon et al, Neurosci. letts, 24.65, 1981). Fifteen min. after cannula insertion ^{3}H-GABA was perfused for 30 minutes (4 x 10^6 cpm in 750 μl Ringers) and 15 min-fractions were collected thereafter. ^{3}H-GABA washout and release was determined by direct scintillation counting and endogenous enkephalin release was measured by a Leu-enkephalin RIA. Six perfusion sessions were carried out from 8.00 AM to 2.30 PM (daytime) and six from 4.00 PM to 10.30 PM (evening). Resting enkephalin release increased 100% between 2.00 PM and 10.00 PM, while ^{3}H-GABA resting release decreased 60%. Similarly, K^+-stimulated enkephalin release increased 6 fold while K^+-stimulated ^{3}H-GABA release decreased more than 10 fold within that time. In relation to the opposed diurnal rhythms of enkephalin and GABA release from the globus pallidus it is noteworthy that enkephalin and GABA transmission in this region also have opposite actions on the motor activity of the rat. Bilateral injection of enkephalin analogs in the pallidum leads to hyperactivity, an action which is blocked by naloxone (Joyce et al, Brain Res, 221.359, 1981). In contrast, bilateral pallidal administration of drugs promoting GABA-ergic transmission produces akinesia, while the opposite is found for drugs antagonizing this transmitter (Pycock et al, Brain Res, 116.353, 1976). Being the rat a nocturnal animal, the evening increase of enkephalin release and the decrease of GABA release in the pallidum parallel these psychopharmacological observations. Thus, we suggest that the coordinated GABA-enkephalin transmission in the pallidum may be an important link in the neural control of the motor activity rhythms in the rat. (Supported by grants from CONACYT, Mex. the FONEIN, Mex. and the Instituto Mexicano de Psiquiatria).

170.20 THE OPIOID OCTAPEPTIDE MET^5-ENKEPHALIN-ARG^6-GLY^7-LEU^8 IN RAT SPINAL CORD: BIOCHEMICAL CHARACTERIZATION, DISTRIBUTION, RELEASE AND ANALGESIC ACTIVITY. M. J. Iadarola, J. Tang*, P. Panula*, J. Chou*, H.-Y.T. Yang and E. Costa. Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

The content and biochemical characteristics of met^5-enkephalin-arg^6-gly^7-leu^8 (met-enk-arg-gly-leu) immunoreactive material were analyzed in rat spinal cord using a sensitive and specific antibody directed against the C-terminal of this octapeptide. Sacral and lumbar cord contained two-fold more immunoreactive material than thoracic or cervical cord. The concentration of immunoreactivity in dorsal cord was greater (3-4 fold) than that in ventral cord at all spinal levels. Lowest levels of met-enk-arg-gly-leu were observed in ventral thoracic cord (0.7 picomol/mg prot); highest levels were observed in dorsal lumbo-sacral cord (5-6 picomol/mg prot).

Biochemical characterization of the immunoreactive material by gel filtration revealed two distinct species: that with high molecular weight (~8,000d on Biogel P-30) represented half of the total immunoreactivity measured in acid extracts of cord tissue; the low molecular weight material was shown to be identical with synthetic met-enk-arg-gly-leu by reverse phase HPLC.

Finer details of met-enk-arg-gly-leu localization were obtained by peroxidase-antiperoxidase immunohistochemistry using the same antiserum as in the RIA. The densest staining was observed in laminae I-II. Numerous densely stained fibers were observed in the dorsal lateral fasciculus. This pattern is similar to observations made with antisera against met^5-enkephalin and met^5-enkephalin-arg^6-phe^7. Cell bodies could be observed in Lamina I through V and around the central canal but not in the ventral horn. Ventral horn contained only fibers. These fibers mainly innervated neuronal somata which in some cases were clearly outlined by the reaction product.

The octapeptide was released in vivo into a spinal perfusate containing 10^{-6}M substance P using the intrathecal perfusion system described by Yaksh et al. (Brain Res. 242:279, 1982). The subarachnoid space was perfused at a rate of .1 ml/min with artificial CSF containing 10^{-6}M bestatin and 10^{-6}M captopril. Under these conditions both the octapeptide and its high molecular weight component were recovered in the perfusate. This suggests that both high molecular weight material and met-enk-arg-gly-leu are released from nerve terminals.

Met-enk-arg-gly-leu was a potent analgesic. Intrathecal injection of 270 ng (in 30μl of artificial CSF containing bestatin and captopril) increased tail flick latency within 5 min; peak activity occurred by 10 min. Tail flick latency returned to predrug values by 25 min. Analgesia produced by met-enk-arg-gly-leu was reversed by naloxone (30μl of $2x10^{-5}$M intrathecally). These data suggest that the opioid octapeptide met-enk-arg-gly-leu which is derived from preproenkephalin A may function as a neuromodulator.

171.1 AUTORADIOGRAPHY OF RECEPTORS FOR BOMBESIN IN MAMMALIAN BRAIN
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The peptide bombesin (BN) is a potent hypothermic and hyperglycemic agent after central injection. These biological activities may be mediated by the endogenous BN-like peptides which have been detected in discrete brain regions (Moody, T. et al., Peptides, 2: 75, 1981). Upon release from certain brain neurons, endogenous BN-like peptides presumably activate specific receptors which have been demonstrated using rat brain homogenate (Moody, T. et al., PNAS, 75: 5372, 1978). Here, using in vitro autoradiographic techniques, the discrete regional distribution of binding sites for BN-like peptides was investigated.

Binding studies were conducted using the method of Wolf et al. (Eur. J. Pharm., 87: 163, 1983). Unfixed 12 μm coronal slices derived from the forebrain bound (^{125}I-Tyr^4)BN reversibly and with high affinity (K_d, 5 nM) to a single class of sites (B_{max}, 130 fmol/mg protein); the ratio of specific/nonspecific binding was 6/1. Also, pharmacological studies indicated that only peptides which have a C-terminal similar to that of BN compete for specific (^{125}I-Tyr^4)BN binding sites.

In vitro autoradiographic studies were conducted using the method of Young and Kuhar (Brain Res., 179: 255, 1979). Highest grain densities were present in the olfactory bulb and tubercle, nucleus accumbens, suprachiasmatic nucleus, medial and anterior amygdaloid nuclei, periventricular nucleus of the hypothalamus, dorsomedial thalamic nucleus, hippocampus, locus coeruleus, facial nuclei and nucleus tractus solitarius of the hindbrain and substantia gelatinosa of the spinal cord. Moderate grain densities were observed in the pyriform cortex, deep layers of the neocortex, rhinal sulcus and striatum. Negligible grain densities were present in the cerebellum, white matter and sections treated with 1 μM unlabeled BN. The presence of high concentrations of binding sites and immunoreactive BN in discrete regions, such as the suprachiasmatic and solitary nuclei as well as the substantia gelatinosa, suggests that BN-like peptides may function as important neuroregulatory agents in these brain loci. This research supported in part by grants GM 07309, MH 00053, MH 25951 and NS 17073.

171.2 PICOMOLAR AFFINITY SOMATOSTATIN RECEPTORS IN THE CNS AND PERIPHERY LABELLED WITH A METABOLICALLY STABLE SS-28 ANALOG.
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Somatostatin, originally identified as a somatotropin release inhibiting factor, has since been demonstrated to be a potent modulator of numerous endocrine and neuronal functions. Several molecular forms have been localized to neurosecretory cells in the hypothalamus, neurons in the CNS and periphery and endocrine cells in the pancreas and G.I. tract. Using ^{125}I-labeled SS-14 analogs, several groups have identified SS binding sites in preparations from brain, pituitary, pancreas and adrenal; (Reubi et.al., Life Sci. 25:2191-8, 1981) used a metabolically stable analog of SS-28 (^{125}I-[Leu^8, D-Trp^{22}, Tyr^{25}]-SS-28, to label sites in brain, pituitary and pancreatic β-cells.

We have refined the binding assay with this probe and now report the presence of a very high affinity site in rat cortex (K_D 20 pM, B_{max} 200 fmole/mg protein). A lower affinity site (K_D > 1 nM) is also observed. These results were obtained by increasing the incubation volume while lowering both the receptor and ligand concentrations.

Briefly, aliquots of crude membrane preparation (0.33 mg wet weight tissue) were incubated with 1-5 pM ligand in 4 ml Hepes buffer (10 mM, pH 7.6) for 90 min at room temperature. The mixture was filtered over GF/B filters pre-soaked in 0.25°/° polyethyleneimine HCl; the filters are rinsed with 3 x 4 ml cold buffer and radioactivity on the filters is measured with a gamma counter. Specific binding was defined as that remaining in the presence of 10^{-9} or 10^{-7} M somatostatin-28.

In a typical assay with rat cortical membranes, 32,000 cpm were added; total bindng was 3600 cpm, and non-specific binding 1200 cpm (of which 800 cpm represented non-displaceable binding to filters). Using this assay, we have specifically labeled sites in several brain regions (highest in cortex), as well as membranes from pituitary, pancreas, adrenal, stomach and intestine.

Through the use of this refined binding assay we will examine the potency of a number of SS analogues to determine if more than one distinguishable SS receptor exists in the CNS. The ability to label receptors in the periphery now provides the opportunity to compare the properties of central and peripheral receptors. Comparison studies have already revealed pharmacological differences between pancreatic pituitary and CNS SS receptors (Reubi et al., Endocrinol., 110:1049-1057, 1982).

171.3 DISTRIBUTION OF SOMATOSTATIN RECEPTORS IN RAT BRAIN AS DETERMINED BY IN VITRO RADIOAUTOGRAPHY.
P. Leroux* and G. Pelletier (SPON: A. Dupont). MRC Group in Molecular Endocrinology, CHUL, Quebec, Canada.

Somatostatin is a neuropeptide which is widely distributed throughout the brain. By radioreceptor assays performed in macroscopically dissected brain structures, somatostatin receptors have been shown in many brain areas, especially cortex, thalamus, hypothalamus and striatum (Srikant, C.B. and Patel, Y.C., Proc. Natl. Acad. Sci. USA 78:3930, 1981). In an attempt to precisely determine brain localization of somatostatin binding sites, we have used in vitro radioautography performed on slide mounted frozen brain sections as previously described for brain opiate receptors (Herkenham, M. and Pert, C.B., J. Neurosci. 2:1129, 1982). The somatostatin agonist, [Tyr^0, DTrp^8]somatostatin (supplied by Dr. D.H. Coy), was iodinated by the lactoperoxydase method and purified on a Vydac C18 RP-HPLC system using a gradient TEAP-acetonitrile as mobil phase. The specificity of the binding was investigated by competition with 10^{-6}M/l native somatostatin within the incubation buffer. High specific binding was observed mainly in the claustrum, the median and lateral amygdalian nucleus, the olfactory bulb, the deep layers of the cerebral cortex, the periventricular nucleus of the thalamus, the hippocampus, the gyrus dentatus and the locus coeruleus. Moderate binding was detected in the septal medial nucleus and amygdalian anterior area whereas low binding could also be observed in the caudate-putamen nucleus. Worthy of note is the complete lack of binding in the hypothalamus and ventral premamillary bodies, two areas known to contain very high concentration of immunoreactive somatostatin in the rat brain. However, with a few exceptions, our data show a good correlation between somatostatin binding sites and immunohistochemically identified somatostatin pathways in rat brain.

171.4 EVIDENCE THAT [D-Trp^8]-SOMATOSTATIN INHIBITS SYNAPTIC PLASMA MEMBRANE PROTEIN PHOSPHORYLATION BY INTERACTION WITH A SPECIFIC MEMBRANE BINDING SITE.
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Somatostatin and related peptides inhibit protein phosphorylation in the synaptic plasma membrane (SPM) fraction from rat brain with characteristics that suggest a receptor-mediated mechanism (Dokas et al., Eur. J. Pharm. 88:185). We now report further characterization of this effect, using [D-Trp^8]-somatostatin, ([D-Trp^8]-SS), the most potent of the peptides examined previously. The SPM fraction was prepared from rat hippocampus and incubated with ATP^{32} to phosphorylate endogenous proteins. The standard assay was an incubation of 25 μg of membrane protein for 20 sec at 30°C in an acetate buffer, pH 6.5, containing 1 mM calcium and 7.5 μM ATP^{32}. The major phosphorylated protein of the SPM was B50 (48,000 daltons). When 10^{-4}M [D-Trp^8]-SS was added for 15 sec prior to addition of ATP^{32}, a subsequent inhibition of -44.3±2.6% was seen in the phosphorylation of B50. Increasing the preincubation with [D-Trp^8]-SS to 30 min resulted in virtually complete (-92.9±1.0%) inhibition of B50 phosphorylation. This time course is consistent with that required for somatostatin-related peptides to reach maximal binding to membrane sites. Preincubation of the SPM fraction for 30 min in the absence of [D-Trp^8]-SS had no effect on basal protein phosphorylation. Similar inhibition by [D-Trp^8]-SS after 30 min was seen in the presence of 200 μM leupeptin or 1 mg/ml bacitracin, suggesting the loss of B50 phosphorylation was not due to substrate protein degradation during the preincubation. The effect of [D-Trp^8]-SS was compared to that of 10^{-4}M ACTH or β-endorphin. After 15 sec of preincubation ACTH inhibited B50 phosphorylation by -68.5±7.3%. This apparently was a maximal effect, since increasing the preincubation with ACTH to 30 min produced no greater inhibition (-69.9±8.1%). β-endorphin inhibited B50 phosphorylation slightly (-9.3±6.3%) after 15 sec of preincubation. Increasing the preincubation with β-endorphin to 30 min resulted in more inhibition of B50 phosphorylation (-23.8±6.4%), but the % effect was much smaller than that seen in response to [D-Trp^8]-SS. The effect of [D-Trp^8]-SS on protein phosphorylation was also examined in the cytosolic fraction. Preincubation for 15 sec with [D-Trp^8]-SS had no effect on the phosphorylation of a protein of approx. 85,000 daltons. The phosphorylation of an approx. 52,000 dalton band was inhibited by [D-Trp^8]-SS equally after a 15 sec or 30 min preincubation. This evidence suggests that [D-Trp^8]-SS interacts with specific SPM binding sites in the rat hippocampus to produce inhibition of B50 phosphorylation. Supported by BRS Grant 5 SO1 RR 05700 13, NS17118 and AM 18370.

171.5 ^{3}H-BRADYKININ RECEPTOR LOCALIZATION IN SPINAL CORD AND SENSORY GANGLIA - EVIDENCE FOR A ROLE IN PRIMARY AFFERENT FUNCTION. D.C. Manning and S.H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Bradykinin (BK), an endogenous nonapeptide, is a extremely potent algesic substance causing stimulation of small diameter afferent fibres and is often used for experimental production of pain. The great potency of BK suggests that specific BK receptors exist on primary afferent fibres. We have investigated this proposal using ^{3}H-BK receptor autoradiography.

^{3}H-Bradykinin receptor binding sites were localized autoradiographically in nervous tissue of the guinea pig and dog using a modified version of Young and Kuhar's converslip method (Young, W.S. and Kuhar, M.J. Br. Res., 179:255-270, 1979).

Tissues were perfusion fixed with 0.1% formaldehyde/10% sucrose in phosphate buffered saline adjusted to isotonicity. Ten micron frozen tissue sections were cut and incubated for 120 min at 4°C in a media containing 25 mM TES pH 6.8, 1 mM 1,10 phenanthroline, 0.2% BSA 1 mM dithiothreitol, 140 µg/ml Bacitracin, 1 µM captopril (SQ14,225), 300 mM sucrose and 0.2 nM ^{3}H-BK (52 Ci/mmole). Nonspecific binding was defined in the presence of 1 µM unlabelled BK. After incubation the tissue sections were dried rapidly and NTB3 emulsion coated coverslips were apposed and exposed for 3 months.

^{3}H-Bradykinin receptors in the guinea pig spinal cord are concentrated in the dorsal horn in the approximate area of Rexed's lamina I and II. Bradykinin receptors in the dorsal root and trigeminal ganglia of the guinea pig are highly concentrated on a small subpopulation of ganglia cells. Receptors for ^{3}H-BK in the dog stellate ganglia display a streaking pattern consistent with ganglionic fibre tracts.

The nervous system localization of ^{3}H-BK binding sites supports the contention that bradykinin plays a role in pain perception. The dorsal horn localization of sites is coincident with the terminations of primary afferent fibres. The primary afferent nerve fibre role for bradykinin is further supported by a localization of binding sites in the dorsal root and trigeminal ganglia. The ganglia receptors may represent newly synthesized binding sites ready for axonal transport. The stellate ganglion contains the cell bodies of sympathetic afferent fibres that supply the heart. The localization of ^{3}H-BK binding sites to the stellate ganglion may support a role for bradykinin in cardiac afferent function.

171.6 CHOLECYSTOKININ RECEPTORS IN THE STRIATUM AND SUBSTANTIA NIGRA ARE FOUND ON INTRINSIC NON-DOPAMINERGIC NEURONS. P. Gaudreau, R.T. Jensen, S. St-Pierre, C.B. Pert and A. Pert. (SPON: C.T. Bennett). Biological Psychiatry Branch and Clinical Neuroscience Branch, NIMH; Digestive Diseases Branch, NIADDK; Department of Pharmacology, University of Sherbrooke, Sherbrooke, Canada.

Comparative autoradiographic distribution of cholecystokinin (CCK) receptors in rat brain using [^{3}H]pentagastrin or N$^{\alpha}$[^{125}I desaminotyrosyl] CCK-33 has been studied. The two probes showed high densities of CCK receptors in the nucleus accumbens, especially its medial part, moderate levels in the striatum and substantia nigra, and very low levels in the ventral tegmental area. Since these brain areas contain dopamine terminals and cell bodies, respectively, it was of interest to ascertain the precise localization of CCK receptors in relation to mesolimbic and nigrostriatal dopamine neurons.

Nigrostriatal dopaminergic projections were lesioned unilaterally by intranigral injections of 6-hydroxydopamine (6-OHDA) (9 ug). Mesolimbic dopaminergic projections were lesioned unilaterally by injection of 6-OHDA (7.5 ug) into the ventral tegmental area. Unilateral lesions of the medial forebrain bundle (MFB) were also made by injections of 6-OHDA into this fiber bundle at the level of the lateral hypothalamus. Intrinsic neurons in the striatum and substantia nigra (SN) were destroyed by unilateral injections of ibotenic acid (10 ug). One month later the animals were killed and their brains removed. Serial 25 um sections were taken through the forebrain region encompassing the nucleus accumbens and caudate nucleus and the midbrain region encompassing the substantia nigra and ventral tegmental areas. These sections were then exposed to [^{3}H]pentagastrin or N$^{\alpha}$[^{125}I des- aminotyrosyl] CCK-33 and processed for autoradiography using a tritium-sensitive film.

6-OHDA lesions of the SN failed to alter CCK binding in either the ipsilateral striatum or SN. A-10 lesions were also ineffective in this regard. MFB lesions, which produce retrograde degeneration of DA neurons in the zona compacta of the SN, were also ineffective in modifying CCK binding in the SN. Injections of ibotenic acid into the striatum and SN, on the other hand, effectively decreased CCK binding at the site of injection.

Altogether, these results suggest that CCK receptors are localized on intrinsic non-dopaminergic cell bodies in the striatum and SN and that DA terminals in the striatum and nucleus accumbens are devoid of CCK receptors.

171.7 HIGH AFFINITY BINDING OF GLUCAGON TO RAT BRAIN MEMBRANES. N. M. Hoosein* and R. S. Gurd. Medical Sciences Program and Dept. of Chemistry, Indiana Univ., Bloomington, IN 47405.

Glucagon, a 29 amino acid peptide usually found in the pancreas and gastrointestinal tract, has been detected in canine brain extracts by radioimmunoassay (Unger, R. et al., Diabetes 28:700, 1979). Also immunocytochemical studies have localized higher molecular weight forms of glucagon in the rat hypothalamus and thalamus (Tager, H. et al., Proc. Natl. Acad. Sci. U.S.A. 77:6229, 1980, and Loren, I. et al., Histochemistry 61:335, 1979). To determine the possible presence of glucagon receptors in rat brain, in-vitro binding of radiolabeled glucagon to rat brain membranes was investigated.

Mono[^{125}I]iodoglucagon bound to rat brain membranes with high affinity (K_d = 5.0 nM). Regional distribution studies indicated higher specific binding to olfactory tubercule, hippocampus and pituitary membranes with somewhat lower binding to those from medulla, thalamus, olfactory bulb and hypothalamus. Regions whose membranes did not bind significant amounts of radiolabeled glucagon were cerebral cortex, cerebellum, colliculli, striatum and mid-brain. The specific binding per mg. of protein was of the order of that for insulin but differed in distribution (Havrankova, J. et al., Nature 272:829, 1978). Binding assays performed with subcellular fractions showed a greater density of binding sites in the synaptosomal fraction relative to mitochondrial or myelin fractions. Insulin, vasoactive intestinal polypeptide and vasopressin did not inhibit binding of radiolabeled glucagon to lysed P_2 fractions obtained from pituitary gland.

To further characterize brain glucagon binding sites the effects of time, pH, GTP and glucagon analogues were examined. At 30°C, steady state binding was attained within 20 min. at an optimum pH of 6.8 - 7.5. In analogy to the regulation of hepatic glucagon receptors by nucleotides, inclusion of 0.1 mM GTP in the binding assay reduced the binding affinity (K_D = 44.5 nM). Competition assays performed with 3 different glucagon analogues revealed lowered affinities with the order of affinity differing from that for hepatocyte receptors.

These data suggest the existence of receptors in rat brain which bind glucagon with characteristics that may differ from those of hepatic glucagon receptors. (Supported by NIH Grant AM21121.)

171.8 SOLUBILIZATION AND CHARACTERIZATION OF THYROTROPIN-RELEASING HORMONE RECEPTORS FROM RAT BRAIN. W.A. Johnson and A. Horita* (SPON: P. Prinz). Dept. of Pharmacology, University of Washington, Seattle, WA. 98195.

The binding site for thyrotropin-releasing hormone(TRH) was successfully solubilized from rat brain in a stable unbound form. The three amino acid neuropeptide, TRH (pyroGlu-His-Pro-NH_2), has been shown in membrane binding studies using the labeled analogue ^{3}H-(3MeHis^{2})TRH to bind to specific saturable sites in the extrahypothalamic brain of various mammalian species. The natural glycoside, digitonin, in 20 mM TrisHCl, pH 7.4, was the only agent out of a variety of ionic and non-ionic detergents tested capable of solubilizing the binding site for ^{3}H-(3MeHis^{2})TRH in its unbound form. The solubilized site exhibited binding kinetics virtually identical to the membrane preparation and responded with a similar order of potency to a series of TRH-analogues that inhibit binding of the labeled ligand. Gel exclusion chromatography on Sepharose 6B indicated M_r 230,000 for the receptor-digitonin complex. The solubilized preparation was kept frozen at -20°C for nine days with no loss of binding activity, but storage at 4°C for 48 hours resulted in a significant loss of activity.

171.9 EFFECTS OF INTRAVENTRICULAR ANGIOTENSIN II ANTAGONIST INFUSION ON ^{125}I-ANG II BINDING IN RAT BRAIN.
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Our previous studies have demonstrated that brain Angiotensin II (Ang II) receptors are unresponsive to chronic intraventricular (IVT) infusion of [Ile^5] Ang II (Physiologist 25: 297, 1982). Here, we report the effects of IVT infusion of [Sar^1,Ile^8] Ang II, an Ang II antagonist. Male Sprague Dawley rats (350-400 g) were infused with either [Sar^1,Ile^8] Ang II at 500 ng/hr (AIIA, n=10) or 0.9% saline vehicle at 1 µl/hr (SV, n=10) for 6 days using subcutaneously implanted osmotic pumps. Specific ^{125}I Ang II binding to brain and adrenal Ang II receptors was determined at 5 concentrations of ^{125}I Ang II (0.1 nM - 1.0 nM) in the presence and absence of 1 µM unlabeled Ang II. The ^{125}I Ang II receptor binding site density in the hypothalamus-thalamus-septum-midbrain (HTSM) region was 13.1 ± 0.68 fm/mg protein in AIIA rats vs 15.1 ± 1.06 fm/mg protein in SV rats. Although HTSM ^{125}I Ang II binding was decreased by 13.3% in the AIIA group, it was not statistically significant. There was no change in brain ^{125}I Ang II binding affinity. In a separate group of AIIA infused rats we found that the drinking response to centrally injected Ang II (1-2 µg) was blunted through day 7 of infusion, whereas similar injections evoked equal drinking responses in AIIA and SV rats on day 10 when the pumps were spent. In AIIA rats, ^{125}I Ang II binding to the adrenal cortex was significantly reduced (986 ± 151 vs 1972 ± 413 fm/mg protein, $p < 0.05$) with no change in binding affinity. This reduction might be due to leakage of Ang II antagonist into the circulation and subsequent occupation of Ang II receptors in the adrenal cortex. The ^{125}I Ang II binding to the adrenal medulla did not differ between the two groups. Systolic blood pressure, daily water consumption, urine output and urine sodium excretion did not change in AIIA rats as compared to either pre-infusion level or SV rats. Thus, chronic Ang II antagonist infusion did not appear to cause significant alterations in HTSM receptor number or affinity. These results further indicate that brain Ang II receptors do not undergo homologous regulation. Supported by Am. Heart Assn. N.E. Ohio Affil., NIH HL-27568 and NIH HL-6835.

171.10 INFLUENCE OF INTRACEREBROVENTRICULAR Sar^1-Ile^8-ANGIOTENSIN II ON SYSTEMIC ANGIOTENSIN III INDUCED BLOOD PRESSURE CHANGES.
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Investigations utilizing [^{125}I]-Angiotensin II (AII) have indicated significant binding in several circumventricular organs (CVOs) of the rat (Bennett & Snyder, J. Biol. Chem., 251: 7423-7430, 1976; Sirett et al., Brain Res., 166: 139-148, 1979). Our laboratory recently discovered similar high specific binding for [^{125}I]-Angiotensin III (AIII) in rat CVOs (Harding et al., Soc. Neurosci. Abst., 8: 902, 1982). This raised the possibility that AIII or a metabolite, may be the active ligand for centrally mediated blood pressure control. The present investigation initially measured pressor responses to systemically administered AIII in rats. We next attempted to prevent these AIII induced elevations in systemic blood pressure by intracerebroventricular (icv) pretreatment with the specific angiotensin competitive antagonist Sar^1-Ile^8-AII, and several peptidase inhibitors. Previous research has established that icv pretreatment with a functionally equivalent compound, saralasin acetate (Sar^1-Ala^8-AII), prevented the pressor effect of icv infused AII (e.g. Hoffman & Phillips, Brain Res., 109: 541-552, 1976). Presently, Sar^1-Ile^8-AII and several peptidase inhibitors were effective in reducing systemic AIII induced pressor effects.

These results suggest that AIII is a potent peripheral pressor agent, and that its pressor effects can be significantly reduced by icv pretreatment with these compounds. These findings also suggest that the pressor effects previously attributed to AII may be due to its degradation product AIII, or a metabolite.

171.11 EFFECT OF INTRACEREBROVENTRICULAR Sar^1-Ile^8-ANGIOTENSIN II ON INTRACEREBROVENTRICULAR ANGIOTENSIN II AND III INDUCED BLOOD PRESSURE ELEVATIONS.
S.L. Morseth, M. L. Mills*, G. G. Deffenbough*, J.W. Harding, and J. W. Wright. Departments of Psychology and Veterinary Comparative Anatomy, Pharmacology and Physiology, Washington State University, Pullman, WA 99164.

Previous research has demonstrated that intracerebroventricular (icv) pretreatment with the specific angiotensin competitive antagonist saralasin acetate (Sar^1-Ala^8-Angiotensin II), prevented the pressor effects of icv angiotensin II (Hoffman & Phillips, Brain Res., 109: 541-552, 1976). Our laboratory recently discovered high specific binding for [^{125}I]-Angiotensin III in rat circumventricular organs (Harding et al., Soc. Neurosci. Abst., 8: 902, 1982). This raised the possibility that angiotensin III, or a metabolite, may be the active ligand for centrally mediated blood pressure control. In the present investigation pretreatment with icv injections of Sar^1-Ile^8-Angiotensin II and several peptidase inhibitors were effective in blocking the pressure effects induced by icv injections of angiotensin II and angiotensin III in rats. These data suggest that angiotensin III, or a smaller fragment, may be responsible for at least a portion of the centrally mediated pressor effects observed following icv angiotensin II injections.

171.12 LOCALIZATION OF NEUROTENSIN RECEPTORS IN GUINEA PIG BRAIN.
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Recent studies have shown some differences between rat and guinea pig neurotensin (NT) receptors (Quirion et al., Br. J. Pharmacol., 68, 83, 1980; Checler et al., Life Sci., 31, 1145, 1982). Thus, I decided to investigate NT receptor distribution in guinea pig brain using an autoradiographic technique (Herkenham and Pert, J. Neurosci., 2, 1129, 1982), which permits precise anatomical localization of binding sites.

Slide-mounted guinea pig brain sections were preincubated in 50 mM Tris-HCl, pH 7.4, at 4°C for 15 min and then incubated in the same buffer plus 0.5 mg/ml bacitracin, 0.1% BSA, and 4.0 nM [^{3}H]NT at 4°C for 60 min. At the end of the incubation, slides were transferred sequentially through 4 rinses (2 min in each) of cold incubation buffer. Specific binding was calculated as the difference in cpm bound in presence and absence of 1.0 µM NT. Autoradiograms were generated by placing slides against tritium-sensitive film in cassettes for at least 8 wks.

In preliminary experiments, saturation curves and Scatchard analysis show that [^{3}H]NT binds to an apparent single saturable class of receptor (K_D = 4.7 nM) in guinea pig brain. Structure-activity studies reveal a ligand selectivity pattern similar to rat brain (Quirion et al., Peptides, 3, 757, 1982), except for [Trp^{11}]NT which is at least 10 times weaker in guinea pig brain. These results confirmed those obtained by Checler et al. (Life Sci., 31, 1145, 1982) using brain membrane preparations.

The autoradiographic distribution of [^{3}H]NT binding sites in guinea pig brain is similar to the one observed in rat brain (Young and Kuhar, Brain Res., 206, 273, 1981, Quirion et al., Peptides, 3, 757, 1982). For example, high densities of [^{3}H]NT binding sites are found in the external plexiform layers of the olfactory bulb, cingulate cortex, medial and cortical nuclei of amygdala, substantia nigra, pars compacta, and ventral tegmental area. Low densities are observed in caudate-putamen and nucleus accumbens. However, some difference in [^{3}H]NT receptor distribution between rat and guinea pig brain are noted. In guinea pig brain, layer I of cortex and dendate gyrus contain more [^{3}H]NT binding sites than rat brain. Also, very little [^{3}H]NT binding is observed in lateral septum and zona incerta of guinea pig compared to rat brain. In summary, [^{3}H]NT binding pharmacology and autoradiographic localization appear to be slightly different in guinea pig brain as compared to rat brain.

172.1 SPONTANEOUS DISCHARGES IN RAT SENSORY NEURONS CAUSED BY HERPES SIMPLEX VIRUS. P. H. Lima*, G. Anderson-Beckman*, D. J. Forbes, and R. S. Pozos. Depts. of Physiology and Anatomy, Univ. of Minn., Duluth, MN 55812.

Sensory neurons in dissociated cell cultures prepared from embryonic rat dorsal root and trigeminal ganglia were infected with herpes simplex virus (HSV). Changes in the electrical properties were examined by intracellular electrophysiological techniques. The most prominent change is the appearance of repetitive spontaneous discharges in many of the neurons by 10-12 hours post infection. Initially the discharges are small, 5-10 mV spikes which occur at low frequency (20-30/min). Gradually both the frequency and amplitude of the spikes increase. At the maximum discharge rate, full sized action potentials with amplitudes averaging -60 to -70 mV occur at a frequency of 3-4/sec.

This spontaneous activity was associated with infections by both HSV type 1 (HFEM strain) and type 2 (R2 strain). The R2 was somewhat more virulent than the HFEM. Cultures infected with R2 at a concentration of 10^5 pfu rarely survive longer than 30 hours while those infected with HFEM (10^5 pfu) typically survived at least 60 hours. Spontaneous firing could be recorded as long as the neuron was able to maintain a resting membrane potential of at least -35 to -40 mV.

HFEM is the wild parent strain for tsB5, a temperature sensitive mutant deficient in a glycoprotein required for the virus to penetrate through the cell membrane. HFEM grown at 34° and 38.5°C as well as the tsB5 grown at 34°C all cause similar spontaneous activity but tsB5 grown at 38.5 did not cause any spontaneous activity or other changes in the electrical properties of the neurons.

The antiherpetic drug acyclovir (ACG) inhibits HSV by interfering with the HSV DNA polymerase preventing viral DNA replication. Cultures were first infected with the virus and then continuously treated with ACG (20-40 μM). In most of the treated neurons there was no spontaneous activity and the cells demonstrated normal action potentials for up to 60 hours. In approximately 5% of the R2 infected neurons there was some spontaneous firing. Spontaneous discharge would appear in all the cultures 10-12 hours after removal of the ACG.

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172.2 PRESERVATION OF CATECHOLAMINE UPTAKE AND RELEASE IN HERPES SIMPLEX VIRUS TYPE 1-INFECTED PC12 CELLS. R. Rubenstein* and R.W. Price. Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

Although the peripheral nervous system plays a critical role in the ecology of herpes simplex virus type 1 (HSV-1) infections and the central nervous system is a target of severe HSV-1 infection, little is known of the effects of infection on neuronal cell metabolism other than eventual cell death. To address this issue we have undertaken a series of studies evaluating the effects of HSV-1 infection on "specialized" neuronal functions using the PC12 cell as a model system. We now report the effects of infection on catecholamine uptake, content and K^+-stimulated release following inoculation of the PC12 cells with the F-strain of HSV-1. During a 24-hour period of productive infection we found little alteration in these aspects of catecholamine metabolism despite production of viral progeny and marked morphological cytopathology. Thus, uptake of ^{3}H-norepinephrine paralleled that of mock-infected cultures, and in both cases the specificity of this uptake was confirmed by desmethylimipramine inhibition. Similarly, throughout the course of infection, endogenous dopamine and norepinephrine stores evaluated by thin-layer chromatography did not differ between HSV-1-infected and mock-infected cells. Likewise, content of these amines was reduced to a similar extent on exposure of infected and uninfected cells to 51.5 mM KCl added to the medium.

These studies reveal a remarkable preservation of catecholamine uptake, content and release despite conversion of PC12 cells to virus production, suggesting that neurons may retain a measure of functional capacity for an unexpectedly prolonged period during the course of viral infection.

172.3 UNCONVENTIONAL PROPERTIES OF PAIRED HELICAL FILAMENTS IN HUMAN NEURONS IN AGING AND ALZHEIMER'S DISEASE. D. J. Selkoe, C. Abraham*, L. K. Duffy* and C. G. Rasool. Harvard Medical School, McLean Hospital, Belmont, MA 02178.

The principal ultrastructural change of human cerebral neurons during normal aging and in Alzheimer's disease is the formation of helically wound pairs of intermediate filaments, known as paired helical filaments (PHF), in many cell bodies and neurites. We have found that PHF remain insoluble and structurally intact in sodium dodecyl sulfate, urea and other conventional protein solvents. Further studies now demonstrate that certain strongly chaotropic salts, viz. guanidine thiocyanate (7M) and lithium bromide (10M), can denature PHF conformation so that it is no longer recognizable by electron microscopy while apparently failing to depolymerize the fibers and release soluble polypeptide subunits. Furthermore, the insoluble fibrous material remaining after such harsh solvent extraction can still be immunolabeled by rabbit antibodies raised against intact PHF; these antibodies are highly specific for PHF and fail to react with neurofilaments or any other native brain cytoskeletal proteins. PHF also display considerable resistance to digestion by specific and nonspecific proteases (e.g., trypsin, pepsin, pronase). Similarly, the fibers are not significantly digested (as judged by gel electrophoresis) and retain their reactivity with αPHF antibodies following cyanogen bromide cleavage in 70% formic acid. The specificity of our αPHF antibodies recommends them for immunoaffinity purification of PHF to homogeneity, a prerequisite to breaking them down by limited acid hydrolysis and elucidating their composition and origin. Initial results using this strategy will be presented.

Our results to date indicate that the paired helical filaments which accumulate progressively with age in selected human neurons are unconventional, highly stable macromolecules that (1) contain determinants which are highly modified from normal neuronal proteins and (2) are held together by strong noncovalent interactions and apparently by covalent crosslinks, properties without precedent among known neuronal proteins.

172.4 EFFECTS OF EXPERIMENTAL SUBARACHNOID HEMORRHAGE (SAH) ON THE MORPHOLOGY AND METABOLITE UPTAKE IN THE RABBIT CHOROID PLEXUS T.M. Liszczak, P.McL. Black, L. Foley,* A. Tzouras,* Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, Mass. 02114. Autologous nonheparinized blood (0.5 ml) was injected into the cisterna magna of anesthetized adult rabbits on days, 1, 7, and 14. Control animals had injections of saline or artificial CSF but were otherwise treated identically. The animals were sacrificed 7 days after the last blood injection. Isotopes 0.5 ml. of 14C linoleic acid specific activity of 900 mCi/mmol and 0.2 ml. 3H leucine specific activity of 158 ci/mmol were injected into the cisterna magna in 9 animals. Two hours later the animals were sacrificed by aldehyde perfusion and tissues were examined for isotope incorporation by scintillation counting. The choroid plexus from 15 different animals using the same experimental protocol was examined by scanning and transmission electron microscopy. Linoleic acid incorporation was reduced in all tissue after SAH. The results expressed as nanomoles linoleic acid incorporated/gram dry weight before and after SAH are: basilar artery from 1731 ± 123 to 148 ± 14, arachnoid from 1079 ± 3 to 100 ± 8, optic nerve from 24 ± 3 to 1 ± 0, choroid plexus from 417 ± 71 to 2 ± 0, frontal gray matter from 12 ± 2 to 0, and the frontal white matter from 1 ± 1 to 2 ± 0. Leucine incorporation was significantly increased in basilar arteries from 2268 ± 103 to 8292 ± 1011 nanomoles/gram dry weight and choroid plexus from 42 ± 4 to 244 ± 22 after SAH, ($p<0.01$, 2 tailed t test). The leucine incorporation rates for the other tissues remained similar after SAH: arachnoid from 1170 ± 74 to 1124 ± 18, optic nerve from 75 ± 5 to 40 ± 1, frontal gray from 37 ± 4 to 95 ± 15, and frontal white matter from 114 ± 12 to 349 ± 34. Pathological changes were observed in 50% of the animals. Dilation of the choroid plexus lateral and basal intercellular spaces were seen in 25% and erythrocytes in lysosomes were present in the choroid epithelium of 50% of the experimental animals when compared with controls.

172.5 RECOVERY OR MALFORMATION (HYDROCEPHALUS) FOLLOWING X-IRRADIATION: NEUROEPITHELIAL-MESENCHYMAL BASAL LAMINA RELATIONS. R. A. Glover*, C. J. D'Amato and S. P. Hicks. Depts. of Anatomy and Cell Biology, and Pathology, Univ. of Michigan Med. Ctr., Ann Arbor, MI 48109.

We showed earlier that 6-14 somite-pair rat fetuses recover substantially from devastating cell-killing by 150R rivaling amphibia in regulative regenerative capacity, and also demonstrated that 35 somite-pair fetuses sometimes recover so well as to be indistinguishable as adults from normals (D'Amato 1982). We now report that 16-24 somite-pair fetuses (11th day) recover remarkably well after 150R, with substantial regeneration accomplished in 12-18 hours. Maximum regulation is attained by 48-72 hours, but complete success is marred by eye defects. Fetuses are removed by successive caesarian sections for light and electron microscopy. Regulation failed after 225R given on the 11th fetal day, characterized by late fetal stenosis of the aqueduct with hydrocephalus due to thickening of the midbrain-thalamic junction (MTJ) (D'Amato, Hicks 1981). Because a mutant rat also displays stenosis with hydrocephalus, which we attribute, in part, to an incomplete basal lamina (BL) between the MTJ neuroepithelium and mesenchyme appearing around the 11th fetal day (Glover, D'Amato, Hicks 1983), we explored whether a similar situation occurred in 225R 11th day fetuses. Several hours after 225R, the BL of the MTJ showed discontinuities, allowing contact between neuroepithelium and mesenchyme. By 24 to 48 hours extrusions of neuroepithelium into the mesenchyme developed leading to minute neural ectopias, as in the mutant. Focally disturbed orientation of neuroepithelial cells lining the MTJ ventricle also occurred. In contrast, there was no disturbance of the BL in 150R 11th day fetuses, despite cell-killing. We conclude, with Hay (1982), that the integrity of the BL is crucial to normal development and, in our experiments, appears essential for regulative success of the neuroepithelium and mesenchyme. The precise mechanisms involved in the two forms of failed regulation characterized by the aqueduct stenosis syndrome are unknown; they take on added interest because B12 and folate deficiency also produce the syndrome (Newberne, O'Dell 1961). USPHS 10531, Univ. of Michigan Biomedical Research Council 2S07RR05383-22.

172.6 IMMUNE DEVELOPMENTAL PATHOLOGY OF THE FRONTAL CORTEX AND SELECTED AREAS OF THE VISUAL SYSTEM FOLLOWING LCMV INFECTION. M.F. Kritzer and M. del Cerro (SPON: J. Ison). Center for Brain Research, University of Rochester, Rochester, N.Y. 14642.

Lymphocytic choriomeningitis virus (LCMV) induces autoimmune disorders in the nervous and sensory systems of mice and rats. The present study concerns histopathological changes in the frontal cortex, the superior colliculus, and the lateral geniculate nucleus of developing rats neonatally infected with the virus. Preliminary results indicate a characteristic evolution of pathology depicting non-specific and specific immune responses in these central nervous system regions.

In early development (10 post-natal days (PND)) marginated monocytes in superficial vessels and activated macrophages in meninges and superficial neuropil are evident. From that time on, macrophages from the meninges infiltrate the superficial neuropil and continue migration in a ventro-medial direction. In deeper neuropil, these cells become satellite to neuronal somas. Macrophages then migrate to the ventral limits of the structures and appear to exit brain tissue via the ventricular system. As development continues, fewer macrophages enter the regions, neuronal-macrophage contacts are lost, and ventral migration of these cells increases. With this waning of macrophage involvement, a concommitant increase in plasma cell populations is observed. Plasma cell distributions display an evolving pattern similar to that of macrophages found earlier, as an apparent site for site replacement of macrophage populations by plasma cells takes place.

One interpretation of data depicts LCMV associated pathology in structures studied as being representative of an immune reaction within the brain to the spreading of surface and cytoplasmic neo-antigens of viral origin. The LCMV disease offers a safe, and highly reliable animal model for the study of inflammation in the developing brain.

Supported by National Eye Institute grant EY 02632.

172.7 OPTIC NERVE CHANGES IN THE SPONTANEOUSLY HYPERTENSIVE RAT. R. L. Bondar* (SPON: J. Fullard). Div. of Neurology, McMaster Univ. Med. Ctr., Hamilton, Ont., Can. L8N 3Z5.

Hypertension frequently has been found in association with idiopathic ischemic optic neuropathy (ION). Human clinical pathological correlation is lacking in these "non-arteritic" forms. Alterations in optic nerve morphology-physiology in genetic models of hypertension have received little investigation to date. The present study was initiated to assess morphological changes in blood vessels in the optic nerve of spontaneous hypertensive rats and to document pathological changes in the optic nerve secondary to altered vascular supply. Spontaneous hypertensive rats (SHR) 28 weeks old and age-matched Wistar Kyoto (WKY) rats were perfused through the carotid artery with glutaraldehyde and paraformaldehyde following a vascular rinse under nembutal anesthesia. The optic nerves were removed, post-fixed with osmium tetroxide and processed for electron microscopy. Toluidene blue stained 1 μ sections and silver-gold thin sections were analyzed. Blood vessels within the septa of the optic nerve of SHR rats showed a marked increase in cross-sectional diameter, with changes in the endothelial cells, thickening of the basement membrane and accumulation of collagen. Infarction of the optic nerve was present around several of the severely affected blood vessels. These changes were not present in the control rats. At least in this genetic model of hypertension, optic nerve infarction can occur as a consequence of alterations in the vascular supply of the optic nerve.

172.8 THE CHRONIC EFFECTS OF METHYLMERCURY ON THE INTRAMUSCULAR NERVES AND MOTOR ENDPLATES OF THE EXTENSOR DIGITORUM LONGUS MUSCLE OF THE RAT. R.K. Yip and D.A. Riley. Dept. of Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226.

Methylmercury (MeHg) is a potent neurotoxin. Due to the severity of the neurological symptoms, many studies have focused attention on the central nervous system (CNS) as the primary target after MeHg exposure. The peripheral nervous system (PNS) has not been thoroughly investigated. Ataxia and paretic gait observed in humans and experimental animals after MeHg poisoning are generally attributed to the lesions in the cerebellar cortex. Recent electrophysiological studies revealed that MeHg inhibits neuromuscular activities, raising the possibility that the ataxia and paresis are due in part to neuromuscular dysfunctions. The present study sought to examine the effects of chronic MeHg treatment on the motor and sensory axons of mammalian skeletal muscle.

Six male Sprague-Dawley rats (175-200 g) were gavaged daily for approximately six weeks with an aqueous solution of methylmercuric chloride (2 mg/kg body weight). Controls received equal volumes of 0.9% saline solution. When the MeHg-treated rats were sacrificed, they exhibited abnormal crossing reflexes of their hindlimbs, ataxia, and paresis. Intramuscular nerves and extrafusal motor endplates were examined in longitudinal sections (100 μm) of extensor digitorum longus (EDL) muscles stained with a combined acetylcholinesterase (for extrafusal motor endplates) and silver impregnation technique (for nerve fibers).

In the MeHg-treated animals, coagulative degeneration was found in 16% of the extrafusal motor endplates (n=1000/muscle). In these animals, nerve fiber fragmentation and axonal debris were found in 22% of the I_a afferents while frank necrosis was observed in 90% of the I_b afferents. Moreover, there were varying degrees of fiber loss and degeneration within the intramuscular nerve bundles of EDL from the intoxicated animals. All of the aforementioned changes were absent in the controls.

Present results demonstrated that chronic MeHg administration caused degeneration of the intramuscular motor and sensory axons. These changes could explain the ataxia and paresis associated with MeHg poisoning as impairment of neuromuscular function rather than attributing these deficits solely as a result of the CNS lesions.

This work is supported by NASA Ames University Consortium NCA-OR665-102.

172.9 THYROID STATUS IN AN INHERITED CANINE NEUROMUSCULAR DISORDER ACCOMPANIED BY TYPE II SKELETAL MUSCLE FIBER DEFICIENCY.
G. A. Hegreberg, M. J. Hamilton*, R. H. Abhold*, and J. A. Magnuson*. Depts. Veterinary Microbiology and Pathology and Chemistry, Wash. State Univ., Pullman, WA 99164.

Several parameters were examined to evaluate thyroid status in a genetically transmitted neuromuscular disorder of Labrador retriever dogs which is characterized by muscle weakness and atrophy, intolerance to exercise and cold, loss of glycolytic fibers from skeletal muscle, and an autosomal recessive mode of inheritance. Total and free T3, reverse T3, total and free T4, and total serum levels of cortisol, cholesterol, calcium, and lactate dehydrogenase (LDH) were measured on 14 adult affected dogs (7 males and 7 females) and 7 adult nonaffected dogs (4 males and 3 females). Total and free levels of both T3 and T4 were measured by radioimmunoassay methods in fasting serum. Reverse T3 was measured by radioimmunoassay in plasma. Free T3, reverse T3, and free T4 levels were significantly elevated in the affected dogs as compared to the nonaffected dogs (free T3, affected - 305 pg%, nonaffected - 205 pg%; reverse T3, affected - 0.41 ng/ml, nonaffected - 0.24 ng/ml; free T4, affected 1.73 ng%, nonaffected - 1.02 ng%). No significant differences were found in total T3 and total T4 levels comparing affected with nonaffected dogs. Total LDH was significantly elevated in the affected dogs as compared to the nonaffected dogs (affected - 127 IU/dl, nonaffected - 80 IU/dl). Total serum cholesterol was also significantly elevated in the affected dogs as compared to the nonaffected dogs (affected - 231 mg%, nonaffected - 164 mg%). No significant differences were found in the total serum calcium and cortisol levels comparing affected dogs with nonaffected dogs.

Muscle atrophy, weakness, and cold intolerance are often associated with hypothyroidism. Elevated serum cholesterol and serum LDH are also indicators associated with a hypothyroid state. On the other hand, the elevated free T3, reverse T3, and free T4 levels in the affected dogs indicate that they are hyperthyroid. The dichotomous results suggest an enhanced thyroid hormone production but improper utilization of the hormone with the subsequent clinical appearance of a hypothyroid state. Current studies are underway to further define the role of the thyroid in this disorder.

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172.10 HYPOPHYSECTOMY-INDUCED GROWTH RETARDATION MITIGATES THE PREVALENCE OF SKELETAL MUSCLE FIBER NECROSIS IN GENETICALLY DYSTROPHIC HAMSTERS. G. Karpati, P. Jacob*, S. Carpenter* and S. Prescott*. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

Peripheral nerve section and high thoracic cordotomy performed in the prenecrotic stage prevent muscle fiber necrosis in the appropriate muscles of genetically dystrophic hamsters (Muscle & Nerve 5: 369, 1982.). We have suggested that these procedures cause an immature state of muscle fibers in which the necrotizing effect of the dystrophic gene is negated, possibly by the persistence of a fetal isoenzyme or isoprotein which is functionally equivalent to the product of the gene that is the site of the mutation. We have created impaired growth (and presumably immature state) of muscle fibers in dystrophic hamsters by growth hormone deficiency induced by surgical ablation of the pituitary at 22 days of age. Corticotropin and thyroid stimulating hormone were injected daily into these animals. Sham-operated dystrophic hamsters served as controls. At 33 and 45 days, the prevalence of centronucleated fibers (which is a valid cumulative index of all necrosis that had taken place) was found to be markedly reduced in quadriceps muscles of effectively hypophysectomized dystrophic hamsters as compared to controls (5% and 12.5% versus 23% and 60%). In those hypophysectomized animals whose growth retardation was only partial (as ascertained by several indices including length of femurs) the prevalence of centronucleated fibers appeared directly proportional to growth. These experiments indicate that mitigation of the necrotizing effect of the dystrophic gene can be achieved without apparent reduction of the mechanical activity of muscles. This avenue may be exploited therapeutically in human muscular dystrophy.

172.11 INTERACTIONS BETWEEN MACROPHAGES AND MOUSE CNS MONOLAYER CULTURES AFTER NEURITE AMPUTATION VIA LASER MICROBEAM SURGERY.
C.R. Gardner* and G.W. Gross, Dept. of Biology, Texas Woman's University, Denton, TX 76204 (SPON: J.F. Hines).

We have cocultured macrophages with neurons and glia to investigate interactions of those cells after neuronal or glial injury. Mouse macrophages were isolated via peritoneal lavage and seeded on established embryonic mouse spinal cord cultures grown on glass coverslips. Within 2 hours the macrophages were firmly established on the substrate but produced no obvious morphological damage within a seven day observation period. Cell process transections were carried out at 337nm either with single 12ns shots from a nitrogen laser at 4-6$\mu J/\mu m^2$ or multiple shots at 4Hz and energy densities ranging from 1-3$\mu J/\mu m^2$. The latter technique resulted in a slow pinching of the process followed by transection or recovery, depending on the cumulative energy density applied (1). The minimum focus diameter of the laser beam was 2.2μm. Preliminary data indicate that many macrophages within 25μm of a transection site will migrate to the site of the lesion and begin to phagocytize the process. Within minutes, other macrophages in the vicinity of the target process will approach and attack seemingly intact segments of the process both proximal and distal to the actual lesion. Maximal response distances of 50μm have been observed. Initial response times from the time of lesion to detected macrophage movement towards the lesion range from 30sec. to 5min. The length of time of attack for any given macrophage is based partially on its size and can last between 15 and 45min. Thereafter, the macrophage retracts and enters a refractory period during which it is unable to respond to another lesion in the vicinity. Macrophage attack has also been observed after process lasing that resulted in cytoplasmic pinching without actual process transection. This indicates that a chemotactic substance may be released during cytoplasmic contraction at the laser impact site even though the process may not transect and may actually recover to its original diameter. Furthermore, macrophages also show interest in beading processes at locations (along the process) of 100 to 200μm from the actual lesion. It appears that quantitative data on neuron-macrophage interaction can be gathered in culture after precise laser-induced lesions. Such systems could represent simple models for CNS trauma and may help determine if macrophages interfere with cell recovery from injury.

1. Gross, Lucas and Higgins. J.Neurosci., 1983, (in press).

172.12 HISTOCHEMICAL STUDIES OF PHOSPHATASE ACTIVITIES IN NERVES UNDERGOING WALLERIAN DEGENERATION. G. Szumanska*, A. Vorbrodt*, H. M. Wisniewski* and R. M. Gould. Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314.

Wallerian degeneration of peripheral nerves includes the destruction of myelin sheaths surrounding degenerating axons. This destruction includes the breakdown of myelin into ovoids and the digestion of myelin lipids and protein components. In order to characterize the sites where hydrolytic activity is increasing, we are using histochemistry with water soluble phosphate-containing substrates, including some related to the breakdown of myelin phospholipids. We are comparing the hydrolysis of phosphorylcholine, myo-inositol 2-phosphate, and cytidine 5'-monophosphate with other substrate commonly used in histochemical studies, β-glycerophosphate, inosine diphosphate, thiamine pyrophosphate, adenosine monophosphate, and pyridoxyl phosphate.

The sciatic nerves of adult rats were crushed and ligated. After 4, 24 or 72 hrs, the rats were perfused with 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer pH 7.3. The degenerating nerve and contralateral control were removed and left in fixative for 90 min, before storing overnight (4°C) in cacodylate buffer. The reactions were carried out for 1-3 hrs on longitudinally-sectioned (40 μ) pieces of nerve. Saponin (0.025%) was included in some incubations and lead was the primary trapping agent. Cerium was used in a few studies. Liver, brain and dorsal root ganglia were used in some experiments to test enzyme activities, and incubations without substrate were included as controls.

Our results so far demonstrate that some but not all activities appear or are enhanced in degenerating nerve compared with control. Among those that appear are acid phosphatase for β-glycerophosphate and cytidine monophosphate, but not phosphorylcholine (results with myo-inositol 2-phosphate are equivocal). Activities that are enhanced include alkaline phosphatase (β-glycerophosphate), inosine diphosphatase, thiamine-pyrophosphatase and 5'-nucleotidase (adenosine monophosphate). We plan to report the localization of these activities at the light microscopic level in teased fiber preparations and in electron micrographs.

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173.1 GLYCEROL INHIBITS THE SYNTHESIS OF THE 71 KILODALTON "STRESS PROTEIN" IN BRAIN SLICES. F.P. White, S.R. White, and R.W. Currie*. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland. A1B 3V6

Telencephalon slices, incubated in vitro, synthesize a 71 kilodalton protein which can constitute up to 20% of the newly synthesized protein produced by the slices. This protein has been referred to as a 'stress protein' (SP71) because, although it is not normally present in mammalian brain or most other organs in vivo, it is induced in vivo following stresses such as hyperthermia and ischemia, as well as beng induced when brain or other organs are sliced and incubated in vitro (White & Currie, in Heat Shock From Bacteria to Man, ed. Schlesinger et al, 1982). In rat brain, SP71 synthesis in response to hyperthermia or slicing appears to be restricted to endothelial cells of the microvasculature.

We are currently investigating the effects of various manipulations of the brain slice incubation media in an attempt to determine what factors influence SP71 synthesis in stressed tissue. Salicylate (10 mM) nonspecifically inhibits the synthesis of all proteins including SP71. Altering concentrations of ionic gradients, known to be important as cellular signals (Ca^{++}, Na^{+}, H^{+} and K^{+}), and drugs which inhibit those systems (papaverine, chlorpromazine, and trifluoperazine), altering levels of cyclic nucleotides (caffeine, dBcAMP, dBcGMP and theophylline), and addition of anti-inflammatory agents (dipyridamole, ibuprofen, indomethocin and PGI_2) had little or no specific effect on SP71 synthesis. However, 1M glycerol specifically inhibited SP71 synthesis in brain slices. Glycerol (1M) also prevents induction of SP71 in rat embryo cell cultures stressed with cadmium, heat, or canavanine (S. Whelan & L. Hightower, personal communication). Interestingly, glycerol has been used to treat cerebral edema. It is possible that there is a relationship between this therapeutic effect of glycerol and glycerol's ability to prevent induction of SP71 in stressed tissue. We are currently examining whether intraperitoneal injections of glycerol can inhibit the synthesis of SP71 in response to hyperthermia. (This research is supported by grants from the Canadian Heart Foundation and MRC (Canada)).

173.2 PROTEINS OF THE BRAIN EXTRACELLULAR FLUID (ECF): EVIDENCE FOR RELEASE OF S-100 PROTEIN INTO ECF OF RAT HIPPOCAMPUS. B. W. Moore*, V. E. Shashoua and G. W. Hesse*. (SPON: A. Pope). Dept. of Biol. Chem., Harvard Med. School, Mailman Res. Center, McLean Hospital, Belmont, MA 02178, & Dept. of Psych., Pharm. and Anat., Washington Univ., School of Medicine, St. Louis, MO 63110

S-100 is an acidic brain protein that is highly localized in a certain class of astrocytes. It has been reported to bind to synaptic membranes in vitro. Such a property, if it occurs in vivo, requires the availability of S-100 in the extracellular fluid. We have devised a procedure for isolating proteins from the brain extracellular fluid (Neurochem Res. 6:1129, 1981; J. Neurochem. 40:1448, 1983). The method uses isotonic media to selectively extract ECF proteins from perfused brains at 0°C without disrupting the integrity of the tissue. The use of the low temperature conditions avoids leakage of cytoplasmic proteins into the extracts from which the cytoplasmic fraction marker, tyrosine hydroxylase, is virtually absent.

Electroblots of ECF proteins isolated from rat brain hippocampus were found to contain a single staining band with antiserum to S-100. Immunoelectrophoresis and RIA measurements indicate that ECF contains about 2.5 µg S-100/mg protein. This is 5-fold higher than the S-100 protein content of the cytoplasmic fraction and suggests that the protein may be released into the ECF.

Direct confirmation of this possibility was obtained in studies of protein release from viable slices of rat brain hippocampus in vitro. Superfusion of the slices results in a release of about 2 µg protein/mg tissue/hr. That the released proteins were not due to products of cell lysis was established by assays of the cytoplasmic enzyme marker, lactate dehydrogenase. Using an ELISA procedure and specific antisera for S-100, the superfusion fluids were found to contain 4.1 µg S-100/mg protein (the cytoplasmic fraction of the tissue had 0.8 µg S-100/mg protein). Thus, S-100 in the released fraction is again 5-fold higher than that retained in the tissue cytoplasm.

ELISA analyses show the presence of 1.8 µg S-100/mg protein in rat brain CSF, indicating that in vivo release of the protein also occurs. This result is of the same order of magnitude as that obtained by RIA of ECF and the in vitro studies of protein release. These findings suggest that S-100 is normally released into the brain extracellular fluid and raise the possibility that its functional role in the CNS may involve loci other than the site of its synthesis. (This research was supported by NINCDS grant No. 09407.)

173.3 PROTEIN RELEASE FROM HIPPOCAMPUS IN VITRO. G. W. Hesse* and V. E. Shashoua (SPON: G. Hauser). Dept. of Biological Chemistry, Harvard Medical School, Mailman Research Center, McLean Hospital, Belmont, MA 02178

There is growing evidence that the proteins released into the extracellular spaces of the CNS may have a variety of significant roles in brain function. We have begun to investigate this process in mammalian brain using the hippocampal slice preparation as a model system. Using physiologically viable slices of rat hippocampus we find that, after an initial rinse and recovery period, protein release from unstimulated slices is relatively constant for several hours at about 2 µg/mg tissue/hr. Assays of a cytoplasmic marker enzyme (lactate dehydrogenase) indicate that this material is not the result of cell lysis.

Pulse-chase experiments using [^{3}H]valine indicate that a substantial fraction of the newly synthesized proteins eventually appears in the incubation medium (18.7% ± 3% of the total TCA precipitable radioactivity during a 6-hr superfusion) and that the releasable protein pool has an apparent half-life of about 4 hr. This apparently rapid rate of export is comparable to export rates reported for proteins released from various cell cultures derived from nervous system tissue.

Simultaneous labeling of newly synthesized proteins with [^{3}H]-fucose and [^{14}C]valine showed a 3-fold higher ratio of [^{3}H]fucose to [^{14}C]valine in the released protein fraction in comparison to the soluble cytoplasmic fraction and the crude membrane fraction of the tissue. This suggests that the soluble released fraction is more highly glycosylated than either the soluble or membrane proteins of the tissue.

Electrophoretic patterns on SDS-polyacrylamide gels show differences between the soluble cytoplasmic proteins of the tissue and the released proteins. While the released fraction contains a broad spectrum of molecular weights, several bands between 42 kD and 68 kD appear to be characteristic of the released protein fraction.

These results suggest that a distinct group of proteins and glycoproteins exists in hippocampal tissue which is destined to be selectively released into the extracellular space. (This research was supported by NINCDS grant No. 09407.)

173.4 MODULATION OF BRAIN PROTEIN PHOSPHORYLATION BY THE S-100 PROTEIN. J. Patel.* and P. J. Marangos. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

The S-100 is an acidic protein localized primarily in the nervous system of mammals. Although S-100 protein has been implicated in the process of learning and memory, little is known of its interactions at a biochemical level. The S-100 protein characteristics of particular interest are that it shares a number of properties with calmodulin, including similarities in amino acid sequence and the ability to specifically interact with calcium. In this report, we have investigated the effect of the S-100 protein on the phosphorylation of brain proteins. Our results demonstrate the S-100 protein to be an effective inhibitor of phosphorylation of a number of proteins. The most potent effect exhibited by S-100 was on the phosphorylation of a protein having a molecular weight of 73,000. S-100 concentrations of 5 µg/ml caused significant inhibition of the phosphorylation of this protein. The S-100 mediated inhibition of the 73,000 molecular weight protein was strictly dependent on the presence of calcium. The phosphorylation of the 73K protein was not cyclic AMP, calmodulin or calcium dependent and was found most predominantly in the cytosolic fraction of the nervous system. More recent studies lead us to believe that the inhibitory effects of S-100 protein are widespread, affecting not only the phosphorylation of cytosolic protein, but also potently inhibiting the phosphorylation of a number of membranal proteins. The data suggest that the putative actions of the S-100 protein may involve a calcium dependent modulation of phosphorylation of selected brain proteins. This finding should serve to provide much needed direction for future studies of S-100 function.

173.5 EFFECTS OF PROTEIN CARBOXYLMETHYLATION ON (α^{32}P)-8-AZIDO-ATP BINDING IN RAT BRAIN. Melvin L. Billingsley, Paul A. Velletri*, Donald M. Kuhn, Robert A. Levine and Walter Lovenberg. Section on Biochemical Pharmacology, NHLBI, NIH, Bethesda, MD 20205.

Cytosol prepared from rat brain exhibits Ca^{++}-Calmodulin (CaM)-dependent protein kinase activity. Our laboratory has investigated the possibility that carboxylmethylation of Ca^{++}-CaM protein kinase by the enzyme protein-O-methyltransferase (PCM; E.C. 2.1.1.24) modulates Ca^{++}-CaM-dependent phosphorylation. Using purified PCM and 100 μM S-adenosylmethionine (AdoMet), we found that methylation of whole brain cytosol reduced subsequent Ca^{++}-CaM-dependent protein phosphorylation. Since CaM itself is methylated by PCM, we depleted whole brain cytosol of CaM using chromatography over fluphenazine-Sepharose or DEAE-Sephacryl. CaM-depletion was determined using SDS-gel electrophoresis. CaM-deficient cytosol was incubated with PCM and AdoMet (100 μM), and the reaction stopped with 100 μM S-adenosylhomocysteine, a potent competitive inhibitor of PCM (K_i = 1.0 μM). (γ^{32}P)-ATP (5 μM) was added to this reaction, along with native CaM (1-5.0 μg) and Ca^{++} (150 μM), to initiate phosphorylation. CaM-depleted cytosol phosphorylations were inhibited by carboxylmethylation, presumably due to the inactivation of protein kinase as other factors participating in the phosphorylation reaction. We utilized the photoaffinity probe [α^{32}P]-8-azido-ATP to examine possible effects of carboxylmethylation on ATP binding in the protein kinase preparations. CaM-depleted and whole brain cytosol had several prominent bands that bound the photoaffinity label. Exposure of these cytosolic preparations to carboxylmethylating conditions significantly inhibited the binding of [α^{32}P]-8-azido-ATP to various proteins with a prominent reduction in a 49,000 dalton protein common to all preparations. Further purification of cytosol by chromatography over CaM-Sepharose yielded a preparation highly enriched in Ca^{++}-CaM-induced protein kinase activity. This preparation contained one band (M_r = 49,000) that bound [α^{32}P]-8-azido-ATP. Carboxylmethylation of this specific CaM-binding fraction inhibited the binding of the photoaffinity probe to the 49,000 dalton protein. Thus, this 49,000 dalton protein may be a subunit of Ca^{++}-CaM protein kinase, and its state of activity may be regulated by carboxylmethylation.

173.6 ENZYMATIC DETERMINATION OF SUBPICOMOLE QUANTITIES OF PHOSPHO-L-ARGININE AND ADENOSINE 5'-TRIPHOSPHATE IN AXOPLASM. E.M. Lieberman and J. Pascarella*. Dept. of Physiology, East Carolina U. Sch. of Med., Greenville, NC 27834.

An ultramicro ATP and phospho-L-arginine (Arg-P) assay and techniques for handling nanoliter volume biological samples are described. A glass cannula attached to an oil-filled syringe was used to remove nanoliter samples of axoplasm from an intact functioning medial giant axon of the crayfish, Procambarus clarkii. The sample was ejected onto a 25 μm wire held under oil and its volume measured optically. The sample, while still in the oil-filled chamber, was prepared for the enzymatic determination of ATP and Arg-P. The assay for Arg-P is based on the ATP:L-Arginine phosphotransferase (E.C. 2.7.3.3.) catalyzed phosphorylation of ADP to ATP. The generated ATP is measured with the luciferin-luciferase bioluminescence method. Standard curves of ATP vs. scintillation counts and the generated [ATP] vs. [Arg-P] in the reaction medium were linear when plotted on full log paper over a 3 decade range, representing ATP and Arg-P contents of 10^{-14} moles per sample. ATP and Arg-P concentrations of pure axoplasmic samples, from resting nerve, were found to be approximately 2 mM and 13 mM, respectively. Following Iodoacetic acid and cyanide poisoning, preliminary studies have shown that the axon consumes approximately 50%/hr of its available high energy phosphate (sum of ATP + Arg-P) for all cellular functions. The high energy phosphate consumed was shown to be at the expense of the axoplasmic concentration of Arg-P. The ATP concentration was relatively constant, near 100% of its control value, until Arg-P was significantly reduced. It is presumed that ATP is the directly utilized energy substrate but is regenerated by the conversion of ADP to ATP using Arg-P as the phosphate donor. After addition of ouabain, energy consumption falls to 20%/hr indicating that Na-K exchange accounts for approximately 60% of the energy utilized by this axon. There was no evidence that substances present in the biological sample interfered with the assay. Recovery of added ATP and Arg-P was approximately 100%. ATP and Arg-P concentration of isolated biological samples was found to be stable for one hour at room temperature. The assay and method was demonstrated to be reliable and reproducible for the detection of subpicomole quantities of ATP and Arg-P in serial samples of cytoplasm from single isolated giant nerve fibers. The techniques described here could be easily modified for the study of a variety of metabolic intermediates in situ in functioning tissues and organs. Supported in part by grants from the US Army Research office #DAAG29-82-K-0182 and NSF grant INT-8117183.

173.7 EFFECTS OF MEMBRANE FATTY-ACID MODIFICATION ON [^{3}H]ADTN BINDING AND DOPAMINE-SENSITIVE ADENYLATE CYCLASE OF N1E-115 NEUROBLASTOMA. M.G.Murphy* and P. Pehtla*. (Spon:J.G. Rutherford) Dept. of Pharmacology, Dalhousie University, Halifax, N. S. Canada B3H 4H7

Cultured neuroblastoma cells provide an excellent model for studying the role of membrane lipids in cell-surface receptor function. Complex lipid components can be extensively modified by media supplementation without compromising cell viability and radioligand binding properties and intracellular responses can be examined in the intact cell system. Mouse neuroblastoma (clone N1E-115) have been supplemented with fatty acids which increase membrane fluidity. Subsequently, the binding of the dopamine agonist, [^{3}H]ADTN, and the activity of dopamine-sensitive adenylate cyclase have been examined. Linoleic (18:2ω6), linolenic (18:3ω3) and cis-vaccenic (19:1ω7) acids were all taken up by the cells and incorporated into membrane phospholipids (either directly or after metabolism). Fatty-acid profiles of the supplemented cultures were distinct for each culture condition, with major differences being observed in relative proportions of individual PUFA and of 18:1 isomers. The density of dopamine receptors on N1E-115 was significantly ($p < 0.03$) increased in cells fed linoleic acid, with an average B_{max} of 352.9 ± 54.96 pmol [^{3}H]ADTN specifically bound/mg protein. Linolenic acid-fed cells also had a higher B_{max} than control cells (275.1 ± 53.8 and 156.6 ± 9.1 pmol/mg protein, respectively). By contrast, the binding profile in cultures supplemented with cis-vaccenic acid (17% phospholipid fatty acids were 18:1ω7) was the same as that for controls, suggesting that non-specific "fluidizing" of a membrane does not enhance the extent of ligand-receptor interaction. The affinity of [^{3}H]ADTN for binding sites on N1E-115 did not change with culture conditions, and membrane fatty-acid modification did not alter the pattern of displacement of the agonist (apomorphine > dopamine > noradrenaline). Supplementation with 18:2 and 18:3 resulted in elevated (1.5 to 2.5 fold) basal levels of intracellular cAMP; adenylate cyclase in 18:2-fed cells was less sensitive to stimulation by dopamine than was the control enzyme. (Supported by the Medical Research Council of Canada).

173.8 INCREASED ADENYLATE LEVELS IN TERMINAL AXON REGIONS COUPLED TO SYNAPTIC TRANSMISSION. C. A. Lindgren, C. T. Gibson*, and D. O. Smith. Department of Physiology, University of Wisconsin, Madison, WI 53706.

High-energy phosphates were studied in single identified axons. After exposing the excitor axon innervating the opener muscle, the entire preparation was placed in a bath of well-oxygenated saline for 20 min. The tissue was then quick frozen in liquid nitrogen either immediately (nonstimulated) or after nerve stimulation (stimulated) for 1 min. at 50 Hz. After lyophilization, the terminal axon region was removed by microdissection and weighed. High-energy phosphates were then assayed using the luciferin-luciferase method.

Following stimulation, the ATP content increased from 13.34 to 18.16 nmol/mg dry weight; this difference, 36%, is statistically significant at the 0.05 level. The ADP and AMP levels also rose. These changes are expressed as a significant increase in the total adenylates. In contrast, the calculated energy charge did not differ significantly; average values were 0.86 and 0.88 in the nonstimulated and the stimulated tissue, respectively. The rise in ATP could not result from rapid glycolysis or oxidative phosphorylation, for there was not a corresponding decrease in ADP levels. An immediate source of the increased ATP might be the phosphagen, phosphoarginine (PArg). Thus, its levels were also assayed. The results indicated that the PArg content did not change significantly during the 1 minute stimulation. Thus, some other less direct pathway must be involved.

To test whether the underlying muscle is the source of the increased adenylates synaptic transmission was blocked and ATP levels again measured. In separate experiments transmission was blocked by addition of the calcium-current blockers Co^{2+}, verapamil, D-600, and Ruthenium Red, and the glutamate antagonist γ-methyl-glutamate to the bath. There were no significant changes in ATP levels following stimulation in any of these cases indicating that synaptic transmission, and consequent muscle activity, is necessarily related to elevated adenosine levels.

Possible release by muscle was tested by measuring ATP levels in the bath saline following nerve stimuation. We estimate that at least 0.70 pmol were released. This increase was not detected when synaptic transmission was blocked. Furthermore, addition of ATP to the bath (1 to 10 mM) caused ATP levels assayed in non-stimulated tissue to rise significantly. Our tentative conclusion is that ATP derived from the muscle becomes associated with the presynaptic nerve. Supported by grants from NIH (NS13600 and NS00380) and the Whitehall Foundation.

173.9 STUDIES ON THE SUBCELLULAR LOCALIZATION OF CNPase IN CAT PERIPHERAL NERVE. S.M. Ross*, M.I. Sabri and P.S. Spencer, Institute of Neurotoxicology, Departments of Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

2',3'-Cyclic nucleotide 3'-phosphohydrolase (CNPase) is present in CNS myelin, in plasma membranes of myelin-free oligodendrocytes and in murine glial tumor cells grown in culture. Little is known about the subcellular localization of CNPase in the PNS. Several previous studies have indicated only marginal enrichment of CNPase in the heavy myelin fraction (0.85M sucrose phase) over that of the crude homogenate (CH) prepared from peripheral nerve. CNS myelin isolation procedures are usually used to prepare PNS myelin, in which the meniscus between the 0.32M:0.85M sucrose interface is collected as the crude myelin fraction. To produce purified light myelin and to facilitate localization of CNPase in peripheral nerve, a 0.6M sucrose phase was introduced into our discontinuous sucrose gradient (DSG) system (0.25M:0.6M:0.85M). Subcellular fractions from cat sciatic nerve were prepared according to the method of Ross et al., (J. Neurochem., 1983). CNPase specific activity was enriched in the particulate (P_2) fraction and was lowest in the soluble (S_2) fraction. After layering the P_2 fraction over a DSG, CNPase specific activity was highest in membranes floating above the 0.6M and 0.25M sucrose phases 2.5- and 2.0-fold over the CH, respectively. Activity in membranes floating above the 0.85M interface was equal to that of the CH, and CNPase specific activity declined in heavier fractions. Taken together, CNPase activity in the material floating above the 0.25M:0.6M:0.85M sucrose phases constituted 70% of the total activity, while membranes above the 0.6M interface accounted for 51% of total activity. A second osmotic shock of these membranes (0.6M sucrose phase) and relayering of the material over a second DSG resulted in reduction of the specific activity by 11% and of total activity to 34% in this fraction. SDS-PAGE of membrane proteins individually harvested from the 0.25M:0.6M:0.85M interfaces indicated the presence of P_0, P_1, and P_2 myelin-specific proteins, and electron microscope examination revealed typical multilamellar myelin membranes. In summary, CNPase specific activity was increased in membranes extracted from above the 0.6M and 0.25M sucrose phases after one osmotic shock and, collectively, represent 58% of total CNPase activity. Typical myelin lamallae and myelin-specific proteins are resident in these fractions. These data indicate, based on morphological and biochemical criteria, that CNPase in cat peripheral nerves is associated with purified light myelin which floats above 0.6M and 0.25M sucrose interfaces.

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173.10 POLYPEPTIDE PATTERNS FROM IN VITRO SYNTHESIS BY BRAIN MITOCHONDRIA AND SYNAPTOSOMES. C.C. Irwin* (SPON: G. Adelman). Dept. of Biochem., Shriver Center for Mental Retardation, 200 Trapelo Rd., Waltham, MA 02254

Protein synthesis in brain mitochondria and synaptosomes has been a matter of some controversy, partly because of difficulty in interpreting the quantitative effects of cycloheximide, an inhibitor of cytoplasmic protein synthesis, and chloramphenicol, an inhibitor of mitochondrial protein synthesis. To help resolve this issue, qualitative aspects of protein synthesis in brain mitochondria and synaptosomes were studied. Mitochondria and synaptosomes were isolated by discontinuous Ficoll-sucrose gradients from 10-14 d. rat brain cortexes and incubated in an amino acid incorporation system with 200 µCi ^{35}S-methionine. After 1 h incubation at 37°, mitochondria and synaptosomes were washed, dissolved in SDS-containing medium and electrophoresed on gradient SDS-polyacrylamide slab gels. Autoradiography of the gels revealed that both mitochondria and synaptosomes synthesize a large population of polypeptides in the absence of inhibitors (50 or more in the molecular weight range 3,000-200,000) with only minor variations in the overall pattern. In the presence of cycloheximide, fewer bands appeared (at least 10-12), primarily in the lower molecular weight region (3,000-43,000) with 5 bands predominating (molecular weights 19000, 25000, 30000, 33000, 40000). Incubation with chloramphenicol reduced or eliminated these bands. Thus, no major qualitative differences in protein synthesis occur in brain mitochondria and synaptosomes by one-dimensional analysis. Furthermore, the polypeptide patterns from cycloheximide-inhibited organelles corresponded closely to patterns obtained from rat liver mitochondria and intact neural cells (cultured neuroblastoma and glioma). The results are consistent with the idea that brain mitochondria, including intraterminal mitochondria, synthesize a conventional array of proteins inhibitable by chloramphenicol. Furthermore, both mitochondrial and synaptosomal preparations, isolated by traditional methods, also synthesize a broader range of proteins, inhibitable by cycloheximide, that may reflect intrasynaptosomal non-mitochondrial, or extrasynaptosomal vesicular, protein synthesis, as suggested by others.

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173.11 RATE OF CHANGE OF OXIDATION OF NADH IN THE RESPIRATORY CHAIN BY CO_2 IN DORSAL ROOT GANGLION NEURONS SOMAS. Carlos Rodríguez-Estrada. Cátedra de Fisiología I.M.E. Facultad de Medicina, U.C.V., Caracas, Venezuela.

Previous reports (Rodríguez-Estrada,Carlos, Neuroscience Abs. 3:321,1977; 8:992,1982) have shown that carbon dioxide blocked the metabolic response after a short period of peripheral nerve stimulation and that it is a critical CO_2 concentration need to block the oxidation of NADH after a short period of anoxia.

In this work the rate of change of oxidation of NADH and the per cent change of NADH was measured. Fluorometric determinations of NADH were done on in vitro preparations of dorsal root ganglion (Rana palmipes spix as previously described, and pH was measured in the Ringer's solution bathing the preparation (Beetrode*), also pO_2 of the gas circulating the chamber was measured simultaneously, (25°C).The preparation in O2 was alternated with N_2 or CO_2-N_2 mixtures for 7 min duration. In half the preparations following this sequence: O_2, N_2, O_2, CO_2-N_2, O_2 (N_2), the other half O_2, CO_2-N_2, O_2, N_2, O_2. Results reported here are from changes observed in preparations subjected either (N_2) or (CO_2-N_2) sequence. The rate of change was the per cent change of oxidation during a period of time measured from the beginning of oxidation to its half oxidation ($t_{1/2}$). In each preparation the oxidation change (%fl) and the rate of change of oxidation ($t_{1/2}$) were larger, without exception, after N_2 than after CO_2-N_2. A group of three series performed in the same preparation (N_2), (2.5%CO_2-N_2), (5%CO_2-N_2)the values observed were: (N_2) "%fl, N=10, M=13.08, SD=2.34, SE=.74,R=9.65 -18.2; $t_{1/2}$, N=10, M=1.454, SD=.579, R=.183, R=.66 -2.52"; (2.5%CO_2-N_2), '%fl, N=10, M=8.28, SD=1.72, SE=1.81, R=6.1-12; $t_{1/2}$, N=10, M=.657, SD=.207, SE=.065, R=.28-.85"; (5%CO_2-N_2), "%fl, N=10, M=5.68, SD=3.75, SE=1.18, R=2.4-11.2; $t_{1/2}$, N=10, M=.338, SD=.260, SE=.082, R=.2-.78".The t(ratio) of $t_{1/2}$ for (N_2) and for (2.5%CO_2-N_2) were 4.10 P>.001,and 5.74 P>.001, respectively. There was no correlation between the oxidation change and the rate of change of oxidation for (N_2) and (2.5%CO_2-N_2), a r=.47 and .06 respectively, but a r=.97 for (5%CO_2-N_2).

The results show a negative correlation between the rate of change of oxidation of NADH of the respiratory chain and the CO_2 concentration.

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173.12 EFFECT OF INTRAVENOUS FEEDING ON BRAIN AND PLASMA METABOLITE ABNORMALITIES AFTER PORTACAVAL ANASTOMOSIS. A. Mans*, J. Biebuyck*, D. Davis* and R. Hawkins (SPON: R. Bryan). Departments of Anesthesia and Physiology, Hershey Medical Center, The Pennsylvania State University, Hershey, PA 17033.

Rats with a portacaval anastomosis show several characteristic metabolic abnormalities: brain content of glutamine, ammonia, tryptophan, tyrosine and phenylalanine are elevated, blood ammonia is high, and there is an abnormal pattern of plasma amino acids. Transport of neutral amino acids into brain is increased. In patients with liver failure and encephalopathy, both oral and intravenous amino acid mixtures have been administered, in an attempt to improve mental status by altering entry of those amino acids into brain which are neurotransmitter precursors. So far, reports on effects on brain function have been conflicting. We have studied the effect of nutrition on brain amino acid content and the altered blood-brain barrier transport systems. Rats with portacaval shunts were deprived of food (except controls) and given either oral dextrose, intravenous dextrose, or intravenous dextrose plus leucine, valine and isoleucine (in amounts adequate to provide caloric need). Four days of treatment lowered blood ammonia concentration especially in the group given oral glucose. Brain glutamine content decreased remarkably from about 14 µmol/g to about 8 µmol/g (normal 6 µmol/g). This effect was seen with all three regimens and is presumably due to the absence of oral protein intake and subsequent production of ammonia. In general, most plasma amino acid concentrations were lower after treatment. The high phenylalanine was reduced whereas tyrosine was unaffected in all three groups. The low branched-chain amino acids were further decreased by administration of dextrose alone, either orally or intravenously. In all three treated groups, the resulting plasma amino acid pattern led, in theory, to decreased competition for tryptophan transport into brain (calculated from the neutral amino acid concentrations and their inhibitor constants). Thus, based on consideration of competition between neutral amino acids, the entry of tryptophan into brain was predicted to be greater after all three treatments. Furthermore, the brain uptake index of tryptophan, which was 45% higher after shunting, was not lowered in the treated groups. Interestingly, in spite of these findings, brain content of tryptophan, phenylalanine and tyrosine was decreased by intravenous dextrose plus amino acids. Thus, a partial reversal of several abnormalities found after portacaval shunting was obtained by removal of oral protein and administration of glucose. The addition of branched chain amino acids to the glucose infusion had the greatest effect on brain aromatic amino acids, although the mechanism remains to be clarified.

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173.13 INCREASED GLYCOLYSIS IN GOLDTHIOGLUCOSE-INDUCED LESIONS OF CIRCUMVENTRICULAR ORGANS. R.J. DiRocco* and E.E. Coons*. Monell Chemical Senses Center, Philadelphia, PA 19104. Psychology Department, New York University, New York, N.Y. (SPON: L.A. Palmer)

We have recently reported that inflammatory brain lesions of experimental allergic encephalomyelitis (EAE) in rats are accompanied by the intense stimulation of glycolysis. One interpretation of this result is that it represents anaerobic glycolysis, as the culmination of inflammatory events beginning with increased capillary permeability followed by exudation, edema, hemoconcentration, hemostasis and ischemic hypoxia in the lesion sites. Another contributing factor may be the high metabolic rate of sensitized T-cells which aggregate to produce these foci of inflammation. Unlike EAE lesions, however, goldthioglucose (GTG)-induced lesions of circumventricular organs (CVO's) are not accompanied by the infiltration of significant numbers of lymphocytes. Moreover, macrophages are not evident in CVO's until several days after GTG injection. Therefore, any increase in glycolysis that occurs before this time could not easily be attributed to high metabolic rates in aggregated leukocytes, microglia or macrophages of peripheral origin.

CBA/Ki mice were injected (I.P.) with GTG (0.4 mg/g). ^{14}C-deoxyglucose (100μ Ci/kg) was injected (I.P.) 1,3,5, and 7 hrs, as well as 2,8 and 12 days after GTG administration. Mice were sacrificed 45 minutes after ^{14}C-2DG injection and autoradiograms were prepared from frozen brain sections obtained with a cryostat. Autoradiograms obtained one hour after GTG were normal, however by 3 hours glycolysis in the ventromedial region of the hypothalamus (VMH) including the median eminence was intensely stimulated. By 5 hours, the involved region was larger but the lesion was characterized by a leading edge of metabolic activity surrounding a central quiescent zone. Seven hours after GTG, all CVO's were involved. This pattern of activity persisted for two days. By 8 days after GTG, however, the region of VMH metabolic involvement had diminished in size to a sharply demarcated area of intense metabolic activity corresponding to scar formation. Other CVO's appeared normal at this time. Twelve days after GTG, all circumventricular regions appeared normal by visual examination of the autoradiograms.

Intense glycolysis in GTG-induced CVO lesions as early as three hours post GTG occurs well before significant accumulation of leukocytes and macrophages, and therefore cannot be explained in terms of a high metabolic rate of these cells. This observation supports the hypothesis that ischemic hypoxia leading to anaerobic glycolysis is a cause of the increased metabolic rate of glucose in inflamed regions of the central nervous system.

173.14 APPARENT ACTIVATION OF [^{3}H]2-DEOXYGLUCOSE UPTAKE BY SLICES OF CEREBRAL CORTEX AFTER EXPOSURE TO LOW GLUCOSE INCUBATION MEDIA. B. I. Gold and J. Kyle-Lillegard. Dept. Pharmacol. Uniformed Services Univ. Sch. Med., Bethesda, MD 20814

We have been using the uptake of [^{3}H]2-deoxyglucose ([^{3}H]2DG) by brain slices to model glucose transport by brain. With this model we have shown that [^{3}H]2DG is taken up selectively and stereospecifically. Uptake is saturable and it displays a temperature dependence that is consistent with a carrier-mediated process. We now report data from preliminary experiments designed to question whether glucose uptake in the brain is regulated in part as a function of glucose availability.

Slices of cerebral cortex from freshly-killed rats were routinely incubated in oxygenated Krebs-Ringer-bicarbonate media (KRB). After a 30 min equilibration in KRB with 10.4 mM D-glucose (D-glc), slices were transferred and incubated for up to 1 h in KRB which contained 10.4 mM L-glucose (L-glc), the inactive stereoisomer of D-glc, or 10.4 mM choline Chloride. Control slices were incubatecd in parallel in 10.4 mM D-glc. After exposure to L-glc or to choline Cl, slices were filtered, rinsed, and resuspended in D-glc-free KRB. Uptake was initiated with 0.5 mM [^{3}H]2DG and after 2 min slices were filtered again, rinsed, solublized in Protosol and radioactivity was estimated. These steps have been described in detail (Neurochem. Res. 6:949; 8:473).

[^{3}H]2DG uptake by slices exposed to L-glc or choline Cl, was increased up to threefold as compared to controls. In separate experiments, [^{3}H]2DG uptake was halted by filtration but samples were extracted in 0.4N $HClO_4$. The extracts were neutralized with KOH, they were centrifuged and the supernatants were applied to small anion exchange columns. Two fractions were collected, a neutral fraction containing [^{3}H]2DG and an acidic fraction containing [^{3}H]2-DG-6-phosphate ([^{3}H]2DGP). In slices that had been exposed for 1 h to 10.4 mM choline Cl, not only was total ^{3}H uptake increased, but the amount of ^{3}H that eluted in the acid fraction increased almost fivefold as compared to slices incubated for 1 h in 10.4 mM D-glc. In similar experiments, [^{3}H]2DG uptake by slices exposed to 10.4 mM 3-O-methylglucose, an analogue that is not a substrate for hexokinase increased twofold as compared to control but uptake by slices exposed for 1 h to 10.4 mM 2DG was not increased and actually showed a 40% decrease. [^{3}H]2DG uptake by slices exposed for 1 h to 2.0 mM D-glc, analagous to hypoglycemic plasma levels, increased about 30% as compared to control slices exposed for 1 h to 10.4 mM D-glc.

These results suggest that brain glucose uptake at the cellular level responds to decreased glucose availability by increasing the rate or the capacity of glucose transport. New data are required, however, to determine if increased uptake is secondary to or driving the increased glc metabolism. (USUHS protocol #TO7528)

173.15 AN IN VIVO MODEL FOR ISOLATING THE PHOTORECEPTOR CELL LAYER IN THE RAT. S. Y. Schmidt*, J. C. Blanks and M. A. Sandberg*. Berman-Gund Laboratory, Harvard Medical School, Massachusetts Eye and Ear Infirmary, Boston, MA, and Estelle Doheny Eye Foundation, Los Angeles, CA.

A model system was developed to obtain a retina rich in photoreceptor cells. Neonatal rats (postnatal days 5-11) were subjected to a series of 12-13 subcutaneous and intraperitoneal injections of a combination of neurotoxic agents, including glutamate, aspartate and 6-hydroxydopamine. The photoreceptor cells were not affected by these injections and underwent normal differentiation and growth. On day 26, photoreceptor cell function was evaluated by electroretinography (ERG) and photoreceptor cell metabolism by the capacity for RNA synthesis in vivo and in vitro and by the cells' ability to restrict the entry of sodium in light but not in dark. ERGs showed a normal a-wave and a greatly reduced b-wave; the capacity of photoreceptor cells for RNA synthesis and excluding sodium in light was comparable to that in uninjected littermate controls. In some experiments, rats were given an additional intraocular injection of alpha-aminoadipic acid (AAA); 20 hours later ERG responses showed further reductions in the b-wave, a normal a-wave and the retina was composed almost entirely of photoreceptor cell layers with nearly complete disappearance of inner retinal neurons and Müller cells. The photoreceptor cells remained intact and their capacity for RNA synthesis was normal as shown by biochemical and autoradiographic analyses.

These studies establish that such photoreceptor-rich retinas provide an in vivo system for electrophysiological analysis of the isolated a-wave of the ERG and an in vitro system for defining RNA synthesis and exploring the molecular biology of photoreceptor cells.

173.16 METHIONINE SULFOXIMINE EFFECT ON N-ACETYL-L-ASPARTATE LEVELS IN SYNAPTOSOMES. G. Massieu and K.Aoki. Department of Neuroscience. CINVESTAV del IPN. Apartado Postal 14-740, México 07000, D.F.

There is growing evidence that N-acetyl-L-aspartate (NAA) is not an inert metabolite, but rather is utilized in the lipid biosynthesis in the developing rat brain (D'Adamo et al, 1968). On the other hand, Tapia et al (1967) showed that administration of methionine sulfoximine (MS) to mice decreases the brain levels of glutamic and aspartic acid, GABA and glutamine. It was therefore of interest to examine the effects of MS on the NAA levels in the mice and rat cerebral cortex as well as the influence of MS-treatment on NAA content of synaptosomes obtained from rat brain cortex. In the normal cortex of mice, the contents of NAA are of 81.1 $\pm$ 9.38 (mean $\pm$ S.D. n=4) mμmoles per mg of protein. 24 hours after the injection of 150 mg/kg of MS, the content of NAA in the cerebral cortex was changed to 66.5 $\pm$ 4.42 mμmoles per mg of protein. This difference was not significant. However, in the remainder of the brain there was a significant reduction of NAA ($P<0.02$) from 67.9 $\pm$ 3.08 control (n=4) to 53.2 $\pm$ 2.52 (n=5). In the rat cortex, MS also reduced the levels of NAA (from control values of 101.5 $\pm$ 3.59 n=4 to 89.4 $\pm$ 8.09 (n=5) after MS), but was without significant effects in the remainder of the brain (79.6 $\pm$ 1.61, n=4 control to 77.4 $\pm$ 4.84, n=5 after MS).

Similar decreases of NAA after (100 mg/Kg MS) were observed in synaptosomal preparations obtained from rat cerebral cortex. Control values were 78.01 $\pm$ 1.74 (n=7) and were changed to 54.7 $\pm$ 1.92 (n=7) after 100 mg of MS.

In another series of experiments we compared the action of MS (100 mg/Kg) administered together with methionine (372 mg/kg). There was practically no difference between the NAA content of the synaptosomes of MS-treated rats (54.9 $\pm$ 1.65, n=18) as compared with the MS + methionine treated rats (58.2 $\pm$ 2.14) which were significantly smaller ($P<0.05$) than controls (76.0 $\pm$ 2.09, n=30).

It is concluded that NS-treatment induces a moderate decrease of the NAA content of brain tissue. In the case of synaptosomes the falling of NAA is more significant. These findings support the view that NAA metabolism is active.

173.17 ESTIMATION OF THE LEVEL OF NOREPINEPHRINE AND ASCORBATE AUTOXIDATION INHIBITING ACTIVITY IN THE CNS AND SERUM OF GUINEA PIG, RAT AND HUMANS. G.B. Kovachich* and O.P. Mishra* (Spon: A. Winokur). Dept. of Pharmacology and Inst. for Environmental Medicine, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

During the past year evidence has been presented that the rat brain cortex contains 2 heat stable, nondialyzable substances with potent inhibitory effect on the in vitro autoxidation reactions of ascorbate (ASC) and norepinephrine (NE) (Kovachich and Mishra, Neurosci. Lett. 34:83, 1982, Neurosci. Lett. in press.) Data presented below indicate that these antioxidants are distributed widely in the CNS and beyond, with uniform activity levels.

ASC autoxidation was determined by spectrophotometric measurements at 265 nm, NE autoxidation was estimated by spectrophotometric detection of noradrenochrome at 480 nm in buffered reaction media. Rat and guniea pig brain areas (brain stem, midbrain, cerebellum and cortex) and spinal cord samples were dissected from adult animals, human frontal lobe sections were obtained from the University Hospital morgue 6-8 hours post mortem. Tests showed no change in rat brain antioxidant activities post mortem for at least one day. Serum samples were prepared from rats and guniea pigs after Nembutal anesthesia directly from the heart, human serum through I.V. puncture. CNS samples were homogenized in 10% w/v 10mM TRIS or 16mM NaPi pH 7.4 and 100,000g supernatant was used for assay. The choice of buffers did not effect the assay results. Serum samples were diluted 10-fold with buffer and all samples were dialized overnight (m.w. cut off at 8,000).

The inhibitory activities on ASC and NE autoxidation were determined using 1-100 ul aliquots in 2 or 4 ml buffered reaction mixtures against various concentrations of ASC and NE. All samples were active. Although there were minor variations in the potency of the samples, the uniformity of the results were more striking than the differences. Experiments with EDTA verified previous findings that the inhibitory effect of the extracts is far greater than the maximum effects that could be obtained by the use of EDTA.

Precise quantitative generalizations are difficult to obtain without further measurements, however, the inhibitory effect of tissue extracts on both the ASC and NE autoxidations were discernable at up to $5x10^4$-fold dilution of the original tissue activity. Maximal inhibitory effect was obtained at approximately 10^3-fold dilution. The substances responsible for these effects appear to be in considerable excess of what is required for inhibition of autoxidation. This suggests that the physiological role of these entities is yet to be identified. Supported by NIH Grant HL-08899 and ONR Contract N00014-7-00248.

173.18 **EFFECTS OF EXPERIMENTAL HEAD INJURY ON TISSUE DENSITY AND VASCULAR PERMEABILITY TO PROTEIN IN WHITE MATTER.** P. A. Tornheim, K. R. Wagner, R. L. McLaurin*. Depts. of Anat. & Cell Biol, Neurol., and Surgery, Univ. of Cincinnati; VAMC, Cincinnati, OH 45267.

Although the characteristics of the spread of edema fluid from thermal insults to cerebral cortex (cold injury) have been well described, the early development of edema after mechanical injury to the brain has received little investigative attention. The purpose of the present study was to determine the topography of white matter edema following experimental mechanical head trauma. Ketamine-anesthetized cats were injected intravenously with Evans Blue dye (25 mg/kg), followed by impact to the exposed skull with a Remington Humane Stunner. At 15 min and 6 hr after injury the head was frozen in liquid nitrogen, followed by coronal slicing on a band saw. Only head-injured cats with unilateral contusions of the lateral frontotemporal area were studied. Frozen slices were examined for white matter Evans Blue dye, indicating the location of protein-rich (vasogenic) edema fluid. Each slice was thawed under kerosene and serial white matter samples were taken 1-10 mm medial to the edge of contusion as well as bilaterally from the deep white matter (centrum semiovale) 0-15 mm caudal to the level of tissue hemorrhage. Samples were measured for tissue density with an organic gradient. Density data from head-injured animals were compared with those from uninjured control cats to estimate change in tissue water content (edema).

At 15 min after injury, a narrow rim (0.2-1.5 mm) of Evans Blue dye was visible in white matter deep to contusions. White matter from this area had substantial decrease in density from control values. At 2.5-10 mm deep to contusion, outside of the vasogenic edema front, there was a small but significant decrease in density from control values. Deep white matter 0-7 mm caudal to contusion had slight hypodensity. Extremely caudal and contralateral white matter had normal density.

At 6 h after injury there was a substantial Evans Blue territory medial to contusion, usually with sparing of dorsal subcortical white matter and arcuate fibers. The deep white matter was stained up to 8 mm caudal to contusion. Substantial decrease in density occurred in the subcortical and deep white matter medial and up to 8 mm caudal to contusion, but not contralaterally. The present results document, at 15 min after injury, changes in white matter density in a widespread area peripheral to the vasogenic edema front surrounding contusions. These findings contrast with those seen with cold injury. We conclude that events in addition to spread of fluid from sites of tissue hemorrhage contribute to the white matter edema of mechanical trauma.

This study was supported by NIH grant R01 NS-17975.

173.19 **ACUTE CHANGES IN REGIONAL CEREBRAL METABOLITE CONCENTRATIONS FOLLOWING EXPERIMENTAL BLUNT HEAD TRAUMA.** K. R. Wagner and P. A. Tornheim. Departments of Neurology and Anatomy and Cell Biology, University of Cincinnati, VA Medical Center, Cincinnati,OH 45220.

The present study asks whether mechanical head injury alters cerebral metabolite concentrations and, if so, whether these effects: 1) relate topographically to tissue hemorrhage; and 2) vary for gray versus white matter. Ketamine-anesthetized cats monitored for arterial and intracranial pressures and blood gases were subjected to impact to the skull with a Remington Humane Stunner. Controls were handled identically except for the injury. Brain metabolite concentrations were fixed by in situ freezing of the head at 15 min after impact. Only injured animals with unilateral cerebral contusions were studied. Metabolite concentrations were assayed by enzymatic-fluorometric methods on samples from cerebral cortex and deep white matter neighboring, distant (ipsilateral), and contralateral to tissue hemorrhage. Tissue density was measured with an organic gradient to correct for dilution of metabolite concentrations by edema fluid.

For 5 control cats, concentrations of ATP, phosphocreatine (PCr) and lactate were 2.20±0.06, 4.10±0.25 and 1.29±0.15 µmoles/g (Mean±SEM) respectively in white matter and 2.57±0.09, 5.76±0.48 and 2.14±0.41 µmoles/g respectively in cortex.

For 8 head-injured cats, white matter neighboring contusion had substantial metabolic changes (corrected values for ATP, PCr and lactate of 1.37±0.33, 1.79±0.49 and 8.26±1.55 µmoles/g respectively). Distant white matter had smaller alterations; contralateral white matter values were similar to controls. By contrast, cortex neighboring contusion had lesser changes in metabolites (corrected ATP, PCr and lactate of 1.93±0.39, 3.56±0.79 and 6.94±2.53 µmoles/g respectively) than white matter, with variable responses distant and contralateral to contusion. Systemic responses to trauma do not explain the topography or magnitude of metabolic changes seen with our model.

The present results demonstrate, at 15 min following head injury, marked metabolic changes in deep white matter that decrease with distance from contusion. We conclude that the metabolism of the white matter compartment is particularly vulnerable to trauma and that acute metabolic insults may contribute to the white matter edema frequently seen following severe head injury in man.

This study was supported by NIH Grant #R01 NS17975.

173.20 31-PHOSPHORUS NUCLEAR MAGNETIC RESONANCE OF DECAPITATED RAT BRAIN, J.A. Helpern, J.R. Ewing*, K.M.A. Welch, S.J. McGee*. Nuclear Magnetic Resonance Lab., Dept. of Neurology, Henry Ford Hospital, Detroit, MI 48202.

31-Phosphorus Nuclear Magnetic Resonance (NMR) has been used to measure high-energy phosphate metabolism during global ischemia in the decapitated rat brain. Brains from rats (male, Sprague-Dawley, 325-350 gm) were isolated post-decapitation and immediately placed over an insulated NMR surface coil, tuned to the 31-P resonance frequency of 32.5 MHz. NMR parameters (pulse intervals) were optimized for the detection of phosphocreatine. Data acquisition began within 40 seconds of decapitation, at a rate of 120 scans per 49 seconds, and continued for at least 5 minutes. No phosphocreatine or ATP/ADP peaks were detectable in any of the subsequent spectra. These parameters were also used to detect phosphocreatine in anesthetized rats with a signal-to-noise ratio >4. Since our first data point is the accumulation of individual scans during the time frame of 40-89 seconds post-decapitation, we conclude that any phosphocreatine in rat brain after global ischemia of > 1 minute in duration is not detectable by NMR. These results seem to contradict the findings of Norwood et al (Norwood WI, et al, Am J Physiol, 244(3):205, 1983), who recently demonstrated the presence of phosphocreatine in perfused adult and neonatal rat brain well after 5 minutes of ischemia. In order to clarify these findings, whole decapitated rat heads, as well as decapitated rat heads which had been cleaned of extraneous skin and muscle, were placed in the NMR with the surface coil located directly over the calvarium. Whole decapitated rat heads as well as those that had been cleaned of some but not all extraneous tissue demonstrated the presence of phosphocreatine and ATP/ADP peaks lasting for up to 25 minutes post-decapitation. However, rat heads which had been thoroughly cleaned of extraneous tissue showed no indication of any presence of phosphocreatine or ATP/ADP. As further evidence that any detectable levels of phosphocreatine or ATP/ADP after global ischemia of > 1 minute in duration result from tissue other than brain, the brains were removed from the skull, replaced with a sack of water, and the head placed in the NMR. Subsequent NMR spectra confirmed that the phosphocreatine and ATP/ADP peaks originally thought to be resulting from brain actually result from the surrounding tissue of the skull.

175 SYMPOSIUM. THE DYNORPHIN PEPTIDES. A. Goldstein, Stanford Univ. (Chairman); S. Numa*, Kyoto Univ.; E. Weber*, Stanford Univ.; F. Bloom, Salk Institute; A. Herz*, Max Planck Institut fur Psychiatrie.

The dynorphin peptides comprise the third known family of opioid gene products in the mammalian nervous system. There are three main opioid peptides within the dynorphin message -- neo-endorphin, dynorphin A, and dynorphin B (rimorphin).

The structures of the messages and genes encoding the opioid peptide families will be compared, and their physiological and evolutionary significance will be discussed (Numa).

The evidence for a high degree of selectivity of the dynorphin peptides for the kappa opioid receptor will be presented (Goldstein).

The several pathways of post-translational proteolysis will be discussed, especially the unusual cleavages that lead to dynorphin A-(1-8) and dynorphin B (Weber).

The hippocampus provides an interesting opportunity to compare release and function in dynorphin and enkephalin circuits, and to study specificity of action of these peptides on their target neurons (Bloom).

Finally, the possible involvement of dynorphin peptides in gross physiologic and behavioral functions -- e.g., pain modulation, neuroendocrine effects, food and water intake -- will be discussed (Herz).

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(H)YGGFLRKYPK(OH)
α-Neo-endorphin

(H)YGGFLRKYP(OH)
β-Neo-endorphin

(H)YGGFLRRIRPKLKWDNQ(OH)
Dynorphin A

(H)YGGFLRRI(OH)
Dynorphin A-(1-8)

(H)YGGFLRRQFKVVT(OH)
Dynorphin B ("rimorphin")

(H)YGGFLRRQFKVVTRSQEDPNAYYEELFDV(OH)
Dynorphin B-29 (putative)

176 SYMPOSIUM: THE ACIDIC INTERIOR OF SECRETORY VESICLES: MECHANISMS AND IMPLICATIONS FOR NEUROBIOLOGY. J.T. Russell, NIMH, and R.W. Holz, Univ. Michigan (Chairmen); D. Njus, Wayne State Univ.; R.L. Pedersen, Johns Hopkins Univ; I. Mellman, Yale Univ.

In recent years it has become clear that many subcellular membrane systems possess H^+ translocating ATP-ases. Among these systems are mitochondria, chloroplasts, secretory vesicles, lisosomes, endosomes, and sperm acrosomes. In secretory vesicles and other vesicular organelles, this enzyme electrogenically transports protons into the lumen rendering them acidic with respect to the cytosol. Such a phenomenon has been found in secretory vesicles from various neuronal and nonneuronal tissues, eg. chromaffin vesicles, neurosecretory vesicles, anterior pituitary vesicles, secretory vesicles from platelets, pancreatic islets, parathyroid, etc. This proton translocating enzyme is a Mg^{++}-dependent ATP-ase and is similar, but not identical, to the F_1/F_0 type proton pump found on mitochondria. Unlike the mitochondrial enzyme, the H^+-ATP-ases in secretory vesicle membranes are not fully biochemically characterized as yet. With the mitochondrial enzyme as the model, the relationship between the macromolecular structure of the protein and the proton translocation will be discussed. In the chromaffin vesicle the pH gradient (ΔpH) and the membrane potential ($\Delta\psi$) drive the uptake of catecholamines which are transported into the vesicles in exchange for H^+. The ΔpH and $\Delta\psi$ also drive electrons in, through a membrane-bound cytochrome b_{561}. In the neurosecretory vesicle the acidic interior provides the optimal environment for post-translational modification of newly synthesized secretory peptides. The proteolytic enzymes involved in this process have acidic pH optima (pH<5.5). The binding of neuropeptides vasopressin and oxytocin to their respective neurophysins also is optimal at pH 5.5. Thus the internal organization of these vesicles may also depend upon the acidification. The question of whether the large H^+ electrochemical potential plays an important role in exocytotic secretion will be addressed in the chromaffin cell model system. The H^+ pumps identified in endosomes, liposomes and coated vesicles are similar to the H^+-translocating enzyme found in secretory vesicles. The ΔpH of endocytic vesicles has important implications for uptake and delivery of macromolecules and membrane recycling. Further, numerous pathogens (viruses, bacterial toxins) make use of this acidic emvironment to penetrate into cells. The possible significance of these phenomena for the normal function and pathology of the nervous tissue will be discussed.

177 SYMPOSIUM. FUNCTIONAL RECONSTRUCTION OF NEURONAL SYSTEMS.

Co-chairmen: Donald H. Perkel, Stanford Univ.; Peter A. Getting, Univ. of Iowa. Other speakers: John P. Miller, Univ. of California, Berkeley; Ronald Joyner*, Univ. of Iowa; W. Otto Friesen, Univ. of Virginia; Daniel K. Hartline, Univ. of Hawaii. Panelists: Jack D. Cowan, Univ. of Chicago; John W. Moore, Duke Univ. Med. Center; Wilfrid Rall, National Institutes of Health; Stephen G. Waxman, V.A. Med. Center, Palo Alto.

The availability and power of present-day computers have made practical the realistic simulation of neurons and circuits, taking advantage of the quantitative morphological and physiological data provided by cellular neuroanatomy, voltage-clamp techniques, and neuropharmacological studies. Functional reconstruction is soundly based on mechanisms of gating dynamics for ion channels, neurotransmitter release and uptake kinetics, cytoplasmic current flow in realistically described cellular morphology, and modulation of conductances by neurotransmitters, calcium ion, "second messengers", and the like. Use of functional reconstruction for neuronal systems provides a quantitative, predictive framework for organizing and comparing experimental data and for testing higher-level theories of neural functioning.

Speakers in the symposium will describe a variety of approaches and applications of functional reconstruction. Perkel will discuss overall aims, mathematical formulations, and types of implementation. Getting will describe applications of functional reconstruction to specific neuronal circuits. Miller will illustrate the use of program packages for electrical networks to simulate integrative properties of dendritic structures. Joyner will describe simulations of interacting pacemaker neurons. Friesen will discuss use of analog methods to simulate networks. Hartline will describe digital simulation of lobster stomatogastric ganglion.

Following the speakers' presentations, the panelists will compare approaches, implementations, and techniques for functional reconstruction. They will attempt to evaluate the utility of various degrees of morphological and physiological realism as well as practical considerations of system size and computational speed. This round-table discussion should be illuminating for both the experienced and novice modeler, and should clarify present and future capabilities of this approach.

178.1 ADAPTATION OF ACTION POTENTIAL FREQUENCY IN HIPPOCAMPAL PYRAMIDAL CELLS IS REGULATED BY CALCIUM-ACTIVATED POTASSIUM CONDUCTANCE AND M-CURRENT. D.V. Madison and R.A. Nicoll. Depts. of Pharmacology and Physiology; and Graduate Program in Neuroscience, University of California, San Francisco, CA. 94143.

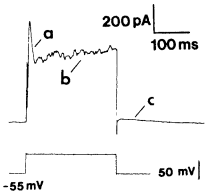
Adaptation of action potential frequency in response to a tonic excitatory stimulus is characteristic of many neuronal cell types. Since adaptation determines the frequency at which the cell discharges, it plays a fundamental role in controlling the output of the neuron. It has been frequently reported that adaptation occurs in neurons of the central nervous system, however, little is known of the mechanisms of this adaptation. Using the in vitro hippocampal slice preparation of the rat, and intracellular recording, we have found that the action potential discharge of CA1 pyramidal cells also shows adaptation in response to excitatory stimuli. While the cells respond to a long depolarizing current pulse (600-800ms) with an initial high rate of action potential discharge, the frequency of this discharge rapidly decays until the cell falls completely silent. Associated with this adaptation is a prominent "sag" in the underlying membrane voltage, and an afterhyperpolarization (AHP), which is known to be due primarily to calcium-activated potassium conductance ($G_{K(Ca)}$). Blocking $G_{K(Ca)}$, either by injecting EGTA into the cell, or by bathing the slice in the calcium channel blocker, cadmium, greatly reduces the "sag", the AHP, and the adaptation seen in response to a depolarizing stimulus. Dentate granule cells in the slice also exhibit adaptation and calcium-activated potassium AHPs, both of which are reduced by cadmium. In pyramidal cells, adaptation, and the remaining "sag", can be further reduced by bathing the slice in low concentrations (1μM) of the muscarinic agonist, carbachol, which is believed to block another potassium current, the M-current (I_M). Thus, we propose that adaptation in pyramidal cells is caused by two potassium currents: The M-current and the calcium-activated potassium current. At least one of these currents, the calcium-activated potassium current, causes adaptation in dentate granule cells. These intrinsic currents are turned on by depolarization, and exert a braking influence on further discharge. Blocking these currents removes their inhibitory influence, increasing the output of the cell to a given stimulus. Thus, these two currents play a critical role in regulating the input/output function of the cell. This role gains much greater significance in light of the fact that I_M and $G_{K(Ca)}$ are blocked by the neurotransmitters acetylcholine and norepinephrine, respectively.

This research was supported by NIH Grant NS-16485, NS-15764, RCDA MH00437 and the Klingenstein Fund to R.A.N.

178.2 THREE COMPONENTS OF OUTWARD CURRENT IN ISOLATED MAMMALIAN CORTICAL NEURONS.
Robert B. Clark* and Robert K.S. Wong, (SPON: W.D. Knowles) Dept. Physiol. Biophys., University of Texas Medical Branch, Galveston, TX 77550.

Single neurons were isolated from slices of adult guinea pig hippocampus (microdissected to remove granule cell layer) using an enzymatic (papain) digestion and mechanical dispersion procedure. The majority of cells, probably pyramidal, had a pear-shaped soma 15-30 μm across and apical dendrite stump of 50-150 μm in length. Intracellular recordings were made using the "whole-cell" gigaseal technique of Hamill et. al. (Pflugers Arch. (1981), 391, 85). Temperature was about 22°C.

Neurons selected had resting potentials in the range -45 to -60 mV and apparent input resistances at rest of 200-800 MΩ. Action potentials, either spontaneous or evoked by injected current, were 80-90 mV in amplitude and 1-2 ms duration (at ½ amplitude). In TTX-containing saline (1 μgm/ml), voltage-clamp currents evoked by depolarizing steps from -35 to +20 mV (holding potentials -45 to -55 mV) were outward (see Fig.). (a) shows transient outward current with an inactivation time constant of ∿ 20ms (Vm = -20 to 0 mV), and similar rate of removal of inactivation by hyperpolarization to -60 to -70 mV. In some cells, transient current was largely inactivated at holding potential but was revealed by pre-hyperpolarization. 4-AP (1 mM) reversibly abolished the current. (b) Delayed outward current appeared to consist of two components. The delayed current was "noisy" in many cells; the noise and amplitude of the current was substantially reduced in Mn^{2+} (1 mM) or Co^{2+} (2 mM) containing salines (which block an inward Ca^{2+} current in these cells), suggesting a Ca^{2+}-dependent K^+ current. A delayed voltage-dependent outward current was also present in 0 Ca^{2+}, 1 mM Mn^{2+} saline. This current activated at about -35 mV, the rate of activation increasing with increased depolarization. Both delayed components were considerably reduced by TEA (10-20 mM), but not 4-AP. In most cells, the current tail on repolarization to the holding potential (c) was outward. The reversal potential of the tail was determined using a two-pulse clamp protocol and was between -55 to -65 mV. All components of outward current were blocked if Cs^+ replaced K^+ in the recording pipette, suggesting that the currents are probably carried by K^+.



Supported by NS-13778, NS-18464 and the Klingenstein Foundation.

178.3 ADAPTATION OF THE ELECTRICAL ACTIVITY OF SINGLE ELEMENTS IN THE OPTIC TECTUM OF GUINEA PIGS IN VITRO: ITS POSSIBLE ROLE IN VISUAL HABITUATION. R. Llinás and J. Lopez-Barneo*. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Intracellular recordings from the large neurons of the stratum griseus medium of the optic tectum in guinea pigs were obtained in vitro following coronal slicing of the brainstem. Direct stimulation produced repetitive firing, the frequency of which reduced rapidly with repeated stimulation. The action potentials generated by these current pulses are characterized by a rapid rate of rise and a break in their falling phase due to a fast after-depolarization which increases in amplitude during the spike train. Replacement of calcium by barium in the extracellular fluid increased this after-depolarization which was blocked by the addition of cadmium to the extracellular medium, indicating that it is a calcium spike, most probably of dendritic origin. Also characteristic of these cells is the observation that with repeated current injections of 500 msec duration at frequencies from 1/sec to 1 every 5 sec for a period of 20 sec, a long-term adaptation of the repetitive response is observed which recovers to control level in approximately 60 sec. The adaptation is accompanied by a small hyperpolarization and a decreased input resistance due to an increased potassium conductance (g_K) which is spike frequency dependent. Adaptation as well as the $g_{K(Ca)}$ are blocked by barium and by ions known to block calcium conductance such as cadmium, all of which indicate that a calcium-dependent g_K of rather long duration is responsible for the long-term excitability change. These results demonstrate that optic tectal neurons possess intrinsic properties which allow them to adapt to repetitive stimuli independently of the properties of the neuronal circuit in which they are embedded. The time course of this adaptation is very similar to that observed for the habituation of the neuronal firing in rabbit tectum following physiological stimulation (Oyster & Takahashi, J. Neurophysiol. 38: 301, 1975). As in their case, four criteria for habituation were observed in these experiments: (a) the responses are the same if separated by sufficient time, (b) the response decreases gradually with each stimulus, (c) the rate of response decrease is stimulus- and spike frequency-dependent, and (d) the response recovers with time after the cessation of the stimuli. We propose that this adaptation is a significant component of the visual habituation in the tectum. Supported by USPHS grant NS13742 and NIH international postdoctoral fellowship 0303201.

178.4 LOCUS COERULEUS ACTIVITY IN VITRO: INTRINSIC REGULATION BY A CALCIUM-DEPENDENT POTASSIUM CONDUCTANCE BUT NOT α_2-ADRENOCEPTORS. R. Andrade and G.K. Aghajanian Depts. of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06508

Previous in vivo studies have shown that locus coeruleus (LC) neurons exhibit a pronounced afterhyperpolarization (AHP) and a long-lasting suppression of spontaneous activity following a burst of spikes. Two mechanisms have been proposed to account for the AHP: the activation of α_2-adrenergic "autoreceptors" through the release of norepinephrine by recurrent collaterals or dendrites or the activation of a Ca^{2+}-dependent K^+ conductance. The present study was undertaken to distinguish between these mechanisms in the in vitro brain slice.

Pontine brain stem slices from albino rats (150-250 g) were cut using a Sorvall Tissue Sectioner. They were continuously perfused in a slice chamber with ACSF (in mM: NaCl 130; KCl 5; $NaH_2PO_4 \cdot H_2O$ 1.25; $NaHCO_3$ 24; $CaCl_2$ 2.5; $MgSO_4$ 1.5; D-Glucose 10) at 32 to 37°C. 95% O_2 and 5% CO_2 continuously flowed over the slices. Extracellular and intracellular recordings were obtained from the visually identified LC using standard electrophysiological techniques.

As previously observed in vivo, LC neurons in vitro exhibit a prolonged AHP and a marked post activation inhibition (PAI) following a burst of spikes. This AHP has a reversal potential (E_{rev}) in the hyperpolarizing direction and is associated with an increase in membrane conductance. Since the E_{rev} for the AHP was virtually identical when recorded with KCl or K-acetate electrodes and shifted in the hyperpolarizing and depolarizing direction with decreases and increases in $[K^+]_o$, it appears that the AHP following a burst of spikes is mediated by an increase in K^+ conductance. Moreover, since the AHP was greatly attenuated by reducing Ca^{2+} influx (i.e., by reducing extracellular Ca^{2+} or blocking Ca^{2+} channels with Mn^{2+} or Cd^{2+}) LC neurons appear to possess a Ca^{2+}-dependent K^+ conductance whose activation by calcium entry during the burst results in the observed AHP and PAI. In contrast to in vivo where the α_2-antagonist piperoxane increases spontaneous activity and reduces the AHP, its administration in vitro failed to reduce the AHP or the PAI even when norepinephrine synthesis was stimulated by supplementing the ACSF with L-tyrosine. Thus it appears that the AHP and postactivation inhibition in the LC in vitro is mediated principally by a Ca^{2+}-dependent K^+ conductance. Supported by USPHS Grants GM-07527, MH-17871, MH-14276, and the State of Connecticut.

178.5 CONTROL OF EXCITABILITY IN ISOLATED HIPPOCAMPAL DENDRITES. Leona M. Masukawa and David A. Prince. Neurology Department, Stanford University School of Medicine, Palo Alto, CA. 94305.

The apical dendrites of CA1 pyramidal cells were isolated from their cell bodies by making cuts through proximal stratum radiatum of transverse hippocampal slices from the guinea pig. This lesion separated the distal apical dendritic elements from the somata, basal dendrites, and 50-100 microns of the proximal apical dendritic tree. Orthodromic stimuli in stratum radiatum evoked excitatory synaptic responses in isolated dendrites, but no inhibitory components could be detected. In spite of this surgically produced disinhibition, orthodromic stimuli did not elicit burst activity at the resting membrane potential. However, isolated dendrites and intact dendrites could generate multiple slow spike activity when directly stimulated with depolarizing current pulses. When isolated dendrites were depolarized by DC current, EPSPs could evoke subthreshold intrinsic slow depolarizations, or repetitive slow spikes, similar to responses elicited by depolarizing current pulses alone. Afterhyperpolarizing potentials associated with an increase in conductance occurred in isolated dendrites following repetitive slow spikes. After exposure to bicuculline(5uM), both intact and isolated dendrites generated bursts of activity following synaptic activation. A possible mechanism for this action of bicuculline is blockade of a residual GABA-mediated inhibition which was not expressed as a postsynaptic hyperpolarization in isolated dendrites. This bicuculline-sensitive event was capable of depressing dendritic excitability in the absence of the recurrent inhibitory synaptic input and was very effective in controlling burst activity. Dendritic behavior is therefore dependent on a complex interaction between synaptic and voltage sensitive events.

This work was supported by NIH grant NS 12151.

178.6 DEPOLARIZATION-INDUCED EFFECTS OF INTRACELLULARLY APPLIED CALCIUM-CALMODULIN DEPENDENT PROTEIN KINASE IN NEURONS OF THE MOTOR CORTEX OF CATS. C. D. Woody[a], D. L. Alkon and B. Hay*, Laboratory of Biophysics, Natl. Inst. Health at the Marine Biological Laboratory, Woods Hole, MA 02543.

Sixteen cells of the motor cortex of awake cats were given intracellular ionophoretic application of calcium-calmodulin dependent protein kinase[b] (CaPK) followed by a 30 sec period of steady depolarization (1.0 nA). These cells showed an increase in input resistance in comparison with a control group of fifteen cells given depolarization only, without application of CaPK. The difference between groups was significant ($p < 0.05$, Student's t test, two-tailed). The increase of input resistance did not occur after injection of CaPK alone. Post-ionophoretic measurements of input resistance in twenty-six cells given CaPK alone were no different from those in seven cells given equivalent negative currents through electrodes containing only KCl, nor were levels of resistance increased after injection of CaPK in the cells that subsequently showed increases in input resistance after CaPK plus depolarization. We conclude that intracellular injection of calcium-calmodulin dependent protein kinase followed by depolarization and depolarization-elicited impulse activity transiently increases input resistance of neurons of the motor cortex of cats. The magnitude of increase in input resistance in cells responding to CaPK plus depolarization averaged +5.0 ± 2.5 (s.e.m.) megohms. Protein kinase alone or weak depolarization in the absence of protein kinase did not produce this effect. An analogous increase of input resistance can be produced in the Type B photoreceptor of Hermissenda by applying protein kinase and sufficient depolarization paired with light to increase calcium conductance and internal calcium concentration. This observation led us to perform the present experiments. Given previous studies linking changes in both types of neurons to the development of conditioning, the results suggest the possibility of shared biochemical steps during the acquisition of conditioned behavior by vertebrate and invertebrate species. (Supported by AFOSR and USPHS.)

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[b] ~2.5 nA, 1 min (5-40 units/ml of phosphorylase kinase, Sigma Chemical, in 1.5 M KCl and 0.025 M TRIS buffer and 0.475 M K^+ acetate)

178.7 CELLULAR PHYSIOLOGY OF THE DEVELOPING RAT NEOCORTEX IN VITRO. A.R. Kriegstein*, T. Suppes and D. Prince (SPON: D. Purpura). Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

We have investigated voltage-dependent membrane properties and synaptic physiology of developing rat neocortex which is relatively immature at birth and undergoes considerable structural change during postnatal stages. Sensorimotor cortex slices (300-400 μm) were prepared from rat pups aged 3-26 days postnatal (PN) and maintained in vitro at 37°C using standard techniques. Slices from animals under 5 days were maintained at 34°C. Stable intracellular current clamp recordings were obtained from neurons of all cortical layers (n=42) in slices from animals as young as 3 days PN.

Very immature cells, < 6 days PN had resting potentials (V_ms) from -40 to -70 mV and peak action potential (AP) amplitudes from 40 to 78 mV (n=11) which were followed by hyperpolarizing afterpotentials. Cells with the highest V_ms had AP amplitudes greater than 65 mV and AP durations < 2 msec.[m] Young neurons also tended to have high input resistances (mean R_N = 70 MΩ). The steady-state current voltage curves in cells < 6 days PN were strikingly linear with a steep slope around the resting potential. In cells 12-20 days PN (n=26) the V_m ranged from -52 to -70 mV, peak AP amplitude ranged from 60 to 80 mV, and mean R_N was lower (35 MΩ). The I-V curve became less steep, and non-linearities were present. Tetrodotoxin (10^{-6}M) blocked the APs in cells at all ages suggesting that a significant Na^+ current underlies the AP. Labelled cells from recordings with HRP-filled electrodes (n=7) were mostly pyramidal neurons, suggesting that our data applies primarily to this cell type.

We examined synaptic responses to stimulation at the pial surface or in the subcortical white matter. Extracellular stimulation in animals < 1 week PN evoked long-lasting post-synaptic responses that underwent marked attenuation with repetitive stimulation even at frequencies as low as 0.1 Hz. The PSP attenuation to stimulus frequency decreased with age. No IPSPs were seen in cells < 5 days PN (n=11). Stimulation in cells older than 10 days PN often led to complex responses that contained IPSPs. It is noteworthy that GABA receptor binding and GAD staining become prominent in rat brain at 7-10 days PN (Coyle, J.T. and Enna, S.J., Brain Res. 111:119-133, 1976).

These results indicate that physiological properties of rat neocortical neurons undergo maturational changes during the postnatal period, and may be studied in vitro.

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178.8 SINGLE K^+ CHANNEL CURRENTS FROM HIPPOCAMPAL PYRAMIDAL CELLS OF ADULT GUINEA PIG. R.K.S. Wong and R.B. Clark*. Dept. of Physiology and Biophysics U.T.M.B. Galveston, TX 77550.

Experiments were carried out using individual neurons isolated from guinea pig hippocampus. Single channel currents were recorded from giga-seal patch recordings from whole cells. When the recording pipettes were filled with isotonic (150mM) KCl or K-gluconate solutions, inward current steps of unitary size could be observed from the membrane patch. The amplitude of these single channel currents increased markedly when the transmembrane potential across the patch was hyperpolarized. The single channel conductance derived from this I-V relationship was 250 pS. The conductance of these single channels was not significantly different when recorded from K^+ KCl or K-gluconate containing electrodes suggesting that K^+ is the charge carrier. We observed that the kinetic properties of these single channel current showed no obvious dependency on the transmembrane potential. When the patch membrane was depolarized, the inward current reduced in size and often with an application of -30mV to the pipette (effectively depolarizing the patch membrane by 30mV to about -20mV transmembrane potential) current event can no longer be observed. Additional depolarization beyond -20mV transmembrane potential (Vm) did not cause an inversion of the single channel current and no events were recorded when Vm was in the range of -20mV to +60mV. Often, when Vm was depolarized above +60mV, large amplitude unitary outward currents were again recorded. These unitary outward current events differ from the events recorded at the resting or hyperpolarized membrane potentials in that (1) the open time of the depolarization-activated single channels markedly increased with additional depolarization and (2) their frequency of occurrence also increased with depolarization. The data suggest that single K^+ channels recorded at resting and hyperpolarized Vm may show inward rectification.

178.9 ALTERED TRANSMISSION AT SPARED SYNAPSES AFTER MAUTHNER-CELL AXOTOMY. M. Titmus and D.S. Faber. Div. Neurobiology, Dept. Physiology, SUNYAB, Buffalo, NY 14214.

Each goldfish Mauthner (M-) axon monosynaptically activates 3 or more cranial relay neurons (CRN) (Hackett & Faber, Brain Res. 264:302-306,1983). These in turn project to interneurons which mediate feedback inhibitions of the M-cell, including a chemically-mediated IPSP, called the late collateral inhibition (LCI). We were interested to see if long term axotomy (35-211 days), at a level which spared output synapses to CRNs, altered LCI.

In control and spinal cord-transected fish (8-10mm caudal to the M-cell soma) the LCI was evoked by either 1) antidromic (AD) impulses elicited by current injection into the M-axon, or 2) surface stimulation of the medulla 5-7mm caudal to the M-cell. LCI magnitude was quantified as the % reduction in amplitude of AD spikes or EPSPs (produced via posterior VIII nerve stimulation) evoked during the LCI relative to controls.

In 10 cells from control fish, LCI magnitude ranged from 30-75%; for any one cell, it was essentially equal for both methods of stimulation. Low intensity medullary surface stimulation revealed stepwise gradations in inhibition, with some components occurring at voltages subthreshold to M-cell activation. This fractionated LCI may result from direct activation of individual CRNs. In contrast, intra-axonal or more caudal spinal cord stimulation activated the LCI in an all-or-none fashion at threshold for an M-cell spike which is consistent with the notion that CRN axons do not project to the spinal cord. Three cells tested before and after acute axotomy (up to 2 hrs) showed no change in LCI strength.

In long term axotomized fish, 10 of 15 M-cells tested exhibited frequent failure of LCI following AD stimulation, while surface stimulation still evoked maximal inhibition comparable to that observed in controls. As in controls, low intensities of stimulation produced graded steps of inhibition. For 2 M-cells, AD-evoked LCI was less than half that produced following surface stimulation.

These results demonstrate there is a decrease in LCI in long term axotomized M-cells which most likely occurs at the level of M-cell synapses onto cranial relay interneurons. The apparent destabilization of M-axon output synapses is not a simple consequence of axonal dieback and may reflect loss of a trophic influence of the distal axon on regional specialization at more proximal sites. (Supported by grant NS 15335.)

178.10 REPETITIVE FIRING PROPERTIES OF CAT NEOCORTICAL LAYER V NEURONS IN VITRO. C.E. Stafstrom, P.C. Schwindt, and W.E. Crill. Depts. of Physiology & Biophysics, and Medicine, Univ. Wash. Sch. Med. and V.A. Med. Ctr., Seattle, WA 98195.

Repetitive firing characteristics of layer V neurons were studied in slices of cat sensorimotor cortex, using current clamp techniques and a single-electrode voltage clamp. In response to steps of intracellularly injected current, neuronal firing rate adapts to a steady level with an approximately exponential time course. The relation between firing rate and injected current was linear (range of slopes 11-36 imp/s/nA) for steady firing and for early interspike intervals (ISIs) during adaptation.

The behavior of voltage threshold for spike initiation (V_T) and mean depolarization during the ISI (V) were examined during adaptation and steady firing. V_T and V increase with injected current (and thus with firing rate). Even at the slowest firing rates, V during the ISI is sufficient to activate a previously described persistent subthreshold sodium current (I_{NaP}; Brain Res. 236:221, 1982). Thus I_{NaP} is available to counter outward currents and to boost depolarization throughout the whole firing range of the neocortical neurons.

Injection of current ramps showed that layer V neurons are sensitive to the rate of change of current (dI/dt) as well as its amplitude (I). The use of ramps followed by steady currents demonstrated that the repetitive response lags behind changes in stimulus parameters and does not reach a steady state, even during slow ramps; i.e., the response depends on time as well as on I and dI/dt. Instantaneous firing rates during the ramp increase linearly with time for a wide range of ramp slopes (dI/dt). The instantaneous firing rate of early interspike intervals is also linearly related to ramp slope, for small ramp slopes. Quantitative analysis indicated that the repetitive response during ramp stimulation cannot, in general, be described by a linear combination of amplitude- and rate-dependence, but the use of a time-varying rate-dependence roughly approximates the ramp response in many cells.

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178.11 MECHANISMS OF IPSP DEPRESSION INDUCED BY REPETITIVE ACTIVATION STUDIED IN THE RAT HIPPOCAMPAL SLICE. N. McCarren and E.F. Alger, Dept. Physiol., Univ. Maryland Sch. Med., Baltimore, MD 21201.

Depression of GABA-mediated IPSPs has been postulated to be responsible for the marked increases in hippocampal neuronal excitability which occur during repetitive synaptic stimulation. Hypotheses which might explain IPSP depression include: 1) presynaptic factors, e.g., blockade of GABA release or interneuron firing and 2) postsynaptic effects, e.g., desensitization of GABA receptors, shifts in GABA reversal potential, etc. We used intracellular recordings from hippocampal CA1 pyramidal cells to evaluate the role of IPSP plasticity in tetanic potentiation induced by stimulus trains of 10 Hz lasting 5 sec.

Since GABA-mediated IPSPs can be evoked by orthodromic (O) or antidromic (A) stimuli, repetitive O or A stimulation should produce similar changes of the IPSP if these changes are specific to the IPSP pathways. Responses to A or to subthreshold O trains were similar (peak IPSP amplitudes decreased from approximately 5 mV to 2.5 mV). However, during a suprathreshold O train, the IPSP was usually abolished or inverted and the cell often became depolarized with respect to the resting membrane potential (RMP). Because antidromic IPSPs are relatively uncontaminated by EPSPs, the depression of this IPSP indicates the maximal specific depression of IPSPs.

To assess what factors can account for the greater changes in suprathreshold O IPSPs we first prevented occurrence of depolarizing potentials during the train by recording with CsCl filled electrodes and depolarizing the cells to approximately 0 mV. In these cases IPSPs persisted throughout the train and were depressed from approximately 35 mV to 24 mV. This suggests that alteration of suprathreshold O IPSPs at RMP is not entirely specific.

The use of potassium sensitive microelectrodes showed that suprathreshold O trains were associated with much greater increases in extracellular K ($[K]_o$) than A trains and that increases in $[K]_o$ were directly proportional to concurrently measured shifts in peak IPSPs during a train. Changes in both $[K]_o$ and suprathreshold O IPSPs were augmented by increases in stimulus intensity, frequency of stimulation and length of trains.

We conclude that alterations in the efficacy of GABA mediated synaptic transmission can account for not more than a 50% decrease in IPSP. The greater apparent effects on suprathreshold O IPSPs are probably due in part to their being obscured by burst potentials, and to the effects of K_o on the cells. We are investigating the degree to which K_o contributes to changes in A and subthreshold O IPSPs. Supported by NIH Grant NS17539 and the McKnight Foundation.

178.12 EFFECTS ON INPUT CURRENTS OF LOCAL INCREASES IN MEMBRANE RESISTANCE IN CORTICAL PYRAMIDAL CELL DENDRITES EXPLORED USING A PASSIVE CABLE MODEL FOR DETERMINING THE TRANSIENT POTENTIAL IN A DENDRITIC TREE OF KNOWN GEOMETRY. W. R. Holmes* and C. D. Woody (SPON: R. G. Pay). Depts. of Biomathematics and Biobehavioral Sciences, UCLA, Los Angeles, CA 90024.

Changed neuronal responsiveness observed after conditioning may result from increased membrane resistance since the neurons show increased excitability to intracellularly injected constant (+) current pulses. Rall has proposed that changes in the spine stem resistance may alter the potency of spine synaptic input response and so affect neural excitability. Changes in membrane resistance in the dendritic trunks themselves might also alter excitability. The present study explores this latter possibility by modeling the effects of injecting constant currents in dendrites of known morphology. The morphology was obtained from a montage composed of photomicrographs taken at different, overlapping areas within serial sections of an HRP-injected, layer V pyramidal cell of the cat motor cortex.

A passive cable model which can determine the transient potential in dendritic trees of arbitrary geometry was used to examine the efficacy of different loci of increased membrane resistance for given loci of current injection. The model used the passive cable equation to express the potential for each interbranch segment of the dendritic tree. By matching boundary conditions at branch points and terminations, a system of equations was readily obtained for the Laplace transform of the potential at the ends of each segment. The inverse transform could then be quickly computed for any arbitrary time point. Since only one equation is required for each interbranch segment, this approach uses far fewer equations than the compartmental approach. The method can be used to model voltage clamping or any of a number of current inputs including constant current, δ-function current, or repetitive trains of δ-function current into any or all terminal segments or branch points.

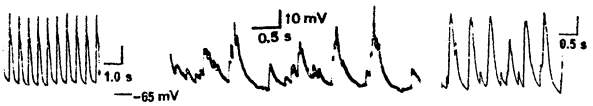
Using the model described above, it was found that an increase in membrane resistance in the region immediately proximal to the point of current input was more effective in increasing soma potential than an increase in a comparable membrane area of a more proximal dendritic region. Under certain circumstances a distal increase in membrane resistance could be more effective than a comparable proximal increase depending on the locus of current injection and the morphology of the dendritic tree. (This research was supported in part by AFOSR F49620-83-C-0077.)

179.1 EFFECT OF EYESTALK REMOVAL ON GLUCAGON-INDUCED HYPERGLYCEMIA IN CRAYFISH. R. H. Leinen* and A. J. Giannini. Northeastern Ohio College of Medicine, P. O. Box 2169, Youngstown, Ohio 44504.

Administration of 0.5 mg glucagon by injection at a coxal membrane caused significant (p 0.05) hyperglycemia in intermolt-stage crayfish Orconectes virilis within two hours (increase to 17.40 + 5.53 mg glucose/100 ml blood filtrate, over normal and vehicle-treated values of 3.87 + 1.87 and 3.98 + 1.32 mg glucose/100 ml blood filtrate, respectively). Early premolt-stage animals, in which stage transformation had been initiated by eyestalk removal 20-24 hours previous to treatment, did not respond to glucagon. In contrast, epinephrine produced a hyperglycemic effect in both stages, showing the availability of a carbohydrate reserve. It is suggested that the action of glucagon depends upon intact eyestalk neuroendocrine function.

179.2 HEART ARRHYTHMIAS IN JUVENILE HORSESHOE CRABS (LIMULUS POLYPHEMUS) MAY BE CAUSED BY POSTEMBRYONIC INCREASES IN THE NUMBER OF CARDIAC NEURONS. D. Gibson. Woods Hole Oceanographic Institution, Woods Hole, MA 02536.

Intracellular recordings from heart muscle fibers of Limulus larvae and juveniles show that the neurogenic heart rhythm is well coordinated when the larvae hatch, but becomes irregular after the first juvenile molt. The rhythm stabilizes after subsequent molts but out-of-phase EJPs are still more frequent than they were in the larval heart.



Larva 1st juvenile molt 3rd juvenile molt

The arrhythmia at the first juvenile molt may occur as additional neurons become wired into the cardiac ganglion. At the first juvenile stage, the ganglion contains about 75 neurons; by the next molt, the number has doubled (whole mounts, cresyl violet Nissl stain), still short of the average 231 neurons in the adult cardiac ganglion (Bursey and Pax, 1970. J. Morph. 130:385).

In the lobster embryo, all four pacemaker and five follower neurons are present when heartbeat begins, and these cells grow larger during embryogeny (Burrage and Sherman, 1978. Cell Tiss. Res. 188:171). In contrast, a first-molt Limulus juvenile has less than a third of the adult number of cardiac neurons, and these do not increase in size with the next few molts; instead, their numbers increase. Neurons at these stages are too small to be recognized as pacemakers or followers by size as they can in the adult (40 μm vs. 60-120 μm; Bursey and Pax, 1970).

It is clear that many Limulus cardiac neurons arise during post-embryonic growth; exactly how this occurs has not been determined.

179.3 RECEPTOR POSITION ON THE CERCUS DETERMINES SYNAPTIC CONNECTIVITY Jonathan Bacon and R.K. Murphey, Department of Biology, SUNY, Albany, NY 12222.

The cercus-to-giant system of the cricket has become a focus of considerable attention for developmental neurobiologists, the problem being the familiar one of connecting an array of sensory neurons to their interneuronal targets in an orderly fashion. In this system, the presynaptic elements are the sensory neurons of the wind-sensitive cercal filiform hairs, and the postsynaptic elements are the giant interneurones. Previous work had established that there are 4 main types of filiform receptor, each of which responds to wind from a different direction; the location of these hair types on the cercus was not known. However, it was known that the interneurons derive their directional selectivity by making specific connections with some of these cells (Tobias and Murphey, J.c.Physiol. 129;51, 1979).

With this knowledge, we began our analysis by mapping the location of receptors of different type and found that receptors of the same directional preference are localised on the cercus in longitudinal strips. Straight borders occur between fields of different hair type; adjacent receptor fields can be sensitive to wind from polar opposite directions. We've known for some time that afferents project into the cercal glomerulus in a topographic fashion. We now provide a functional interpretation of this topographic mapping function by showing that each receptor type terminates in a different region of the glomerulus - in other words, the glomerulus is functionally divided into 4 regions concerned with the processing of wind information from different directions.

In order to test the significance of this finding, we needed to show monosynaptic connections between the sensory cells and wind-sensitive interneurons in the CNS. We have used the anatomy to guide the search for connections. We made simultaneous recordings from interneuron 10-3 and from a sensory neuron whose terminal overlaps the interneuron's dendrites. In approximately 25% of the cases where we knew there was anatomical overlap, we observed unitary synaptic potentials synchronised with the sensory neuron action potentials. These synaptic potentials occurred at a constant latency and were highly susceptible to fatigue. In cases where sensory neurons did not anatomically overlap 10-3's dendrites, we saw no such potentials. We conclude that the position of an interneuron's dendrites within the sensory projection determines synaptic connectivity. When sensory neuron and interneuron overlap, connections are made on a probabilistic basis. These connections shape the receptive fields of the first order interneurones and ultimately shape the behavior of the animal.

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179.4 POSITIONAL INFORMATION DIRECTS SYNAPTOGENESIS BETWEEN SENSORY NEURONS AND THEIR TARGETS. W.W. Walthall, J.P. Bacon, and R.K. Murphey. Neurobiology Research Center, SUNY Albany, Albany, N.Y. 12222.

The theory of positional information attempts to explain how biological patterns are formed. It is based upon the idea that a series of chemical gradients uniquely specifies cells within a developmental field.

The positional information model predicts certain types of regeneration where tissues from non-adjacent locations are brought together. Where the appostion occurs epidermal cells begin to proliferate and, through a process called intercalation, restores normal neighbors. The cricket cercus is such a developmental field and the sensilla on the cercus form a highly ordered receptor pattern. Work from this laboratory has established that sensory neurons project from the periphery to the central nervous system in an orderly way. Here we provide evidence that positional information not only determines the organization of the periphery but also determines the formation of orderly synapses between sensory neurons and their interneuronal targets.

In order to test the positional information hypothesis, strips of cercal tissue from a black cricket were transplanted to a different position on the cercus of a white cricket. These chimera make it possible to distinguish graft from host derived receptors. Such grafts induce the generation of new receptors near the graft margin. The transplant contained receptors that would never synapse with a particular interneuron. However, the graft was selected so that if the positional information hypothesis were true, the graft tissue should generate new receptors that would synapse with the interneuron. After the graft had healed and intercalation occurred, newly generated afferents near the graft boundary were examined. When assessed physiologically unitary postsynaptic potentials were detected between a newly generated, graft derived sensory neuron and the appropriate target. When assessed anatomically, the terminal arbor of the sensory neuron was seen to overlap the interneuron of interest.

These results are consistent with other experiments that suggest positional information underlies the construction of orderly patterns of receptors. They go one step further by directly implicating positional information in the formation of orderly synaptic connections. Supported by NIH grant #NS15571.

179.5 CHOICE OF SYNAPTIC PARTNERS BY SENSORY NEURONS IN THE CRICKET. R.K. Murphey, Neurobiology Res. Ctr., SUNYA, Albany, Albany, N.Y. 12222.

Numerous studies of the insect nervous system focus on the way in which sensory systems are assembled: either by examining the initial choices the growth cones of sensory neurons make embryonically or by observing the effects of surgical or genetic maneuvers which displace them. Studies of the cercal system of crickets have revealed two main regions of neuropil which receive cercal sensory afferents. By staining the sensory cells with cobalt, it was possible to show that all of the "bristle hairs" on the cercus, which are multiply innervated, project to an anterior, ventral region of neuropil. It has been shown elsewhere that the other hair types (filiform and clavate) project to a separate region which has become known as the cercal glomerulus. I have located six previously unidentified interneurons whose dendrites are located in the neuropil innervated by the bristle hairs. These newly discovered interneurons are sensitive to touching the body surface and are insensitive to stimulating other receptors on the cercus such as the filiform hairs. Thus when cercal sensory neurons first reach the nervous system during development, they make a crucial choice between two target areas. This dichotomous choice determines their synaptic partners since different subsets of interneurons are available for synaptic contact in the two regions.

This choice is repeated in every ganglion. For example, when cerci were transplanted to the thorax, bristle hair sensory cells from the cercus arborized in a ventral region of neuropil which is distinct from the region which receives the filiform afferents (Johnson and Murphey, this volume).

Thus a multitude of external insect receptors can be assigned to only two categories based on their target area. What is it about these two categories of sensory cells that distinguishes them? One obvious difference is that most hairs which I have called "bristles" are multiply innervated while the filiform and clavate hairs are singly innervated. Thus they have different lineages; bristles have an extra round of division in their lineage. This lineage difference may determine the growth of their processes and thereby their choice of synaptic partners.

Supported by NIH Grant #NS 15571

179.6 CELLULAR INTERACTIONS BETWEEN A MUSCLE AND ITS MOTOR NEURON ARE NOT INVOLVED IN THEIR ENDOCRINE-MEDIATED DEATHS DURING METAMORPHOSIS IN THE TOBACCO HORNWORM MANDUCA SEXTA. Janis C. Weeks and James W. Truman. Dept. Zoology, Univ. of WA, Seattle, WA 98195.

Metamorphosis in Manduca involves the reorganization of the musculature as well as of central motor circuits. Some muscles persist unchanged throughout the insect's life, some undergo programmed death at specific times, and some muscles arise anew from retained myoblasts during adult development. Individual motor neurons also undergo different metamorphic fates: some innervate the same muscle throughout whereas others experience the death of their original larval target muscle. In the latter case, the targetless motor neurons either persist and come to innervate newly generated muscles or else they die(Levine & Truman, 1982). We have examined an example in the last class, in which a motor neuron and its muscle die together. The muscle is the principal planta retractor muscle (PPRM) of the caterpillar, contraction of which retracts the planta (the distal tip of the abdominal proleg) and disengages the proleg from the substrate. PPRM is innervated by a homosegmental motor neuron, PPR. During the larval-pupal transformation the prolegs are lost and both PPRM and PPR die. Using electrophysiological and anatomical techniques combined with hormonal manipulations we have investigated the cues responsible for these events in order to determine if the fates of the muscle and motor neuron are linked, or whether their deaths are triggered independently. Our results show that a rise in the blood ecdysteroid titer 3 d. prior to pupation triggers the degeneration and death of PPRM as well as some initial dendritic loss in PPR. However, PPR survives the death of its target muscle and does not die until 2 d. after pupation. This delay occurs because PPR becomes committed to die only after it sees the falling phase of the prepupal ecdysteroid peak. Neither dendritic loss nor death in PPR appear tied to the death of PPRM, as chronic removal of the muscle does not affect the morphology of the motor neuron. We conclude that the programmed deaths of the muscle and its motor neuron result from their differential sensitivity to the rising and falling phases, respectively, of the prepupal ecdysteroid peak, and not from any interactions between them. Possible functional reasons for the staggering of the time of their deaths are under consideration.

Levine, R.B. & Truman, J.W. (1982) Nature 299:250-252.

179.7 DEVELOPMENT OF LEECH SEROTONIN NEURONS EXAMINED WITH SEROTONIN ANTIBODY, CELL LINEAGE TRACER, AND CELL KILLING. Duncan K. Stuart, Joel C. Glover*, Seth S. Blair*, and David A. Weisblat. Dept. of Molecular Biology, University of California, Berkeley, CA 94720.

Antisera against serotonin was used to follow the origin and differentiation of identifiable serotonin-containing neurons in two glossiphoniid leeches. Antibody staining combined with cell lineage tracers shows that all these cells are derived from a bilateral pair of blastomeres, the N teloblasts. These neurons start staining in a specific order, but some subsequently disappear and are not seen in the adult. Ablation studies suggest that competitive interactions determine which cells disappear.

Each of the first three unfused segmental ganglia of the adult (Haementeria ghilianii or Helobdella triserialis) has nine serotonin neurons: one giant Retzius cell pair; three smaller pairs, the anteromedial, dorsolateral and ventrolateral pairs; and one posteromedial unpaired neuron. The remaining 18 unfused ganglia lack the anteromedial pair, the sex ganglia (5&6) lack one lateral pair, and the posterior 14 (8-21) lack the unpaired neuron. The embryonic origin of these neurons was determined by injecting early blastomeres with a rhodamine-labeled lineage tracer, then raising the embryos to nervous system differentiation (stage 10-11), staining for serotonin with a fluorescein labeled second antibody and examining the nerve cord for double labeling; we find all serotonin cells are derived from the N teloblast pair.

During neurogenesis (stage 9) the serotonin neurons begin to stain in a specific order. In any given ganglion the Retzius cells appear first, followed shortly thereafter by the lateral cell pairs and, in ganglia 1-3, the anteromedial pair. The posteromedial neuron appears last, at the end of stage 9. However, initially a pair of posteromedial neurons is seen in the first seven ganglia, one of each pair ceases to stain shortly thereafter. Also, one of the two lateral pairs disappears in the sex ganglia. Thus, some neurons which first show serotonin staining either die or cease to synthesize or sequestor serotonin.

Two lines of evidence suggest that competitive interactions may be involved in determining which of the two embryonic posteromedial neurons survive to become the unpaired cell of the adult. First, lineage tracer studies show that in each of ganglia 1-7 the unpaired cell of the adult arises with equal likelihood from either N teloblast. So, this disappearance is not determined strictly by lineage. Secondly, if a single N teloblast is killed by DNase I injection, so that only one posteromedial cell appears, this cell always survives and contains serotonin. This implies that interactions between the N-derived cells on the two sides of the nerve cord, possibly between the pair of posteromedial neurons themselves, are involved in the disappearance of one of each pair.

179.8 A MONOCLONAL ANTIBODY SPECIFIC TO THE LEECH PRESSURE-SENSITIVE MECHANOSENSORY NEURONS. C. Loer*, C. Schley*, B. Zipser, and W. Kristan. (SPON: J. Lamborghini) Dept. of Biology, Univ. of California, San Diego, La Jolla, CA 92093 and Cold Spring Harbor Laboratory, NY 11724.

Two pairs of pressure-sensitive mechanosensory neurons (P cells) are found in the standard midbody ganglion of the glossophoniid leech, Haementeria (Kramer and Goldman, J. Comp. Physiol. 144:435, 1981). We have produced a monoclonal antibody specific to the somata and neurites of these cells and to a small number of unidentified fibers. The antibodies were prepared by immunizing Balb-C mice with lightly-fixed, homogenized Haementeria nerve cords. Their spleen cells were fused with myeloma cells to produce hybridomas. After a standard HAT selection, the clones were screened on paraformaldehyde-fixed ganglia of adult Haementeria using an HRP-conjugated secondary antibody and staining with DAB and H_2O_2. Four large cell bodies stain in the standard adult ganglion along with their neurites in the neuropil, peripheral nerves, and connectives. A few as yet unidentified fibers are found more medially in the neuropil and connectives; these also sometimes appear to be sending branches out the nerves to the periphery. Some specialized ganglia stain differently: the two sex ganglia have only one pair of cell bodies staining; the last two unfused ganglia have one pair of large cell bodies and one pair of small cell bodies which stain. The tail brain, composed of 7 fused ganglia, has cells staining that appear to be the segmental homologues of the P cells; the head brain, composed of 4 fused ganglia and a supraesophogeal ganglion, segmental homologues are again apparent as well as a few additional cell bodies.

We have found as well that the antibody cross-reacts with another glossophoniid leech, Helobdella triserialis, in which experimental manipulations of P cell progenitors are possible. We are now using the antibody to examine the consequences of ablations and other experimental manipulations of P cell progenitors. We are investigating the possible function of the antigen by a variety of means. First, the antigen appears by light microscopic observations to be located both on the surface and inside the cell; these observations will be confirmed by other means. Second, the molecular weight of the antigen will be sought. Third, the antibody will be applied to live ganglia while recording from the P cells and their postsynaptic targets.

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179.9 DIFFERENTIATION OF SEROTONIN-CONTAINING NEURONS IN THE LEECH. J.C. Glover* & D.K. Stuart (Spon: L. Henderson). Grad. Group in Neurobiology & Dept. of Molecular Biology, UCB, Berkeley, CA 94720.

The leech Haementeria ghilianii contains 5 types of segmentally iterated serotonin-containing neurons in the ventral nerve cord. In the adult, neurons of each type contain serotonin in high concentrations, sequester serotonin with a high-affinity uptake system, and have characteristic morphologies. Determining the temporal sequence of appearance and maturation of such properties lends insight into the mechanisms whereby neurons differentiate. Consequently, the development of these properties has been examined during embryonic life. The appearance of transmitter store has been investigated using commercially available serum antibodies against serotonin. Because these neurons stain very early, it has also been possible to use these antibodies to study morphological development. The appearance of the high-affinity uptake system has been examined with autoradiography following exposure to ^{3}H-serotonin.

As described in the previous abstract (Stuart, et al.) there is a type-specific sequence of appearance of transmitter store in these neurons. The appearance of the uptake system follows this same sequence, and in fact appears nearly synchronously with the antibody staining. This is supported by the observation that bathing embryos in 10^{-5} - 10^{-3} M serotonin before immunohistological processing leads to an overall increase in intensity of staining without labelling any additional cells. At the time antibody staining is first detectable, the Retzius cells are morphologically immature, having extended only a short neurite ending in a growth cone within the ganglion. This entire structure labels with both antibody and ^{3}H-serotonin. It is tempting to speculate that in addition to containing and sequestering serotonin, the Retzius cell may be able to release serotonin at this early stage as well.

Further morphological development of the Retzius cell involves a progressive branching of the initial neurite such that axons are extended in both ipsilateral connectives and all ipsilateral peripheral nerves. Although the Retzius cells in all segments undergo this morphological sequence, in the adult there are segmental differences. In segments 5 and 6 the Retzius cells have much smaller somata and are missing many of their axons. The former difference occurs during embryogenesis and is due to an early termination of somal growth. It is not yet known when or how the latter difference arises, but it is clear that at some point during development the original morphology is modified. The factors responsible for this modification are being sought.

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179.10 DEVELOPMENT OF AFTERDISCHARGE IN THE BAG CELLS OF JUVENILE APLYSIA. D. Leibowitz* and V. F. Castellucci (SPON: C. D. Toran-Allerand). Center for Neurobiology and Behavior, Columbia University, College of Physicians & Surgeons, and New York State Psychiatric Institute, New York, N. Y. 10032.

The bag cells of Aplysia californica reared in the laboratory have been studied with intracellular and extracellular techniques in order to analyze the changes in their electrophysiological properties during maturation.

Individual bag cells of A. californica are responsive at least 2½ weeks prior to sexual maturity. As in the adult, immature bag cells in the isolated abdominal ganglion are normally electrically silent. In marked contrast to the adult, bag cells in immature animals always show depolarizations associated with each suprathreshold shock to the ipsilateral and contralateral connective nerves. Their apparent absence in mature animals may be due to increasing distance between synapses and recording sites, or to a reorganization of the synapses themselves. Also in contrast to the adult, the bag cells of immature animals are not electrophysiologically homogeneous but fall into two classes. Cells of the first class can be caused to spike by injection of depolarizing current. Upon repetitive stimulation of the connectives the evoked depolarizations show potentiation and can give rise to full regenerative spikes of brief duration (less than 10 msec). The firing of these cells can outlast the triggering shock, older animals being capable of longer bursts. This appears to be the precursor of the adult-type afterdischarge. This firing is correlated with extracellular compound spikes, localized to a single cluster, which are of lower amplitude than those of the second class of bag cells which may fire concurrently. These extracellular spikes show a progressive increase in amplitude (from 1 μV to adult values), duration of afterdischarge, and degree of bilateral synchrony during development.

A second physiological class of bag cells, prominent in immature animals and still present in recently-matured animals, shows no potentiation of the post-shock depolarizations. Temporal summation to threshold can trigger an intracellular spike of relatively long duration. These spikes occur only one at a time, never repetitively, and the cells become readily refractory to further spiking. The spikes are correlated with extracellular spikes relatively high in amplitude; both clusters usually participate simultaneously, although the low amplitude spiking by the same cluster is unilateral. Cells of both classes have been observed in a single cluster, and neither shows intracellular depolarization correlated with extracellular spikes due to the other class.

We intend to extend these observations to younger juveniles, characterize the ionic requirements of these two classes of action potentials, and investigate their fate and functional significance.

CEREBELLUM: OLIVO-CEREBELLAR FUNCTION

180.1 SENSORY-OCULOMOTOR INTERACTIONS IN PRIMATE CEREBELLAR VERMIS: A ROLE IN SMOOTH PURSUIT CONTROL. D.A. Suzuki* E.L. Keller. Smith-Kettlewell Inst. Visual Sciences, San Francisco, CA 94115.

Neuronal activity related to the sensory and oculomotor information necessary for an internal construct of angular target velocity has been recorded in the vermis, but the component signals were studied as dissociated vestibular, vision, and eye movement-related activities. Since smooth eye movements seldom occur dissociated from sensory information, it was of interest to clarify how the vermis processes concurrent sensory and oculomotor information.

Monkeys were trained to track a 0.5 deg spot during simultaneous presentation of vestibular or visual stimuli. During the performance of these tasks, extracellular recordings were made from Purkinje cells (Pc) in lobules VI and VII of the cerebellar vermis (vermis-6,7). In addition, the affect of lesions of vermis-6,7 on smooth-pursuit eye movements was investigated. Out of 42 units that were tested, 37 (88%) exhibited some kind of interaction. Nineteen (out of 22) exhibited modulations in discharge rate related to all three components of target velocity, i.e., eye, head, and retinal slip velocities. Fifteen (out of 16) exhibited interactions between eye and head velocity-related signals, and 3 (out of 4) showed intereactions between eye and vision-related signals.

A monotonic relationship was observed between the amplitude of modulation of Pc activity and the magnitude of eye velocity during concomitant smooth pursuit and vestibular stimulation that were in or out of phase to various degrees. Cells that exhibited responses of differring directional preferences to smooth pursuit and background pattern movement, exhibited a predictable, facilitated response during tracking of a target on a stationary, random dot background pattern. Vermis-6,7 is similar to the flocculus in its processing of eye and head movement-related information, but differs in its additional role in the processing of eye and vision-related activity. While the flocculus supplies a gaze velocity signal, vermis-6,7 generates a target velocity signal.

Removal of the posterior vermis resulted in the expected saccadic dysmetria, but also caused a significant drop in smooth-pursuit gain. Over the range 0.1 to 0.75 Hz, ± 10 deg, a 30-50% reduction in smooth pursuit gain was observed. These gain reductions are similar in magnitude to those observed with floccular lesions (Zee et al.). The results compliment the extracellar recording studies in supporting a role for vermis-6,7 in the regulation of smooth pursuit eye movements.

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180.2 TASK-ORIENTED ADAPTATIONS OF COCONTRACTION OF OPPOSING MUSCLES (TUNING) DEPEND ON THE NEOCEREBELLUM. V. B. Brooks and S. Watts*. Department of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

Performance of a step-tracking task made by fascicularis monkeys was studied. The animals guided a handle into alternating target zones by making elbow flexions and extensions in the horizontal plane. During task learning accelerations and decelerations are scaled together, so that velocities assume 'continuous' single-peaked profiles without oscillations. These movements are called 'programmed' because they can be made from memory, once learned.

Accurately made, programmed movements become faster during training, even though the animals are not rewarded for speeding-up. Speed is increased (by about 1 rad/s) through strengthening of initial agonist muscle bursts and weakening of antagonist pre-movement cocontraction. This 'tuning' increase of reciprocal inhibition of accurately programmed flexions and extensions presumably optimizes muscle use, within the limits of task conditions. Changes carry over on average from one training session to the next. Joints are 're-tuned' towards greater or lesser cocontraction (and thus joint stiffness) as the need arises, for instance after alterations of targets or loads.

These task-oriented adaptations of patterns and intensities of reciprocal muscle actions are likely to be mediated through controls descending to spinal Ia-interneurons. It is well known that control of adaptability is lost after neocerebellar damage in human patients. The same was found to be true during reversible neocerebellar dysfunction in monkeys.

Cooling of the neocerebellar output through the dentate nucleus, or input through the principal nucleus of the inferior olive, reversibly diminishes the number of accurately programmed movements. Those that remain during cooling however, are made at speeds reduced by about 1 rad/s, as if the joints have reverted to pre-tuning stiffness. (calculated from data published with A. D. Miller, and with P. R. Kennedy and H.-G. Ross, in Exp. Brain Res. 1982, 45: 328-332 and 47: 95-104.

It is concluded that task-oriented adaptations of joint stiffness before movement onset (tuning) are routine parts of motor learning, which may be a means of acquiring greater motor skill. This depends on neocerebellar adjustments, including the functions of its olivary input.

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180.3 THE RELATIONSHIP BETWEEN SIMPLE AND COMPLEX SPIKE RESPONSES OF CEREBELLAR PURKINJE CELLS LOCATED IN IDENTIFIED CORTICONUCLEAR ZONES. T.J. Ebner, Q. Yu* and J.R. Bloedel. Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Mpls., MN 55455.

In decerebrate, unanesthetized cats the relationship between the responses of Purkinje cells to natural forepaw stimuli and their location in identified corticonuclear zones was examined. Purkinje cells recorded extracellularly with glass microelectrodes on the surface folia of lobules Va-c were identified by the presence of spontaneous complex spikes. Each cell's site of termination was determined by the location within the fastigial (F), anterior interposed (AI) and posterior interposed (PI) nuclei from which they could be antidromically activated. In most experiments cells were sequentially isolated at 100-300 micron intervals in the sagittal direction. Each cell's simple and complex spike responses to a brief flexion of the ipsilateral forepaw were discriminated and used to construct peristimulus histograms. Contour maps of the simple and complex spike responses of several Purkinje cells recorded on the same folium were constructed to visualize the relationship between response amplitudes and the boundaries of the corticonuclear zones. The results obtained from over 350 Purkinje cells reveal an extremely strict boundary between the cells projecting to the fastigial (F zone) and anterior interposed nuclei (AI zone) at 2.1 mm from the midline. The border between the zones projecting to the anterior and posterior interposed nuclei (PI zone) was located between 3.5 and 3.8 mm from the midline. The contour maps of the simple spike responses revealed areas of simple spike modulation beginning at the border of the F and AI zones and extending throughout the AI zone. Usually a second locus of simple spike modulation was present in the F zone. Similarly in the PI zone the simple spike activity exhibited extensive modulation. The complex spike contour maps showed loci of increased activity at the same locations as the regions where simple spike activity was well modulated. These results suggest that Purkinje cells in all of the corticonuclear zones examined, F, AI and PI zones, receive and process information concerning the same forepaw stimulus. Furthermore, the tight spatial coupling between the location of simple spike and climbing fiber responses suggest mutual interaction between these two afferent systems. Supported by NIH Grants RO1-NS 18338 and RO1-NS 09447.

180.4 SIMULTANEOUS SAMPLING OF THE RESPONSES OF MULTIPLE, CLOSELY ADJACENT, PURKINJE CELLS RESPONDING TO CLIMBING FIBER ACTIVATION J. Bower and R. Llinás. Dept. Physiol. Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

In vitro studies of the inferior olive (IO) have demonstrated that changes in the membrane potential in IO neurons generate a sequence of electrophysiological responses that produce oscillatory spiking. This oscillatory behavior is enhanced by the alkaloid, harmaline (Llinás & Yarom, J. Physiol. 315: 549, 1981; Yarom & Llinás, Soc. Neurosci. Abst., 1981). Furthermore, in vitro as well as in vivo experiments have shown electrotonic coupling between IO neurons.

We have investigated the significance of these biophysical properties on the global organization of the IO by sampling the climbing fiber (CF) responses recorded in cerebellar Purkinje cells using a recently developed method for simultaneous sampling and analysis of extracellular action potentials (Bower & Llinás, Soc. Neurosci. Abst., 1982). Recordings have been made of spontaneous as well as tactile evoked activity of multiple, closely adjacent Purkinje cell complex spikes in crus IIA of the cerebellar cortex of rats. Autocorrelations of single CF activations reveal that these cerebellar afferents fire spontaneously with an average minimum interburst interval of 100-125 ms. Harmaline (25 mg/kg, IV) increases the number of such activations without changing the average minimum interval. The spatial distribution of cross correlation between different units demonstrates a preferred parasagittal orientation, indicating a non-isotropic distribution of the relatedness of CF activity over the cerebellar cortex. These crosscorrelelograms have peaks at 0 and 100-125 ms. Thus, CFs projecting to a given area of cerebellar cortex tend to burst simultaneously at regular, repeated intervals. Investigation of interaction between this inherent group oscillation and peripheral tactile stimuli (e.g. upper lip) indicates a phase relation between intrinsic oscillatory pattern and the probability of a CF response. Because adjacent CFs tend to fire simultaneously (same oscillatory phase), a given successfully timed stimulus is likely to be simultaneously activating many CF afferents. Conversely, the intrinsic oscillatory behavior of the IO may in turn be influenced by robust peripheral stimuli which can reset the oscillatory activity of the CF activation to a given Purkinje cell 'group'. Because the specific CFs constituting such a 'group' vary with time and stimulus condition, with some CFs missing a given interval while others join transiently, grouping represents more a functional state than an anatomical constraint of the IO, suggesting modulation of electrotonic coupling in this nucleus (Llinás, The Physiologist 17: 19, 1974). Supported by USPHS grants NS13742 and NS06958.

180.5 INCREASED RESPONSIVENESS OF PURKINJE CELLS TO PARALLEL FIBER STIMULI ASSOCIATED WITH SPONTANEOUS CLIMBING FIBER INPUTS. J.R. Bloedel and T.J. Ebner. Depts. of Neurosurgery and Physiology, Univ. of Minnesota, Mpls., MN 55455.

Recent work in our laboratory has demonstrated that the occurrence of spontaneous or evoked climbing fiber inputs is associated with an increased responsiveness of Purkinje cells to mossy fiber inputs activated by natural peripheral stimuli. These experiments were designed to evaluate the hypothesis that this change in responsiveness to mossy fiber inputs results from interactions occurring in the cerebellar cortex. In decerebrate unanesthetized cats Purkinje cells identified by their depth and presence of spontaneous complex spikes were isolated on the surface folia of lobule V using conventional electrophysiological techniques. The discriminated simple spike responses to a surface electrical stimulus (LOC) were used to construct peristimulus time histograms (N=100) under two conditions. In the first or 'random' paradigm, the LOC stimulus was applied randomly with respect to the occurrence of spontaneous complex spikes in the same neuron. In the second or 'climbing fiber conditioning' paradigm LOC stimulation of the same stimulus intensity was timed to occur at fixed intervals (20-100 msec) after the occurrence of spontaneous complex spikes. Comparing the response amplitude to the LOC stimulus in the random and climbing fiber conditioned paradigm showed that the simple spike response amplitude was increased when the response to the surface stimulus occurred 30 msec after a complex spike. This increase in responsiveness was independent of the type of simple spike modification produced by the LOC stimuli. In Purkinje cells where the response to the LOC stimulation consisted of an increase in the simple spike discharge rate, climbing fiber conditioning augmented the amplitude of the excitatory response. In cells responding to the LOC stimulus with a decrease in simple spike discharge rate, climbing fiber conditioning resulted in a further reduction of activity, accentuating the inhibitory response. These results support the hypothesis that a change in the gain of the simple spike response is associated with a climbing fiber input and that this gain change results from interactions in the cerebellar cortex. Supported by NIH Grants RO1-NS 18338 and RO1-NS 09447.

180.6 Sensory and Motor Properties of the Inferior Olive of Alert Cats, Reuben Gellman, Alan Gibson, and J.C. Houk, Northwestern University Medical School, Chicago, IL. 60611

We have recorded 226 cells in the inferior olive of 5 alert cats, and determined their responses to sensory stimuli. Somatosensory cells, which were found in all major olivary regions, were more responsive than in the anesthetized animal (see Gellman et al., J. Comp. Neur., in press), only 10% of cells being classified as unresponsive. Of somatosensory cells 51% were classified as receiving cutaneous input, and 47% as proprioceptive. It is probable that many of the former received proprioceptive input as well.

Cells responding to light tactile stimuli were found primarily in the dorsal accessory olive (DAO) (72% of 67 cells in this area), and such cells were frequently encountered in the medial accessory nucleus (MAO; 32% of 90) as well as in the principal nucleus (PO; 31% of 51). The response to a stimulus usually consisted of one spike. The receptive fields of 90% of cells with cutaneous input were strictly contralateral, and were usually restricted to a small part of one limb. Although many cells were very sensitive and responded whenever the limb contacted an obstacle, cells with receptive fields on the plantar surface of the paw generally failed to respond when the animal placed its paw on the surface on which it was standing.

Cells responsive to proprioceptive input were usually activated by rotation at a joint or small displacements of the platform on which the cat was standing. For most cells tested one direction of displacement produced an optimal response; the opposite direction failed to evoke a response; and displacements in other directions produced responses of intermediate probability. Input was usually from the contralateral limb, but 18% of cells had ipsilateral or bilateral input. In no case did we note a reliable response to active movement by the cat, although some cells seemed to be weakly modulated during movement.

We microstimulated (20-50 uA, 100-200 usec pulses, 1-5 sec, 10-60/sec) in all olivary subdivisions (20 locations) while the animal was at rest and during active movement. In no case was a movement produced, interrupted or modified in any noticeable way by the stimulus.

It appears that the inferior olive reliably transmits sensory stimuli produced by an external agent, but not those produced as an intentional consequence of the animal's movement. Supported by 1-PO1-NS17489-02.

180.7 EFFECTS OF REVERSIBLE LESIONS AND STIMULATION OF THE INFERIOR OLIVE ON FLOCCULUS PURKINJE CELL ACTIVITY IN THE CAT. D. A. Echelman*, J. L. Demer* and D. A. Robinson (SPON: R. J. Leigh). Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Reversible lesions and electrical stimulation of the inferior olive (IO) or climbing fiber (CF) decussation (dec.) rapidly alter CF input to the cerebellum, and reversibly alter vestibulo-ocular reflex gain. Alteration of Purkinje cell (P-cell) activity during these lesions would be inconsistent with Marr's hypothesis that CF activity selectively modifies the efficacy of parallel fiber synapses on P-cells during motor learning.

We recorded simple-spikes (SSs) and complex spikes (CSs) of isolated P-cells in the flocculus of 13 cats for 1-4 hr periods. Results were obtained using ketamine/acepromazine anesthesia. An electrode cannula assembly was positioned in the IO or CF dec. to evoke CSs at 50-300 μA. Then 1-10 μL injections of saturated lidocaine were made to reversibly abolish CSs for periods of 10-50 min. Electrode and cannula sites were later verified histologically.

Before CF lesions, mean SS rate over 5 min for 11 cells was 23 Hz (range 10-51), but the rate during the lesions was 40 Hz (range 23-84), a mean increase of 98%. These changes are larger than those observed in a study using smaller injections in rabbits (Leonard & Simpson, Soc. Neurosci. Abstr., 1982). To investigate SS rate variability, we computed the standard deviation (SD) of SS rate (10 s bins) for each of these P-cells. SD decreased from a mean of 6.9 Hz (range 3.0-12.0) before to 3.5 Hz (range 0.8-7.2) during the lesions. Peak SS rate (200 ms bins) increased from 63 Hz (range 30-100) before to 86 Hz (range 35-135, n = 9) during the lesions. CSs recovered gradually after lesions, and repeated lidocaine injections reproduced the above effects. As a control, after CF axons were mechanically severed using the cannula for 2 cells in 1 cat, mean SS rates increased from 65 to 71 Hz and 66 to 85 Hz, but did not change further following injection of lidocaine into the CF dec. The effect of evoked CSs on SS rate was studied in 15 P-cells by electrically stimulating the CF dec. Rates of evoked CSs at or above thresholds of 5 ± 2 Hz (mean ± SD) completely inhibited SSs, and threshold had a weak positive correlation to spontaneous SS rate. For comparison with SS activity during anesthesia, 6 P-cells were isolated in the cerebellum of 1 alert cat. Mean SS rate was 39 Hz (range 20-72), with a mean SD for each cell of 10 Hz (range 6-18).

Because P-cell activity and variability are altered during CS rate enchancement or abolition, our results favor a role for climbing fiber activity in modulating ongoing P-cell responsiveness, rather than in plastically altering the efficacy of P-cell synapses.

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180.8 SHORT-TERM AND LONG-TERM EFFECTS OF FLOCCULAR LESIONS ON OPTOKINETIC AND VESTIBULOOCULAR REFLEXES OF RABBITS. N. H. Barmack, Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR.

The role of the cerebellar flocculus in the modulation of optokinetic and vestibuloocular reflexes has been examined in rabbits which have received unilateral suction ablations of the left flocculus using three different surgical approaches: 1) A dorsal approach, which causes partial damage to the ansiform and paramedian lobes, 2) A caudal approach, which damages the paraflocculus and lateral cerebellar nucleus, and 3) A lateral approach, which causes no damage to other cerebellar structures. The horizontal vestibuloocular reflex (HVOR) (±10 deg, .02-.80 Hz) and the monocular optokinetic reflex (HOKR) (70 x 70 deg, 00.1-50.0 deg/sec) were tested in a group of 19 pigmented and albino rabbits before, immediately after (within 24 hr) and at least 50 days after unilateral suction ablations were made of the left cerebellar flocculus. Eye movements were recorded with an infrared light projection technique.

The immediate effect, observed within 15 min following placement of a floccular lesion, was a conjugate nystagmus with the slow phase toward the side opposite to the lesion when the animal was placed in total darkness. This spontaneous nystagmus lasted from several hours to two days depending on the extent of other cerebellar structures damaged. This nystagmus was reversed in sign if the subjacent vestibular nuclei or vestibular nerve were damaged by the operation. The nystagmus did not appear if the unilateral floccular lesions were made in animals which had been previously bilaterally labyrinthectomized. This spontaneous drift of the eyes observed immediately postoperatively caused a bias in measurements of the HVOR and caused a near-normal gain of the HOKR of the left eye measured in the posterior-anterior direction (same direction as the slow phase of the nystagmus) immediately postoperatively. This rightward bias of the eyes was frequency dependent; less evident at higher stimulus frequencies. When measured 50 days postoperatively, the HVOR had a normal gain and normal bias. When measured 50 days postoperatively, the monocular HOKR (posterior-anterior stimulation of the left eye) was significantly reduced in gain at stimulus velocities below 5 deg/sec.

A quantitative anatomical analysis of the floccular lesions revealed that they caused degeneration of neurons in the contralateral dorsal cap of the inferior olive. This cell loss accounted for as much as 65% of the dorsal cap neuronal population. Cell loss was evident in animals sacrificed more than two days after the floccular lesion was placed and the degeneration was complete within seven days.

These data reveal a permanent deficit in the HOKR, but not the HVOR following unilateral floccular lesions and are consistent with the idea that the flocculus contributes to the visual regulation of low velocity eye movements through the inhibitory modulation of the activity of the subjacent vestibular nuclei. (Supported by EY04167).

180.9 ROTATIONAL POLARITY OF PURKINJE CELL ACTIVITY IN THE RABBIT FLOCCULUS. C.S. LEONARD and J.I. SIMPSON. Dept. Physiol. Biophys., 550 First Ave., New York Univ. Med. Ctr., New York 10016

Rabbit floccular Purkinje cells (PCs) receive visual and vestibular input via mossy and climbing fiber (CF) pathways. Recently we identified a striking rotational polarity in the representation of visual world movement by direction and speed selective visual CFs in the rabbit flocculus. CFs with ipsilateral receptive fields and horizontal preferred directions, respond to large, slow moving, textured patterns with an increased activity for nasal movement and a decreased activity for temporal movement. Since no CFs have been found that increase and decrease their firing rate for stimuli moving in the opposite sense, the CFs as a group can be said to have a response polarity. Here we report that PC simple spike (SS) responses to natural visual and vestibular stimuli also have a response polarity, but opposite to the CF polarity. Extracellular recordings were made from floccular PCs in chronically prepared, unanesthetized, paralyzed rabbits during visual, vestibular and combined visual-vestibular stimulation. 78 PCs having CF responses with ipsilateral receptive fields and horizontal preferred directions were studied. Of the 42 PCs tested with a constant speed stimulus (.1°/s to 30°/s), the majority (40/42) increased their SS firing rate for temporal movement and decreased their firing rate for nasal movement, opposite to the response pattern of the CFs. Similarly, of 36 PCs tested with sinusoidal whole-body rotation (0.05 to 1Hz; ± 1° to 30°) about an earth vertical axis in the dark, 35/36 increased and decreased their SS firing rate for contralaterally and ipsilaterally directed rotation respectively (type II). All PCs whose SS were modulated by vision showed markedly greater modulation for sinusoidal rotation in the light than in the dark (n=27). Although floccular PCs are known to inhibit some second-order vestibular neurons of the vestibulo-ocular reflex (VOR), their role in the VOR is unresolved, especially across species. Several lines of evidence make it reasonable to assume that PCs receiving input from 'horizontal' CFs inhibit some second-order vestibular neurons projecting to the ipsilateral medial and lateral rectus motoneurons, and that these vestibular neurons have type I responses to vestibular stimulation. Therefore, it appears that during head rotations about the earth vertical axis with the horizontal canals in the earth horizontal plane, the rabbit flocculus can enhance the modulation of these second-order vestibular neurons. Supported by USPHS Grant NS-13742.

180.10 TWO NEURONAL GROUPS ASSOCIATED WITH THE MEDIAL LONGITUDINAL FASCICULUS (MLF) OF CAT AND RAT THAT CONVEY VESTIBULAR IMPULSES TO THE CEREBELLAR FLOCCULUS. R.H.I. Blanks and Y. Torigoe* Depts. of Anatomy and Surgery (Otolaryngology), Univ. Calif. Irvine, Irvine, CA 92717.

The vestibular and cerebellar connections of neurons intercalated among the pontine and medullary portions of the MLF in cat and rat were studied using anterograde and retrograde horseradish peroxidase (HRP) techniques following iontophoretic injections into the flocculus and medial vestibular (MVN) and prepositus hypoglossi (ph) nuclei. An analysis of Nissl-stained material in both species reveals the presence of three groups of neurons within, or associated with, the MLF. One eye movement-related group termed the rostral interstitial nucleus of the MLF (rostral i MLF) (Büttner-Ennever and Büttner, Brain Res. 151:31, 1978) arises within the MLF rostral to the IIIrd nucleus. In the present report, we identify two other high density populations which retrogradely label with HRP injections into the flocculus and receive collateral projections from axons traveling with the MLF. We term these the intermediate and caudal i MLF to designate their position within, and association with, the MLF. The intermediate i MLF extends anteroventrally (1-3 mm in cat; 1-1.5 mm in rat) from the rostral border of the abducens nucleus and is adjacent to the superior pole of the nucleus reticularis tegmenti pontis (NRTP). The small-to-medium fusiform neurons comprising this population are largely distributed to the medial and dorsomedial portions of the MLF and are distinct in location from the adjacent raphe central superior nucleus, nucleus raphe dorsalis and NRTP. In contrast, the caudal i MLF is of lower cellular density and extends from the rostral pole of the hypoglossal nuclei to the genu of the facial nerve.

Our anterograde studies with HRP reveal that the intermediate i MLF of both species receives a dense collateral projection from vestibulo-ocular reflex neurons of the MVN and ph. These projections densely cover the neurons of the intermediate i MLF along its full extent and are primarily crossed, though an uncrossed projection also exists. The MVN and ph connections of the caudal i MLF are less dense, and there are indications that these are provided by collaterals of MLF axons comprising the descending limb of the MLF.

Receiving projections from the MVN and ph, the intermediate and caudal i MLF may serve as a feedback pathway to the flocculus conveying head velocity and position signals. Interestingly, the region comprising the intermediate i MLF in the cat (Curthoys et al., Brain Res. 222:75, 1981) contains medium-lead burst and burst-tonic neurons, suggesting that this pathway may also convey information related to saccades or eye position to the flocculus.

180.11 VISUAL INPUT FIBERS IN THE MACAQUE FLOCCULUS. M. Ohno*, H. Noda, T. Fujikado* and D. Belknap*(SPON: R. D. DeVoe). Sch. of Optometry, Indiana Univ., Bloomington, IN 47405.

In a class of Purkinje cells in the macaque flocculus, responses to retinal stimulation within 10° of the fixation point were excitatory and stimulation applied to a wide area of the peripheral retina resulted in deep depression of Purkinje cell activity (1). To elucidate the underlying neuronal mechanisms for the dual visual inputs to the flocculus, discharges of mossy and climbing fiber units were tested under the same paradigms as used to study the Purkinje cells. In addition, visual input fibers were tested with vestibular stimulation by rotating the primate chair sinusoidally around the vertical axis.

Among 53 mossy fiber units studied, 13 responded to moving visual stimulation. Five units discharged when the pattern was moved toward the direction of recording side and 6 units responded during contralateral stimulus movement. These units were suppressed when the pattern was moving in the non-preferred direction. Discharges were depressed in 2 units during stimulus movement in both directions. Showing cyclic modulation in activity responding to sinusoidal changes in the stimulus velocity, 11 units did not increase the peak activity with an increase of the frequency in a range from 0.1 Hz (3.1° /sec) to 0.8 Hz. In the majority of the units, therefore, the responses seemed to saturate at a lower frequency. Receptive fields of all 13 units were found in the peripheral retina. Of the 5 units discharging with ipsilateral visual stimulus movements, 3 units showed peak activity during ipsilateral head movements and none responded to movements in the opposite direction. Of the 6 units showing contralateral visual responses, 2 units responded to contralateral and none to ipsilateral head movements. One unit which was depressed by bilateral visual stimulus movements showed also bilateral suppression during head movements. One climbing fiber unit showed peak firing at the peak velocity of the stimulus movements toward the recording side. Its receptive field was located in the peripheral retina. The response was enhanced when the stimulus frequency was increased, but it did not respond to head rotation.

Although we have not yet discovered input fibers which convey visual information from the central retina, it is shown that a sub-population of visual mossy fibers which transmit peripheral retinal information have parent cells in structures which are related to the vestibular function. These neurons may play an important role during optokinetic nystagmus.

(1) Warabi, T and Noda, H. Exp. Brain Res. 41:A35.

Supported by NIH Grant EY04063

180.12 UNIT ACTIVITY AND RESPONSES TO MICROSTIMULATION IN THE MACAQUE FLOCCULUS DURING SMOOTH PURSUIT EYE MOVEMENTS. D. Belknap*, H. Noda and M. Ohno*. School of Optometry, Indiana University, Bloomington, IN 47405

Purkinje cells in the primate flocculus are known to modulate their simple spike activity during smooth pursuit. In order to understand the role of the flocculus in smooth pursuit, we have applied microstimulation to the flocculus in conjunction with single unit recordings from Purkinje cells. We are extending our earlier results to the situation of ongoing smooth pursuit. Slow eye movements were evoked by stimulation of the macaque flocculus. Trains of brief negative pulses of 20 μA or less were effective at approximately 40% of the sites tested in the floccular cortex. This percentage was not increased by choosing sites near isolated units which modulated their activity during smooth pursuit. 10 μA or less was effective at 20% of the sites tested. When the histological layers were identified by their characteristic unit and background activity, microstimulation was found to be most effective in the granular layer and least effective at the Purkinje cell layer.

Pulse trains having a duration of one-half second applied during a steady fixation evoked slow eye movements in all directions with amplitudes of up to 5 degrees and sometimes including quick phases. After the end of a pulse train, the eyes slowly returned in the direction opposite to the evoked eye movement. Eye velocity increased with increasing stimulus frequency and increasing current. Increasing the current above threshold caused the return eye movement to increase in amplitude and velocity and to begin before the end of the pulse train.

Purinje cell simple spike activity was modulated during sinusoidal smooth pursuit in the vertical and horizontal planes. Directional preferences were predominantly downward in the verticle plane. Eye movements evoked by microstimulation usually had upward vertical components. In samples from some regions of several flocculi the direction of the horizontal component of the evoked eye movement was predominantly ipsilateral and in others contralateral responses prevailed, suggesting that the horizontal component is, to some extent, dependent on the area stimulated within the flocculus. Microstimulation during smooth pursuit reduced eye velocity as would be predicted by the vector sum of the intended eye velocity and the eye movement evoked during fixation by the same stimulus.

Supported by NIH grant EY04063.

180.13 THE RELATIONSHIP BETWEEN SIMULTANEOUSLY RECORDED RESPONSES OF ANATOMICALLY RELATED ANTERIOR INTERPOSITUS NEURONS AND PURKINJE CELLS TO NATURAL PERIPHERAL STIMULI. C.J. McDevitt, T.J. Ebner and J.R. Bloedel. Depts. of Physiology and Neurosurgery, University of Minnesota, Mpls., MN 55455.

Several experiments have demonstrated that the projection of Purkinje cells to neurons in the cerebellar nuclei is sagittally organized with a specific region in the nucleus receiving inputs from zones of Purkinje cells localized in narrow antero-posterior strips. The purpose of these experiments was to examine the relationships of the responses of anatomically related Purkinje cells and nuclear neurons recorded simultaneously. In decerebrate unanesthetized cats concentric bipolar stimulating electrodes were positioned stereotaxically within the red nucleus to antidromically identify neurons recorded in the contralateral anterior interposed nucleus with a tungsten electrode (z=6 Mohm). After isolating and identifying an anterior interposed neuron the appropriate zone of lobule V was searched for Purkinje cells responding antidromically to microstimuli (0.5-75 uA) applied with the same electrode in the interposed nuclei used to record the activity of the nuclear neuron. Following successful isolation of a Purkinje cell and nuclear neuron the unitary responses to sinusoidal and square wave displacement of the wrist joint at various amplitudes and frequencies were determined. The simultaneous activity of twenty-six pairs of Purkinje cells and interposed neurons was analyzed using histograms, autocorrelograms and cross correlograms. When reciprocal activity was observed in a pair of neurons in response to square wave stimuli, nuclear neurons responded with a phasic increase in discharge rate rapidly followed by a more prolonged decrease below background level. Coincident with the decrease in the impulse activity of the nuclear neuron, there was an increase in the discharge of the associated Purkinje cell. However, this relationship was highly dependent on stimulus parameters. In general, the relationship between the two cells' responses to sinusoidal stimuli differed from the relationship observed for square wave stimuli. In some cases one cell of a pair was modulated while the other neuron was unmodulated. For pairs whose activity was modulated by the sinusoidal input the modulation of the two cells could be either in or out of phase. Cross correlograms of the spontaneous activity demonstrated little correlation. Although these data are preliminary, the results suggest that the functional relationship between Purkinje cells and anatomically related interposed neurons depends on the features of the afferent input. Supported by NIH Grants RO1-NS 09447 and RO1-NS 18338.

181.1 THE FUNCTION OF EFFERENT PROJECTIONS FROM THE LUMBOSACRAL SYMPATHETIC CHAIN TO THE URINARY BLADDER OF THE CAT. D.C.Kuo, and W.C.de Groat, Dept. of Pharmacology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Previous anatomical studies in the cat identified a prominent efferent postganglionic pathway from the caudal (S_1 and S_2) sympathetic chain ganglia (SCG) to the pelvic nerves which provide the parasympathetic (PSYM) innervation to the urinary bladder and colon. The present experiments were undertaken to identify the destination and function of these sympathetic (SYMP) postganglionic axons in the pelvic nerve.

In chloralose or barbiturate anesthetized cats, various nerves were isolated for stimulation or recording including: postganglionic nerves on the surface of the colon and bladder, pelvic and hypogastric nerves, and lumbar sympathetic chains (LSC) bilaterally. Electrical stimulation (thresholds 2-4V) of the LSC evoked firing in the pelvic nerve and in colon and bladder postganglionic nerves at latencies of 60-150 msec. The responses were unaffected by decentralization, i.e., cutting the chain one segment rostral to the site of stimulation, but were abolished by the administration of ganglionic blocking agents (tetraethylammonium or hexamethonium). The conduction velocity in the sympathetic postganglionic axons was approximately 1 m/sec.

Electrical stimulation of the LSC at L_5-L_6 also elicited responses in the bladder and bladder ganglia similar to the effects elicited by stimulation of the hypogastric nerves (HGN). For example, spontaneous bladder contractions and those elicited by stimulation of PSYM-preganglionic axons in the pelvic nerve were reduced by repetitive stimulation of the LSC (10-30 Hz, 3-30 V). The inhibition was preceded by a transient rise in bladder pressure which most likely represents a contraction of the bladder neck. This inhibition was not affected by decentralization, but was blocked by ganglionic blocking agent (tetraethylammonium) and by beta adrenergic blocking agent (propanolol). Transmission in bladder ganglia was also depressed (30-40%) by stimulation (10-30 Hz) of the LSC or the HGN. The inhibition was unaffected by decentralization, but was completely antagonized by injection of dihydroergotamine (DHE, 20-50 µg/kg, i.v.) an alpha adrenergic blocking agent. After DHE ganglionic transmission was facilitated by stimulation of the LSC.

In summary, electrical stimulation of preganglionic pathways in the LSC elicits postganglionic firing in nerves innervating the pelvic viscera and evokes responses in the urinary bladder which duplicate the effects produced by stimulation of the HGN. Thus, it seems likely that efferent projections from the lumbosacral sympathetic chain may play a similar role as the hypogastric nerve in the regulation of lower urinary tract function.

181.2 DIFFERENTIAL DISTRIBUTION OF TRANSMITTER BINDING SITES IN THE INTERMEDIOLATERAL CELL COLUMN (IML) OF THE RAT. V. Seybold and R. Elde, Dept. of Anatomy, Univ. of MN, Minneapolis, MN 55455

By combining retrograde transport of Fast Blue (FB) with immunohistochemistry, earlier work determined a close relationship of serotonin- and enkephalin-containing varicosities with cells in IML which project to the adrenal gland (Holets and Elde, Neurosci. 7:1155-1174, 1982). As an extension of this work, we determined whether specific binding sites for these and other transmitters could also be correlated with the distribution of sympatho-adrenal preganglionic neurons.

In order to localize sympatho-adrenal preganglionic neurons, the left adrenal medulla of 4 rats was injected with FB. Five days later, the animals were perfused with 0.1% paraformaldehyde, and the left adrenal gland and spinal cord segments T7-10 were rapidly excised and frozen. Cryostat sections (10 µm) were cut from these tissues, and the location of FB-labeled cells in each section was recorded with a fluorescence microscope equipped with an X-Y stage digitizer. The sections containing FB-labeled cells were then processed with ^{3}H-ligands for receptor autoradiography using the in vitro method with emulsion-coated coverslips. D-Lysergic acid (LSD) was used for visualization of serotonin binding sites, dihydromorphine (DHM) for opiate binding sites, quinuclidinyl benzilate (QNB) for muscarinic cholinergic binding sites and p-aminoclonidine (PAC) for alpha-2 noradrenergic binding sites. After development of autoradiograms, the number of silver grains/1600 µm^2 over regions of Fast Blue labeled cells was counted using the previous maps for orientation.

Each of the ligands displayed a unique pattern of binding in the intermediate regions of the thoracic spinal cord. Distinct densities of PAC binding sites were seen within IML with a high correlation with FB-labeled cells such that density of grains over FB-cells>other regions of IML>adjacent intermediate gray matter. LSD binding showed the same general pattern, although dense clusterings of grains were not apparent in IML. Dense clusterings of grains as with PAC were also observed in IML with QNB binding, but there was no correlation with FB-labeled cells and no consistent difference between grain densities over FB-labeled cells and the adjacent intermediate gray matter. DHM binding was most homogeneous with no significant difference within IML or the adjacent gray matter.

These results indicate that receptor autoradiography can be effectively combined with tract-tracing techniques to determine the distribution of transmitter binding sites over retrogradely-labeled cells. In particular, serotonin and alpha-2 adrenergic receptors had a higher association with the sympatho-adrenal preganglionic neurons in T7-10 of the rat spinal cord than with other regions in the intermediate gray matter.(Support: DA-02148)

181.3 THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) REGION IN EXPERIMENTAL SHOCK, G. Feuerstein, R. L. Zerbe#, A. K. Johnson## and A. I. Faden. Neurobiology Research Unit, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814 and #Department of Psychology, University of Iowa, Iowa City, Iowa 52242, ##Eli Lily Lab, Wishard Memorial Hospital, Indianapolis, Indiana 46202.

The AV3V region was previously shown to be a site of central integration of autonomic and sympathetic responses. In addition, the AV3V was shown to be the site for central pressor and vasopressin (VP) release by angiotensin (AII) (Brody & Johnson, Am. Physiol. Soc., 7:105, 1981). Since the AV3V area seems to have an important role in regulation of the three major pressor systems (sympathetic, AII, VP) and in the development and maintenance of several models of experimental hypertension, we investigated the role of the AV3V region in the cardiovascular recovery after severe hemorrhage, the latter being a known stimulus for sympathetic, AII and VP release. AV3V lesioned (n=19) and control (n=14) rats were implanted with indwelling catheters in both femoral arteries. Twenty-four hours later, conscious, unrestrained rats were exposed to hypovolemic hypotension (40%) by bleeding 8 ml/300 gm over 5 min. Mean BP (MBP) and heart rate (HR) were continuously recorded for 60 min and at 24 hr. Plasma catecholamines (CA, radioenzymatic-TLC) and vasopressin (VP, RIA) were determined at the control period and at 5 min, 1 hr and 24 hr after the bleeding. Water consumption and hematocrit were also monitored. The exact site of lesion was confirmed by microscopic examination of thionine (0.1%) stained sections (50 µ). MBP of AV3V lesioned and control rats fell to 28 ± 4 and 47 ± 6 mmHg, ($p < 0.05$) but no further differences were found throughout the rest of the experimental period respectively (ANOVA with repeated measures). Heart rate response consisted of 4 phases: tachycardia (1 min), bradycardia (2-5 min), tachycardia (20-30 min) and normal rate (24 hr) but no differences were seen between the groups. Plasma norepinephrine, epidneprine and VP were highest at the end of the hemorrhage, up to 6.4±1.2, 23.4± 6.3 and 1.5±0.3 ng/ml, respectively, but at each time point there was no significant difference between the two groups; water consumption 24 hr after the hemorrhage was 38±7 and 44±5 ml in the control and AV3V lesioned rats (p = n.s.). Hematocrit dropped from 42±2 in the control period to 31±1, 28±2 and 24±1% at 5 min, 1 and 24 hr after the hemorrhage but no significant differences were found between the groups. The AV3V lesioned rats had a higher mortality rate ($p < 0.05$). These data indicate that the AV3V is important in recuperation and survival from acute hypovolemic hypotension through mechanisms unrelated to sympathetic activation, VP release or water consumption and not directly related to MBP and heart rate responses.

181.4 SYMPATHETIC NERVOUS SYSTEM AND ADRENOCORTICAL INTERACTIONS. M.R. Brown, L.A. Fisher and W.W. Vale. Peptide Biology Lab, The Salk Institute, La Jolla, CA 92037.

Corticotropin releasing factor (CRF) stimulates pituitary secretion of ACTH and is a physiologic regulator of ACTH release following stress. CRF also acts within the brain to increase the activity of the sympathetic nervous system (SNS). Glucocorticoids (GLC) inhibit stress-induced release of CRF from the hypothalamus into the hypophyseal portal system and in addition antagonize CRF's action on the pituitary gland. To assess the physiologic role of CRF in regulating the SNS, studies were carried out to determine the effects of altered plasma GLC levels on CRF- and stress-induced elevations of plasma levels of epinephrine (E) and norepinephrine (NE).

Experiments were carried out in unanesthesized rats bearing chronic jugular venous and brain lateral ventricular cannula (icv). Plasma E and NE levels were determined using a radioenzymatic method. Dexamethasone (dex) was given subcutaneously and CRF was given icv. Dex (0.1-1 mg/kg 2-hour pretreatment) produced a dose dependent lowering of basal levels of plasma (E) and (NE), and suppression of CRF (1.9 nmole)-induced elevation of plasma E and NE.

Restraint and exposure to ether vapor (stress) for 3 minutes resulted in elevations of plasma E and NE. Dex (1 mg/ kg) given 2, 4 and 6 hours prior to stress suppressed plasma levels of E and NE. Dex (0.001-1 mg 2-hour pretreatment) suppressed stress-induced rise in E and NE with a minimal effective dose ca. 0.01 mg/kg. The dose of dex required to produce 50% inhibition of stress-induced E and NE is similar to the dose for inhibition of ACTH secretion.

To determine the effects of decreased endogenous plasma levels of GLC on the E and NE response to stress, animals were pretreated with antisera against CRF. This treatment (CRF antisera given iv) prevented stress-induced elevations of plasma ACTH and corticosterone, but accentuated the stress-induced rise of plasma E and NE. CRF antisera given iv does not gain access to those brain areas where CRF may possibly be involved in regulating the SNS. This conclusion is based on the observation that CRF antisera given iv did not prevent the rise in plasma E and NE following CRF given icv. Removal of endogenous GLC by acute adrenalectomy elevated plasma NE. These results, however, are confounded by the removal of adrenal E. To avoid this problem studies are currently underway using the steroid synthesis inhibitor, aminoglutethimide, to lower plasma GLC levels.

These studies suggest that GLC may exert an inhibitory effect on the activity of the SNS. We propose the hypothesis that this inhibitory effect of GLC on SNS function may be mediated through GLC inhibition of the secretion and/or action of CRF.

181.5 RELATIONSHIPS BETWEEN NEURONS IN THE PARAVENTRICULAR AND SUPRAOPTIC NUCLEI AND SYMPATHETIC PREGANGLIONIC NEURONS. K. Koizumi, M. Kollai*, H. Yamashita* and K. Inenaga*. Department of Physiology, State University of New York, Downstate Medical Center, Brooklyn, New York, and University of Occupational and Environmental Health, School of Medicine, Kitakyushu, Japan.

Morphological studies have shown the presence of monosynaptic connections between cells in the paraventricular nucleus (PVN) and the intermediolateral cell column (ILC) of the spinal cord. In order to clarify their functional implications, we approached the problem with 3 experimental procedures using electrophysiological techniques in chloralose anesthetized cats and dogs. 1) Electrical stimulation of certain regions in and near the PVN and the supraoptic nuclei (SON) with repetitive pulses (20-100 Hz) produced changes in blood pressure, heart rate and muscle blood flow very similar to the pattern observed in the "defense reactions." Such areas were very restricted but mingled with those from which "depressor response" was obtained by similar stimulations. 2) Recordings from the thoracic white ramus (Th II or III) revealed that stimulations of the PVN and SON regions with a short train of pulses evoked clear discharges. The latencies varied between 20 to 70 msec and the threshold 10 to 30 μA, depending on sites of stimulation. 3) Responses of "identified" and "non-identified" neurosecretory cells in the PVN and SON were recorded following microstimulation of the ILC of Th I or Th II with a short train of pulses. Antidromically evoked action potentials were recorded in 9 out of 297 neurons tested. They were located in both anterior and dorsal parts of the PVN as well as near but not within the SON. Among these 9 neurons 2 were antidromically excited also by the pituitary stalk stimulation, 5 were orthodromically excited by the same stimulus and the last 2 were not excited by the stalk stimulation. These results indicate that, although neurons in or near the PVN and the SON are very much involved in cardiovascular reactions, a very few neurosecretory cells send axons to the ILC of the cord and their specific function, if any, must be sought in the future. (Supported by grants from USPHS NS-00847, NSF INT 8006323, and #5637007 from the Ministry of Education, Japan)

181.6 FASTING DECREASES ARTERIAL PRESSURE AND CYTOCHROME OXIDASE ACTIVITY OF THE PARAVENTRICULAR NUCLEUS IN THE SPONTANEOUSLY HYPERTENSIVE RAT. T.L. Krukoff* and F.R. Calaresu. Dept. of Physiology, Univ. of Western Ontario, London, Canada. N6A 5C1

The spontaneously hypertensive rat (SHR) exhibits a genetically transmitted state of elevated arterial pressure (AP). There is experimental evidence suggesting that the CNS, by increasing the activity of the sympathetic nervous system, may play a role in the development and/or maintenance of the elevated AP. The additional observation that fasting in SHRs reduces both sympathetic nervous activity (SNA) and AP (Young & Landsberg, Science 196: 1473, 1977) has suggested that specific CNS sites, in particular the paraventricular nucleus of the hypothalamus (PVH), may play an important role in the control of SNA and the development of elevated AP in the SHR (Zhang et al., Fed. Proc. 42: 495, 1983). These experiments were done to investigate the metabolic activity of hypothalamic nuclei, and particularly of the PVH, in adult SHRs, before and after fasting, by using the technique for the histochemical localization of cytochrome oxidase (COX). Adult male SHRs and normotensive adult male WKYs (250-300 g) were divided into 2 groups each. The experimental group in each strain was fasted for 4 days; water intake, AP, and weight were recorded before and during the fast. Control groups of each strain were treated in the same manner except that they were not fasted. At the end of the fast or control periods, rats were anesthetized and their brains prepared for COX histochemistry as previously described (Wong-Riley, Brain Res. 171: 11, 1979). The decrease in body weight of fasted rats of both strain was similar. However, the water intake in fasted SHRs (SHR-f) was significantly less than that of fasted WKYs (WKY-f). AP of SHR-f was significantly lower than the AP of non-fasted SHRs (SHR-nf). With regard to COX activity in the hypothalamus, it was shown that (1) activity of neurons in the PVH of SHR-nf was greater than that of non-fasted WKYs (WKY-nf); (2) activity in the PVH of SHR-f was decreased to a level between that of SHR-nf and WKY-nf; and (3) no obvious differences in COX activity within other hypothalamic nuclei were found between control and experimental animals. These results suggest that metabolic activity of the PVH is related to the level of AP in the SHR and provide additional evidence for an important role of this nucleus in the control of AP in this model of hypertension. Supported by the Medical Research Council of Canada.

181.7 THE CENTRAL DISTRIBUTION OF VAGAL SUBDIAPHRAGMATIC BRANCHES IN THE RAT. R. Norgren and G.P. Smith, Penn State Univ. Col. of Medicine, Hershey, PA, 17033 and Dept. Psychiatry, Cornell Univ. Med. Col., White Plains, NY 10605.

We used retrograde and transganglionic transport of horseradish peroxidase (HRP) to examine the central distribution of afferent axons and efferent somata from the major branches of the subdiaphragmatic vagus nerve (SDX). Successful transport occurred in 11 preparations: bilateral SDX, n=3; gastric branches, n=3; coeliac, n=2; hepatic, n=3. In each case, the nerve branch(es) was dissected free, cut, and exposed to crystalline HRP for 6 hr. After 60-65 hr. survival, the brains and vagal ganglia were fixed and reacted ice cold. Sections through the medulla and upper cervical cord were mounted in alternate series, one unstained and one stained with neutral red, and stored at 4°C (Kalia & Mesulam, 1980; Hamilton & Norgren, 1981). Cases in which both SDX trunks were exposed to HRP confirmed previous descriptions (Coil & Norgren, 1978; Gwyn et al., 1979). Anterograde label occurred in the dorsal half of the medial nucleus of the solitary tract (mNST) beginning 0.4-0.5mm rostral to the area postrema (AP) and continued caudally ventral to AP. This bilateral distribution connected across the midline in the commissural nucleus. Caudal to AP (obex) only this midline anterograde distribution remained, and it faded within 0.3-0.5mm. Weak label occurred in AP, but not in the lateral NST beyond the labeled axons in the solitary tract. The sparse reaction product in the ventral half of mNST can be attributed at lease in part to labeled dendrites of DMX neurons. Retrogradely labeled somata filled the dorsal motor nucleus of the vagus (DMX) from 2.0mm rostral to 2.0+mm caudal to obex. Nucleus ambiguus contained many fewer labeled neurons. The anterograde distribution resulting from incubating individual branches indicated some degree of localization with the SDX projection area in mNST. First, the anterior gastric and hepatic branches distributed primarily on the left side of the medulla, while the posterior gastric and coeliac branches were concentrated on the right. When the accessory coeliac was incubated along with the main coeliac branch, the right bias was less obvious. Second, gastric branch label concentrated in the lateral and anterior aspects of mNST. Virtually no anterograde reaction product remained at the level of the obex. The coeliac and hepatic branch label began at the same A-P level as the gastric branches, but was more medial and continued caudally under AP to the level of the obex. The vast majority of retrogradely labeled neurons in DMX and nucleus ambiguus sent axons into the gastric branches. The coeliac branch had a few efferents in the lateral tip of DMX, but the hepatic branch labeled fewer than 100 efferent neurons.

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181.8 PROJECTIONS OF THE SUPERIOR LARYNGEAL NERVE TO THE SOLITARY COMPLEX IN THE RAT. L. D'Ippolito* and J. Ciriello (SPON: V. Hachinski). Department of Physiology, The University of Western Ontario, London, Canada, N6A 5C1.

It is well known that the superior laryngeal nerve (SLN) plays an important role in the regulation of respiration and arterial pressure. However, the primary sites of termination of SLN afferent fibers in the brainstem has not been adequately described. In the present study the central projections of the SLN were studied using the transganglionic transport of horseradish peroxidase (HRP). After application of crystalline HRP to the cut central end of the SLN for 6-8 hrs, the rats were allowed to survive for a period of 2-3 days, and then transverse frozen sections of the brainstem were processed according to the tetramethyl benzidine method. Labelled axons were found to enter the ipsilateral medulla by multiple rootlets approximately 2-3 mm rostral to the obex. After joining the solitary tract, the majority of labelled axons turned caudally and coursed within the lateral half of the tract. At this level, a small component of the labelled fibers appeared to terminate within the ventrolateral subnucleus of the nucleus of the solitary tract (NTS). The terminal labelling in the NTS increased in intensity towards the obex, and was densest in the interstitial subnucleus of the NTS. Additional labelling was present in the dorsolateral, ventral and medial subnuclei. Some labelled axons coursing through the dorsolateral subnucleus were traced into the ventrolateral aspect of the area postrema where they appeared to terminate. Further caudally, the intensity of labelling remained greatest in the interstitial subnucleus, although some labelling was also found in the medial and commissural subnuclei. Labelled axons crossed the midline above the central canal in the commissural subnucleus and terminated in the contralateral medial and dorsolateral subnuclei. These data provide evidence that SLN afferent fibers project to restricted regions of the solitary complex and suggest that several regions of the NTS have physiological roles in the homeostatic regulation of respiration and arterial pressure.

(Supported by the Ontario Heart Foundation).

181.9 SINGLE UNITS IN THE AMYGDALA OF THE CAT RESPONDING TO ACTIVATION OF BARORECEPTORS AND CHEMORECEPTORS. D. F. Cechetto and F. R. Calaresu. Dept. of Physiology, University of Western Ontario, London, Canada, N6A 5C1.

Electrical stimulation of the carotid sinus and aortic depressor nerves has been shown to alter the firing frequency of units in the amygdala (Soc. Neurosci. Abs. 7:365, 1981) but the distribution of neurons within the amygdala responding to selective activation of baro- and chemoreceptors and the input to these neurons from other CNS nuclei involved in cardiovascular and respiratory control are not known. Experiments were done to investigate both the specificity of separate projections to different portions of the amygdala and convergence on neurons in the amygdala of inputs from baro- and chemoreceptor afferents and from the paraventricular nucleus of the hypothalamus (PVH) and parabrachial nuclei (PB), CNS locations with known involvement in central control of circulation and respiration. In 12 chloralosed, paralyzed and artificially ventilated cats electrical activity of spontaneously active units in histologically verified sites in the amygdala was monitored for changes in firing frequency during activation of baroreceptors (BA, phenylephrine, 2 μg/kg, i.v.) and activation of carotid chemoreceptors (CA, sodium cyanide 25 μg in 0.1 ml saline into the medial thyroid artery). Units responding to BA and CA were also tested for responses to electrical stimulation of the PVH and PB. CA altered the firing frequency of 23% (35/154) of the units in the amygdala of which 37% (13/35) were excited while 63% (22/35) were inhibited. Of the 13 units excited by CA, 2 were also excited by PVH stimulation and 4 were excited by PB stimulation. Of the 22 units inhibited by CA 5 were excited and 2 were inhibited by PVH stimulation while 6 were excited and 1 was inhibited by PB stimulation. BA altered the firing frequency of 16% (24/154) of the units in the amygdala of which 71% (17/24) were excited while 29% (7/24) were inhibited. Of the units responding to BA only 2 of the inhibited units responded to PB stimulation while none responded to PVH stimulation. No units responded to both BA and CA. Units responsive to BA and CA were located primarily in the central, lateral and basal nuclei. The units responsive to CA were located primarily medial and dorsal to the units responsive to BA. These experiments show that about half of the units responding to CA also received converging input from the PVH or PB, whereas only a very small number of the units responding to BA also responded to PVH and PB. In addition it has been shown that baro- and chemoreceptor information appears to project to separate functional neuronal pools in the amygdala with a certain degree of anatomical separation. This demonstrated separation of inputs may have functional implications in the role these separate areas play in integrating the autonomic components of behavioral responses. (Supported by MRC of Canada).

181.10 MYELINATED RENAL AFFERENTS PROJECT DIRECTLY TO NUCLEUS GRACILIS AND NUCLEUS SOLITARIUS IN THE RAT. L.P. Schramm, O.R. Simon* The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205

A variety of autonomic responses have been attributed to the activation of afferent fibers from the kidney. Several recent studies have shown that the majority of renal afferents synapse in the dorsal or intermediate spinal gray at low thoracic and high lumbar levels. However, we have noticed that renal nerve fibers with conduction velocities characteristic of the A-delta group could be antidromically activated from high cervical levels in the rat. The present study traced these fibers from their site of spinal entry to their termination.

Female Sprague-Dawley rats were anesthetized with alpha-chloralose, paralyzed, and artificially respired. The caudal medulla, cervical spinal cord, and lumbar spinal cord were exposed. Recordings using hook electrodes were made from unsplit bundles of nerves dissected from the left renal artery. Electrical stimulation (2-20 μa, 0.3 ms duration) elicited single (or, more rarely, several) action potentials in the renal nerve at conduction velocities of approximately 15 M/s. Responses had sharp thresholds and constant latencies, and they followed stimulation to approximately 300/s, confirming their antidromic nature.

Antidromic responses to spinal stimulation could be abolished by sectioning a T10, T11, or T12 dorsal root, indicating entry of the myelinated fibers at these segments. At high cervical levels, minimum thresholds for antidromic activation were located medially in the faciculus gracilis, approximately 0.5 mm below the dorsal surface of the spinal cord. Fibers were also located medially at the level of nucleus commissuralis. However, at the level of the obex, minimum thresholds were observed more laterally in nucleus gracilis and nucleus solitarius. Activation threshold vs depth characteristics indicated widely ramifying terminals in these nuclei.

Myelinated renal nerve afferents could be activated by pressure on the renal hilus, and the resulting action potentials collided with potentials antidromically-evoked from cervical levels. Therefore, these afferents project from the renal parenchema itself and not from surrounding tissue.

We conclude that myelinated renal afferents enter the spinal cord between T10 and T12 and project via the medial faciculus gracilis directly to the nucleus gracilis and the nucleus solitarius. Supported by NIH Grant HL16315

181.11 REFLEX RESPONSES OF SPLENIC AND RENAL NERVES TO SELECTIVE ACTIVATION OF SPLENIC RECEPTORS IN THE CAT. F.R. Calaresu, J.C. Tobey*, and L.C. Weaver. Dept. of Physiol., Mich. St. Univ., E. Lansing, MI 48824.

Experiments were done to investigate the reflex responses of splenic and renal efferent nerves to selective activation of receptors within the spleen. Adult cats, under 80 mg/kg of α-chloralose i.v., were paralyzed, artificially ventilated, and the vagus, aortic depressor, and carotid sinus nerves were sectioned. The spleen was exposed, and all vascular connectives to other organs were ligated. The splenic artery and vein were cannulated through branches of these vessels and prepared for occlusion by a snare. Evidence that all vascular connections between the spleen and other visceral organs had been eliminated was obtained at the beginning of each experiment by the injection of Evans blue dye into the splenic artery after occlusion of the splenic vein and artery. The following variables were monitored: 1) integrated electrical activity from efferent splenic and renal nerves, 2) splenic venous pressure (SVP), 3) systemic arterial pressure (SAP), and 4) heart rate (HR). The following substances were injected into the splenic artery while both splenic artery and vein were occluded: 1) warm physiological saline (PS, 3-20 ml) to increase intrasplenic pressure; 2) capsaicin (CAPS, 5-25 μg); 3) bradykinin (BK, 1-10 μg); and norepinephrine (NE, 1 μg). Congestion of the spleen with PS increased splenic nerve activity (SNA) 40 ± 9%, renal nerve activity (RNA) 15 ± 6%, SVP 26 ± 6 mm Hg, SAP 27 ± 8 mm Hg, and HR 4 ± 2 bpm (n = 6). Stimulation of splenic receptors by CAPS increased SNA 139 ± 32%, RNA 49 ± 11%, SVP 14 ± 3 mm Hg, SAP 68 ± 16 mm Hg, and HR 14 ± 3 bpm (n = 7). Responses to BK were similar to those to CAPS. In contrast, although injection of NE caused increases in SVP of 38 ± 6 mm Hg, SNA, RNA, and HR were not changed, and SAP increased 17 ± 9 mm Hg (n = 4). The neural reflexes, HR responses, and a part of the SAP responses caused by these stimuli were demonstrated to be reflex in nature because they were abolished consistently by splenic denervation. These results indicate that 1) splenic receptors are activated by congestion and by algogenic substances; 2) selective stimulation of splenic receptors causes sympathetic excitation leading to cardiovascular responses; this excitation affects SNA more than RNA; 3) contraction of the spleen induced by NE does not evoke reflexes, suggesting that responses to congestion and perhaps to the noxious agents were initiated by stimulation of stretch receptors.

Supported by grant HL 21436.

181.12 SIMULTANEOUS MEASUREMENT OF BEHAVIORAL AND CORE TEMPERATURE: EFFECTS OF PROSTAGLANDIN E1 AND DOPAMINE. Spencer, R. L*., Marques, P. R*., McDougal, J. N., & Burks, T. F. Depts. of Psychology and Pharmacology, Univ. Arizona, Tucson, AZ 85724.

These studies show the autonomic and behavioral temperature effects of compounds believed to be endogenous hypothalamic mediators of temperature regulation. Three days before the experiments, 34 rats were implanted with ICV cannulas and intraperitoneal temperature sensitive AM transmitters. The emitters send a signal to an AM receiver which is converted by a microcomputer to degrees C. Preferred ambient temperature (Ta) is determined in a temperature gradient, that ranges from 8-38°C. Infrared sensors detect the location of a rat within this 5'long opaque thermocline. The change in a rat's location provides a derived measure of motor activity. The computer continuously monitors the rat's position, activity and core temperature (Tc) printing a 5 min mean of 20 15 sec readings. Prostaglandin E1 (PGE1) was studied at 5 doses (0, .1, .2, .5, 1.0 ug) with both gradient ON (G+)and OFF (G-),totaling 10 combinations. PGE1 in 5 uL produced significant dose related increases in Tc ranging from 1.1 to 2.6°C above basal. Time to peak was also significantly dose-related with a low mean peak of 17 min (0.1 ug) and a high peak of 22.5 min (1.0 ug) for both G+ and G- conditions. Analyses of 4 10 min intervals post injection showed a dose related selection of a warm Ta (32.5 °C) for the 3 highest doses (0.2-1.0 ug) while controls selected 27.5°C Ta. During the next 20 min G+ PGE1 injected rats moved back to a neutral Ta. PGE1 did not alter motor behavior in these studies. ICV injections of dopamine (DA) (50, 100, 200, 400 ug in 10 uL water) produced a dose-dependent decrease in Tc ranging from a mean -1.64°C (50 ug) to a mean -2.85°C (400 ug). The time to peak hypothermia increased with dose. The DA induced hypothermia was greater in the G+ condition. In G+, the rats spent the first 10 min after DA at the cold end of the thermocline, at a mean Ta of 16.4°C. This was significantly different from the control selected Ta (23.9 °C). This cold seeking persisted 20 min after 200 and 400 ug. This behavioral response may explain the lower Tc after DA in the G+ condition. At 30-40 min after 200 and 400 ug DA, rats selected a significantly warmer (31.7°C) location than controls (26.9°C). Motor activity increased significantly for the first 20 min after DA; at 400 ug this increase continued for 40 min. A significant hyperthermic rebound was seen in the G+ condition, but not G-, possibly due to an active compensatory selection of heat when the gradient was available. The time to peak rebound hyperthermia increased with dose of DA (49 min for 50 ug, 83 min for 400 ug). The results emphasize the importance of measuring behavior in studies of temperature regulation. Supported by NS15420.

181.13 THE BICUCULLINE-LIKE EFFECTS OF CENTRAL DOPAMINE SULFATE IN THE CONSCIOUS RAT. POSSIBLE ANTAGONISM OF GABA RECEPTOR BY DOPAMINE SULFATE. N.T. Buu*, J. Duhaime*, O. Kuchel* (SPON: A. Barbeau), Institut de Recherches Cliniques de Montréal, Montréal, Quebec (H2W 1R7), Canada.

Dopamine (DA) sulfate and bicuculline belong to two different neuropathways. The first, present in discrete areas of rat brain (J. Neurochem. 36:769, 1981; Karoum, 1983) and in human CSF,is a metabolite of free DA, while the second is an antagonist of central GABA receptors. However, both agents exhibited similar action. At low doses they increase arterial blood pressure (BP); at high doses they become convulsant agents. The present study was undertaken to compare their effects in the conscious rat in order to better understand their mechanism.

Rats were implanted under Nembutal anesthesia with permanent catheter in the left lateral ventricle. BP was measured through a femoral artery catheter. Intraventricular (IVT) injections of low doses of bicuculline (0.2 - 1.0 μg) or DA sulfate (5-30 μg) caused increases in BP (15-40 mmHg) which were not blocked by DA antagonists haloperidol and metoclopramide. Sympathectomy (80%) by 6-hydroxydopamine, or pretreatment with angiotensin II antagonist saralazin did not affect the response to bicuculline or DA sulfate suggesting that the central sympathetic nervous system, and central angiotensin II receptors are not directly involved. Intraventricular injections of larger doses of DA sulfate (60 μg) or bicuculline (1.2 μg) caused generalized convulsions. The effect of DA sulfate was additive to that of bicuculline: half of a convulsive dose of DA sulfate injected after half a convulsive dose of bicuculline provoked seizure, suggesting that their convulsive effect may be through a common mechanism. Both convulsive and cardiovascular effects of bicuculline and DA sulfate could be blocked or reduced by GABA agonists, diazepam and muscimol.

In another experiment, the possible interaction of DA sulfate with central GABA receptor, suggested also by structural resemblance between GABA and DA sulfate molecules, was investigated. The results showed that DA sulfate could displace sodium independent ^{3}H GABA binding to synaptic membranes from rat brain (IC = 2 X 10^{-4}M).

It is concluded that DA sulfate exhibiting bicuculline - like biological activities in rat brain may act as a GABA antagonist in raising BP and causing convulsions. It may therefore serve as a link between central GABAergic and dopaminergic neuropathways. (Supported by grants from the MRC and Canadian Heart Foundation.)

DEVELOPMENT AND PLASTICITY: TROPHIC AGENTS I

182.1 NGF-MEDIATED ENZYME INDUCTION IN CULTURED ADRENAL CHROMAFFIN CELLS: SPECIFICITY AND LEVEL OF REGULATION. A. Acheson and H. Thoenen. Max-Planck-Institute for Psychiatry, Dept. Neurochemistry, 8033 Martinsried, FRG

While many effects of nerve growth factor (NGF) on its target cells have been established (eg. survival, neurite outgrowth, enzyme induction), little is known about the mechanism of action of NGF beyond its interaction with specific plasma membrane receptors and the involvement of a (so far unknown) second messenger. Primary cultures of calf adrenal chromaffin cells have proved to be suitable for investigating such problems. They are available in large quantities, and they respond to NGF with a pattern of enzyme induction which is representative of that found in physiological target cells in vivo. We have examined NGF-mediated tyrosine hydroxylase (TH) and acetylcholinesterase (AChE) induction in more detail, and since cAMP has been suggested as a possible second messenger for NGF, we have compared its effects with those of NGF. The continuous presence of NGF for 48 hrs was required for TH induction, whereas AChE could be induced by shorter (24 hr) exposures. A 12 hr exposure of cells to cAMP was sufficient to elicit the full effect on both enzymes. α-Amanitin, an inhibitor of RNA polymerase II, did not block NGF-mediated TH induction, while in the same cells AChE induction was completely abolished. cAMP-mediated induction of both enzymes was blocked by this treatment. 9-ß-Arabinofuranosyladenine, a polyadenylation inhibitor, blocked the effect of NGF on both TH and AChE, as well as the effects of cAMP. Thus, it can be concluded that the NGF-mediated TH induction is regulated at the post-transcriptional level, perhaps involving a polyadenylation-dependent event. In contrast, the induction of AChE is transcription-dependent. This, in combination with the different time courses of TH and AChE induction, suggests that NGF-mediated enzyme induction involves several mechanisms. cAMP cannot be the second messenger for NGF-mediated enzyme induction for the following reasons: in contrast to the effect of NGF, cAMP-mediated TH induction is abolished by α-amanitin. In addition, both the patterns and the times of exposure required for enzyme induction differ between cAMP and NGF-treated cells.

182.2 NEURITE-PROMOTING ACTIVITIES FOR CHICKEN SPINAL NEURONS: CHANGES DURING DEVELOPMENT AND AFTER DENERVATION. C.E.Henderson*, M.Huchet* and J.-P.Changeux. Neurobiologie Moléculaire, Institut Pasteur, 75724 Paris XV, France.

Spinal motoneurons may depend on muscle-derived growth factors for axon outgrowth and stabilisation at two principal stages of their development: during the initial invasion of the differentiating muscle masses and during the perinatal regression of multiple innervation. Using a bioassay involving the measurement of neurite outgrowth from 4.5-day embryonic chick spinal neurons in dissociated cell culture, neurite-promoting activity was detected both in medium conditioned over embryonic myotubes in vitro (embryonic muscle-conditioned medium, "ECM") and in soluble extracts of chick leg muscle prepared 3 to 5 days after hatching (postnatal muscle extract, "PNME").

The molecules responsible for these two activities had physicochemical properties that distinguished them both from each other and from reported polyornithine-attached neurite-promoting factors. The former (in ECM) was active on uncoated plastic dishes but did not bind to them under cell culture conditions. It was inactivated by trypsin and was essentially found only in media conditioned by muscle and liver cells (spec. act. 550 units per mg protein). The activity in PNME, on the other hand, bound to tissue culture plastic, was more resistant to high trypsin concentrations, and was found in extracts of many tissues.

The levels of this factor in postnatal leg muscle were developmentally regulated: the specific activity increased 10-fold between hatching and day 3 (max. value 3200 units/mg protein) and fell back to nearly its original levels by day 7. Cultures of embryonic myotubes "aged" for 2 weeks in vitro secreted into the medium a factor having some of the properties of that in PNME.

Following sciatic nerve transection in 6-day chicks, the specific activity in extracts of muscles of the lower leg increased up to 15-fold compared to controls (max. value 10,000 units/mg). The time-course of appearance of this activity (3 to 6 days after denervation) correlates well with the period that elapses in partially denervated mammalian muscles before sprouts appear.

Under all culture conditions, more than 92% of total spinal cord cells contained neurofilaments, as revealed by indirect immunofluorescence. Cells cultured for 26 h in nonconditioned medium retained their capacity to develop neurites in response to ECM. When ECM and PNME were assayed together, the maximum value attained in the neurite outgrowth assay did not increase. It is likely, therefore, that the observed effects of the two activities did not result from differential survival in vitro of different cell sub-populations.

182.3 DEPOLARIZING AGENTS INCREASED TYROSINE HYDROXYLASE IN THE DEVELOPING BRAIN LOCUS COERULEUS (L.C.) IN CULTURE. C.F. Dreyfus, K.A. Markey and I.B. Black. Division of Developmental Neurology, Cornell Univ. Med. Coll., New York, NY 10021.

While abundant evidence indicates that differentiation of peripheral neurons is regulated by epigenetic factors, the regulation of brain neuronal development is largely undefined. To begin approaching this problem at the molecular level, we have been examining development of the mouse l.c. in explant culture. Previous studies indicated that tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH), noradrenergic biosynthetic enzymes, increase markedly during development of this embryonic catecholaminergic nucleus over 3 weeks in vitro. In the present study we examined the influence of membrane depolarization on TH.

Exposure to veratridine (1.5×10^{-6} M for 7 days) which elicits depolarization by increasing sodium influx, significantly increased TH activity. This effect was prevented by tetrodotoxin (10^{-7} M) which blocks the sodium channel effects of veratridine, suggesting that depolarization and transmembrane sodium flux increase TH development. To help determine whether depolarization itself evoked the increase in TH, a structurally dissimilar depolarizing agent, K^+ (20mM) was used. Exposure to elevated K^+ also caused a significant increase in TH in the cultured l.c.

Our experiments suggest that membrane depolarization influences development of brain catecholaminergic neurons. More generally, epigenetic factors may regulate the ontogeny of specific transmitter phenotypic characters in brain neurons, as in the periphery (Supported by NSF grant BNS 8024081 and NIH grants NS 10259 and HD 12108.)

182.4 SEPTAL CHOLINERGIC NEURONS IN CULTURE: IMMUNOCYTOCHEMICAL VISUALIZATION OF CHOLINE ACETYLTRANSFERASE, AND REACTION TO NERVE GROWTH FACTOR, GLIAL CELLS, AND THYROXINE. F. Hefti, J. Hartikka*, F. Eckenstein*, H. Gnahn*, R. Heumann*, M. Schwab, and H. Thoenen. Sandoz Ltd., Preclinical Research, Basel, Switzerland, and Max Planck - Institute, Martinsried/Munich, West Germany.

Cholinergic neurons of the forebrain appear to specifically degenerate in Alzheimer's disease. Understanding their cell biology should facilitate the search for cause and treatment of this disease. We therefore decided to study cholinergic neurons in culture and to investigate their requirements for survival, growth, and expression of transmitter-specific enzymes.

The septal area was dissected from rat embryos (E17). Cells were dissociated and plated in poly-ornithin-coated dishes and grown for 10-12 days in a modified L-15 medium. Cultures free of glial cells were obtained by adding cytosine arabinoside to the medium. Under these conditions, 95% of the cells were identified as neurons by positive labelling with tetanus toxin.

Cholinergic neurons were identified by immunocytochemical staining of choline acetyltransferase (ChAT) and histochemical staining of acetyl cholinesterase (AChE). Immunocytochemical visualization of ChAT was obtained with both, a rat serum against pig brain ChAT in conjunction with a FITC-labelled second antibody, and a monoclonal antibody followed by a biotinylated second antibody and avidin-peroxidase. Positive ChAT staining was only observed, when septal neurons were grown in the presence of glial cells. Both immunocytochemical methods stained cell bodies and proximal processes. Staining intensity of cell bodies was increased by NGF (100ng/ml) and thyroxine (10^{-7}M), in confirmation of earlier findings obtained with biochemical measurements of ChAT activity in these cultures (Gnahn et al., Develop. Brain Res., in press).

ChAT-positive neurons identified with the FITC-method were double stained for AChE. The histochemical staining was the more sensitive method and enabled us to visualize cholinergic neurons in cultures free of glial cells. App. 5% of the cells in such cultures were identified as cholinergic neurons. NGF and antibodies to NGF failed to affect survival of or fiber outgrowth from cholinergic neurons.

In conclusion, cholinergic neurons were identified in cultures of dissociated neurons from the rat septum. Glial cells, NGF, and thyroxine have a trophic influence on these neurons, inasmuch as they enhance ChAT levels. The effect of NGF seems to be limited to a stimulation of ChAT synthesis and does not affect survival and fiber outgrowth of these neurons.

182.5 STUDIES ON THE CHOLINERGIC DIFFERENTIATION FACTOR FOR SYMPATHETIC NEURONS. Keiko Fukada, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115

Recent studies by S. Landis and collaborators have shown that certain cholinergic sympathetic neurons innervating specific targets such as sweat glands undergo a transition from noradernergic to cholinergic as part of their normal development. In order to understand the molecular mechanism of such a plasticity in neuronal development, it is essential to study developmental signals which control it. The existence of such a developmental signal has been shown from culture studies on dissociated rat sympathetic neurons that certain non-neuronal cells produce a diffusible factor (cholinergic factor) which induces cholinergic differentiation in the neurons while inhibiting the development of a variety of noradrenergic properties. This factor influences the transmitter decision but has no effect on survival or growth.

The cholinergic factor is being purified from conditioned medium (CM) prepared by incubation of serum-free, hormone-supplemented medium on cultures of rat heart cells (Fukada, Nature 287, 555, 1980). Using conventional biochemical techniques, an increase in specific activity of more than 10,000-fold has been achieved with a reasonable recovery. The most purified activity is highly sensitive to trypsin and chromatographs on Sephadex at a molecular weight of ∿45,000 daltons. This preparation still possesses the ability to both inhibit the development of noradrenergic characteristics as well as to induce cholinergic differentiation.

Since the factor was found not to be inactivated by the treatment with 1% sodium dodecylsulfate (SDS), further purification of the factor was pursued by preparative SDS-polyacrylamide gel electrophoresis under non-reducing conditions. High recovery of the activity was obtained at a molecular weight of ∿50,000 daltons, which corresponds to the molecular weight determined by chromatography on Sephadex under native conditions. Thus the active form of the factor is a monomer (possible subunits may be held together by disulfide bonds, however). Moreover, high recovery of the activity with the good resolution this method affords makes this technique promising as a further purification step.

In addition to these biochemical methods another approach is in progress. A partially purified CM fraction was injected into mice in an effort to raise monoclonal antibodies. Twenty-three hybridomas were selected for their ability to bind preferentially to the most purified fraction. Several of these antibodies were found to bind to major impurities in the CM preparation and may therefore be useful in further purification. One monoclonal antibody specifically precipitated a significant fraction of the cholinergic activity. (Supported by the Dysautonomia, McKnight and Rita Allen Foundations, and the NINCDS)

182.6 BIOCHEMICAL AND ANATOMICAL EFFECTS OF ANTIBODIES AGAINST NERVE GROWTH FACTOR ON DEVELOPING RAT DORSAL ROOT GANGLIA. U. H. Otten, M. Goedert*, S. P. Hunt*, L. L. Iversen*, M. Schlumpf and W. Lichtensteiger. Dept. of Pharmacology, Biocenter of the University, Basel, Switzerland; MRC Neurochemical Pharmacology Unit, Cambridge, UK and Inst. Pharmacol. Univ. Zürich, Switzerland.

The protein nerve growth factor (NGF) is essential for the development of peripheral adrenergic neurons, as shown by the fact that the administration of anti NGF-antibodies to newborn animals leads to an irreversible destruction of their peripheral sympathetic nervous system. The same treatment results in a marked but reversible reduction in substance P-like immunoreactivity in dorsal root ganglia, spinal cord and skin. In the present study, we have examined the development of rat dorsal root ganglia following prenatal exposure to anti NGF-antibodies.

Rat foetuses (day 16.5 of gestation) received a single intramuscular injection of a monospecific NGF-antiserum and were killed at the age of four months. Control animals were treated with preimmune serum. The anti NGF-antibody treatment produced a reduction of greater than 80% in the number of unmyelinated dorsal root fibers. Substance P- and somatostatin-like immunoreactivities were decreased by over 80% in dorsal root ganglia and skin, as determined by radioimmunoassay. Substance P-like immunoreactivity showed a 30% and somatostatin-like immunoreactivity a 12% reduction in the spinal cord. These results were supported by immunohistochemical findings which showed a substantial depletion of both substance P- and somatostatin-like immunoreactivities in dorsal root ganglia and in the dorsal horn. In addition, fluoride-restistant acid phosphatase was depleted in both structures, as shown by a histochemical technique.

These results indicate that NGF is a natural trophic factor for both sympathetic and dorsal root ganglia. However, whereas anti NGF-antibodies produce a destruction of rat sympathetic ganglia up to postnatal day 12 this effect on dorsal root ganglia is limited to the prenatal period.

182.7 GANGLIOSIDE MEDIATION OF NGF ENHANCED NEURONAL MATURATION. Fred J. Roisen, Maurice M. Rapport, Yung-Yu Huang* and Glee Yorke*. Department of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854 and Division of Neuroscience, New York State Psychiatric Institute, NY, NY 10032.

We have reported previously (Science, 214:577, 1981) that exogenous bovine brain gangliosides (BBG) induce in vitro neuritogenesis of chick embryonic dorsal root ganglia (DRG) and murine Neuro 2a neuroblastoma (N_2A). The mechanism through which BBG enhances neuritogenesis is unknown. We and others have suggested that gangliosides incorporated into neuronal plasma membranes may function as "acceptor" molecules for growth factors. To examine this possibility we have been studying the relationship between gangliosides and Nerve Growth Factor (NGF). Four pure bovine brain ganglioside species (GM_1, GD_{1a}, GD_{1b}, and GT_{1b}, Fidia Res. Labs., Abano Terme, Italy) were found to enhance N_2A and DRG neuritic sprouting. Affinity purified antibodies to GM_1 were prepared and used to probe NGF's ability to increase neuritogenesis of DRG in vitro. The antibodies diminished specifically NGF's capacity to increase neurite length and number but had no effect on the radial migration of accompanying non-neuronal cells. Another consequence of NGF's action on DRG, which occurs prior to sprouting, is an increase in the activity of ornithine decarboxylase (ODC) (MacDonnell et al., PNAS, 74:4681, 1977), the rate-limiting enzyme in the polyamine biosynthetic pathway. ODC activity of DRG is independent of neurite outgrowth. Accordingly, we have examined the effects of antibodies to GM_1 alone, or in combination with NGF on the ODC levels of DRG and a cloned NGF-responsive pheochromocytoma cell line (PC-12). NGF's elevation of ODC activity was reduced in a dose-dependent manner when NGF and antibodies to GM_1 were administered simultaneously to DRG or PC-12 cultures. The ODC activities were reduced to levels below that found in untreated controls when antibodies to GM_1 were applied in the absence of additional treatment. Furthermore, NGF-induced neurite elongation was enhanced by exogenous GM_1. These studies are consistent with the possibility that gangliosides in the membrane play a key role in trophic regulation and demonstrate specifically that NGF interacts with GM_1, in some as yet undefined manner, to increase both ODC activity and neurite elongation.

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182.8 PARTIAL PURIFICATION OF A CHOLINERGIC TROPHIC FACTOR FROM ADULT HUMAN MUSCLE. Ken Vaca* and Stan Appel. Dept. of Neurology, Program in Neurosciences, Baylor College of Medicine, Houston, Texas 77030.

Trophic substances which affect spinal and parasympathetic motor neurons have been extracted from a variety of sources, with some differences in their reported properties. If some neuromuscular disorders are a consequence of aberrant trophic interactions, it would be desirable to be able to identify the trophic molecules active in human. Cultured chick ciliary ganglion neurons provide a sensitive system to assay for such trophic factors. The 100,000 g supernatants of muscle extracts from more than 10 human (vastus lateralis) muscle biopsies typically induced a 4 to 5 fold increase in ^{3}H-ACh synthesis after to 3 to 4 days in culture, roughly comparable to that produced by fetal calf or embryonic chick muscle extracts. However, in contrast to the embryonic extracts which elicited tremendous neurite extension, the adult muscle extract had minimal effect on cell morphologies. (Psoas) muscle extracts from autopsy material were usually nearly as active as the biopsy specimens on cholinergic activity and were used for further purification. Activity was precipitated in 60-85% ammonium sulfate. The active material was present in both high (>100,000) and low (<20,000) molecular weight forms, the former being favored when the 60-85% pellet was resuspended at high concentration. The lower molecular weight activity was separated and concentrated by consecutive ultrafiltration steps. By gel filtration, the apparent molecular weight of the cholinergic factor was approximately 10-12,000, which increased ^{3}H-ACh synthesis more than 2-fold at 1 μg/ml. Preparative isoelectric focusing produced activity with a pI of approximately 7.5. The active fraction did not increase ^{3}H-thymidine incorporation and thus was not mitogenic for supporting cells. This cholinergic trophic factor contained in adult muscle may play a role in maintaining motor neurons in their active, fully differentiated state.

(We thank Dr. Scott Stewart for help in obtaining autopsy specimens. Supported in part by grants from the John A. Hartford Foundation and the Robert J. Kleberg and Helen C. Kleberg Foundation).

182.9 INITIAL PARTIAL CHARACTERIZATION OF A GROWTH FACTOR FOR APLYSIA NEURONS IN TISSUE CULTURE. S.M.Fredman and R. Waziri. Dept. of Psychiat., Univ. of Iowa, Col. of Medicine, Iowa City, IA, 52242.

The blood of Aplysia contains a factor or factors that is/are necessary for and promotes the extension of neurites in culture of dissociated neurons. We now report the partial characterization of this factor. Our assay system consisted of the cell bodies of neurons from the CNS of immature Aplysia (weighing 12-50g) in culture. The neurons were mechanically dissociated without the use of enzymes and plated on poly-L-lysine coated culture dishes in L-15 medium (GIBCO) supplemented with additional salts and antibiotics. With 10-40% (vol/vol) blood added, neurons typically put out processes in 12-24 hours. Neurons cultured without blood, or with blood inactivated by boiling or trypsin failed to extend neurites. This suggests that the growth promoting factor(s) is/are protein(s). The growth promoting factor was retained by a membrane filter (Amicon) with a cut-off of 300,000 MW. More precise size estimation by gel chromatography indicted that the factor has a molecular weight of approximately 500,000 Daltons. Further characterization and purification of the growth promoting factor(s) is now in progress.

182.10 PROCEDURES THAT ENHANCE NEURONAL GROWTH PROMOTING FACTOR(S) IN APLYSIA BLOOD. R. Waziri and S.M.Fredman Dept. of Psychiat., Univ. of Iowa, Col. of Medicine, Iowa City, IA 52242

The extension of neurites by Aplysia neurons in tissue culture requires "growth factor(s)" which is normally present in the blood. We have found that surgical manipulation can enhance the growth promoting abilities of Aplysia blood. Neurons from immature Aplysia (27g average weight) were mechanically dissociated without enzymes and grown on poly-L-lysine coated dishes using L-15 medium plus 5-10% (vol/vol) Aplysia blood. Blood was obtained from adult animals which had: i) an additional CNS implanted; ii) bilateral crush of the cerebro-pleural connectives; iii) CNS implant + connective crush. Blood from size-matched sham-operated and unoperated animals served as controls as did blood taken from the experimental animals prior to surgery. The growth promoting ability of blood from animals with nerve crush + implant was > implant alone >than crush alone > controls. The blood from crush + implant animals not only stimulated more neurons to sprout processes but also promoted more extensive neuritic arborization. This indicated that when combined the two procedures had a synergistic and non-linear effect. These results lead to the conclusion that the CNS of Aplysia is the source of blood borne neuronal growth promoting factors which are enhanced in response to injury. Our data are in agreement with results of Wong et al. (J. Neurosci 1, 1008-1021, 1981) that CNS "conditioned" medium supports the growth of cultured molluscan neurons.

182.11 COMPARISON OF THE NEURITOGENIC ACTIVITIES OF CYCLIC NUCLEOTIDES AND STRIATED MUSCLE-CONDITIONED MEDIUM ON CILIARY GANGLIA IN VITRO. Glenn M. Monastersky* and Fred J. Roisen (SPON: R. Duvoisin). Dept. of Anatomy, UMDNJ-Rutgers Medical School, Piscataway, NJ 08854.

We reported recently that cyclic adenosine-3',5'-monophosphate (cAMP) and its dibutyryl derivative [$(But)_2$cAMP] stimulated neurite formation in embryonic chick ciliary ganglia (CG) in vitro in a dose-dependent fashion. Growth cones (gc) produced in response to exogenously applied cAMP were not associated with neighboring non-neuronal cells as they grew out from CG explants on a collagen-coated substratum. This lack of non-neuronal association exhibited by nucleotide-induced gc was in sharp contrast to the consistent non-neuronal association observed for gc produced by treatment with striated muscle-conditioned medium (StCM). Cyclic nucleotides maintained neurites for up to 72h, whereas StCM supported neurites for more than one week. We have examined further the responses of CG to cAMP and StCM. Simultaneous treatment of CG with $(But)_2$cAMP and StCM had non-additive effects on neurite number and length as well as non-neuronal outgrowth. The gc of neurites in these cultures were either directly associated with non-neuronal cells or isolated. Sequential treatment of CG with StCM for 48h followed by treatment with $(But)_2$cAMP resulted in displacement of the non-neuronal-gc associations formed in response to StCM. To examine the effects of cAMP and StCM on polyamine biosynthesis in CG, the levels of ornithine decarboxylase (ODC), the rate-limiting enzyme in this synthetic pathway, were determined. Treatment with $(But)_2$cAMP elevated CG ODC activity whereas StCM, although highly neuritogenic, did not. We have been examining the effects of several substrata on CG neuritogenesis, including primary and established monolayers. To investigate the role of non-neuronal cells in CG neuritogenesis and to test the specificity of the non-neuronal cell association of StCM-stimulated gc, organized and dissociated CG were plated on monolayers of L929 fibroblasts in the presence or absence of StCM. In these experiments, no neuritogenesis was observed. StCM pre-treatment of L929 monolayers for 24h to permit adsorption of neurite-promoting factors did not enhance CG neuritogenesis, as has been shown for poly-DL-ornithine (PORN) substrata. Currently, we are comparing the neurite promoting activity of cAMP with the PORN-adsorbable component of StCM. Although StCM and cAMP both stimulate CG neuritogenesis, our evidence suggests that they are acting via divergent mechanisms.

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192.12 EVIDENCE FOR BIOCHEMICAL SEPARATION OF MORPHOLOGIC AND CHOLINERGIC-ACTIVE TROPHIC FACTORS FROM EXTRACTS OF NEWBORN RAT SKELETAL MUSCLE, R.G. Smith*, J. McManaman*, and S.H. Appel. Dept. of Neurology, Baylor College of Med., Houston, TX 77030.

We have previously reported the existence of trophic substances extracted from newborn rat skeletal muscle that promote neurite elongation and enhance cholinergic activity in cultures of dissociated 14-day embryonal rat ventral spinal cord. We have also described how these factor-stimulated activities are differentially regulated as a function of the age and innervation-denervation state of the rat from which the extract is prepared. We now report that polypeptides mediating morphological and cholinergic effects are biochemically distinct.

The morphological and cholinergic factors are quantitatively different with respect to their sensitivities to inactivation by heat and trypsin. The neurite inducing activity is labile to heating at 60°C with half-maximal loss at 10 minutes, and to trypsin (0.1 mg/ml at 37°C) with a $t_{\frac{1}{2}}$ of approximately 45 minutes. In the same skeletal muscle extracts, at the same test concentrations, the cholinergic factor activity is lost at 60°C with a $t_{\frac{1}{2}}$ of 30 minutes, and inactivated by trypsin with a $t_{\frac{1}{2}}$ of 15 minutes. At pH between 8 and 10, the cholinergic factor is stable, while the morphological factor is rapidly inactivated.

Following chromatography on Sephacryl S-200, a single peak of neurite inducing activity is recovered, with an apparent molecular weight of 33-37,000 d. On isoelectric focusing, this activity peak elutes at a pI of 4.8. There is no cholinergic inducing activity associated with these fractions. At present, the neurite inducing activity has been purified more than 10,000-fold over the crude extract. The cholinergic stimulating activity is eluted in three different peaks, both by gel chromatography, and by isoelectric focusing. These cholinergically active factors are presently being further purified and characterized. (Supported in part by grants from the John A. Hartford Foundation and the Robert J. Kleberg and Helen C. Kleberg Foundation.)

VISUAL CORTEX: INTRINSIC ORGANIZATION II

183.1 UPTAKE AND LAMINAR DISTRIBUTION OF TRITIATED ASPARTATE, GLUTAMATE, GABA AND GLYCINE BY NEURONS IN THE VISUAL CORTEX OF SQUIRREL MONKEYS: CORRELATION WITH LEVELS OF CYTOCHROME OXIDASE ACTIVITY. E.W. CARROLL* and M. WONG-RILEY (SPON: R.L. Curtis). Dept. of Anat. Med. Coll. of Wis., Milwaukee, WI 53226.

The laminar distribution of four putative neurotransmitters was compared in the visual cortex of squirrel monkeys to determine if any correlation existed between the cytochrome oxidase-rich regions and the neuronal uptake of these amino acids. Each of the four tritium labeled (^{3}H) compounds: aspartic acid (ASP), glutamic acid (GLU), GABA and glycine (GLY), at concentrations of 2.5 to 6μCi/0.1-0.2μl/site, was injected into restricted zones within areas 17 and 18 of different animals. Perfused brain tissues were reacted for cytochrome oxidase (C.O.) and subsequently processed for autoradiography.

In general, radioactively labeled neurons that were C.O.-reactive (C.O./^{3}H+) and C.O.-nonreactive (^{3}H+) were present in all laminae with each amino acid tested. The laminar distribution and density of ^{3}H+ and C.O./^{3}H+ neurons were similar for the putative excitatory transmitters ASP and GLU; however, differences existed between ASP/GLU, GABA and another putative inhibitory transmitter, GLY.

ASP/GLU In lam II and III, few neurons (^{3}H+ or C.O./^{3}H+) were labeled with either ASP or GLU. An increase in labeled neurons (^{3}H+ and C.O./^{3}H+) was observed for both amino acids in lam IV. The greatest number of labeled neurons were found in lam V and VI, with C.O./^{3}H+ neurons more abundant in lam VI. Labeled fusiform (7x14μm) and stellate shaped neurons (8-20μm in diameter) were observed within the various laminae, as were large (25μm in diam.) C.O./^{3}H+ pyramidal neurons in laminae V and VI.

GABA-labeled neurons (C.O./^{3}H+ and ^{3}H+) were more prevalent in lam II than III. Preliminary analysis indicated that there were more C.O./^{3}H+ neurons within the cortical C.O.-rich puffs. An increase in GABA labeled neurons (C.O./^{3}H+ and ^{3}H+) was observed in lam IV to lam VI, with the most prominent increase in lam V and VI. Labeled neurons (C.O./^{3}H+ and ^{3}H+) in these and other laminae included fusiform (8x17μm) and stellate neurons (8-15μm).

GLY Significantly more neurons (C.O./^{3}H+ and ^{3}H+) were labeled with GLY than with the other amino acids. These neurons were more uniformly distributed throughout all cortical laminae. They included fusiform and stellate neurons of all sizes from lam II to VI, a distinct row of large fusiform-shaped neurons (C.O./^{3}H+) in lam IVB, and large C.O./^{3}H+ pyramidal neurons at the V-VI border.

Preliminary results in area 18 suggested that the distribution and density of labeled neurons (^{3}H+ and C.O./^{3}H+) paralleled the laminar distribution and differential uptake observed in area 17.

183.2 GLUTAMIC ACID DECARBOXYLASE IMMUNOREACTIVE NEURONS AND TERMINALS IN THE VISUAL CORTEX OF MONKEY AND CAT. D. Fitzpatrick*, J.S. Lund, D. Schmechel. Ophthalmology Research, Medical University of South Carolina, Charleston, South Carolina and Division of Neurology, Department of Medicine, Duke University, Durham, North Carolina 27706.

Considerable evidence supports the idea that GABA mediated inhibition plays an important role in shaping the response properties of visual cortical neurons, yet we know little about the morphology, distribution, frequency and connectional relations of GABAergic cortical neurons. In this study we have examined the distribution of neurons and terminals which are immunoreactive for glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA, with in the visual cortex of squirrel monkey and cat.

GAD immunoreactive terminals and axon dilatations are found in all layers of the cortex and are frequently found surrounding the somata of both immunoreactive and non-immunoreactive neurons. In both species, cytochrome rich geniculate recipient zones are distinquished from other cortical layers by the presence of distinctly larger immunoreactive terminals; and, in the squirrel monkey, this distinction also applies to the geniculate recipient patches of layer 3.

GAD immunoreactive somata are present in all layers and constitute 8-15% of the neurons. No clearly identifiable glial cells appeared to be immunoreactive. The proportion of GAD immunoreactive neurons remains relatively constant across layers. For example, there is no significant difference in the proportion of GAD immunoreactive neurons in geniculate recipient vs. non-geniculate recipient zones (including the layer 3 patches in the monkey). However there are obvious laminar differences in the sizes of GAD immunoreactive neurons, with particularly large neurons occuring in layers 4A, 5 and 6 in the cat, and layers 4Cα and 6 in the monkey. The largest neurons in monkey striate cortex (those in 4B and in the upper part of layer 6) are not found to be GAD immunoreactive.

Laminar differences in the sizes of GAD immunoreactive terminals and cell bodies suggest that GAD neurons may comprise a heterogeneous population and may be the source of an inhibitory circuitry that is unique for different laminae.

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183.3 THE EFFECT OF IMPULSE BLOCKAGE ON CYTOCHROME OXIDASE ACTIVITY IN THE MONKEY VISUAL SYSTEM. M. Wong-Riley and E. Carroll*. Dept. of Anat., Med. Coll. of Wis., Milwaukee, WI 53226.

Neurons in the visual system are particularly sensitive to sensory deprivation and environmental alterations. Recently, we found that the blockage of impulse transmission with intravitreal injections of tetrodotoxin (TTX) could lead to significant though reversible changes in cytochrome oxidase (C.O.) activity in the adult cat visual system (Wong-Riley & Riley, '83). The present study sought to extend the findings to the primate, where C.O.-rich zones have been characterized by others (Horton & Hubel, '80, Humphrey and Hendrickson, '80) and ourselves.

Adult Macaque monkeys were anesthetized and were monocularly injected with 19ug TTX in 10ul D.W. every 3-4 days. Survivals ranged from 3 days to 4 weeks. Perfused brains were then processed for cytochrome oxidase histochemistry. In the normal retina, the large ganglion cells were usually much more reactive than the medium and small ones. Sublamina b (presumed ON-center; Nelson et al., '77) of the inner plexiform layer (IPL) was frequently more reactive than sublamina a (presumed OFF-center), with a relatively clear zone in between. Horizontal cells were highly reactive, as were the photoreceptor inner segments, the cone pedicles and the outer plexiform layer. The pattern of reactivity in TTX-treated retinae was similar to the normal, with a slight decrease in the ganglion cell layer, the IPL and the unmyelinated portions of ganglion cell axons which were previously quite reactive. In the normal lateral geniculate nucleus (LGN), magno layers 1 and 2 and parvo layer 6 were the most reactive, followed by layer 5, while layers 3 and 4 were the least reactive. Thus, the ON-center layers 5 and 6 (Schiller & Malpeli, '78) were more reactive than the OFF-center layers. TTX-treatment resulted in reduced C.O. activity in the LGN laminae innervated by the injected eye. However, changes were more marked on the ipsi- than the contralateral side. Within the striate cortex, TTX treatment induced a pattern of alternating light and darkly reactive bands in lamina IV C, as well as alternating rows of dark puffs and smaller, lightly reactive puffs in laminae II-III. Serial reconstructions revealed the exact alignment of lightly reactive puffs in II-III with light bands in IV A and IV C, and the darkly reactive puffs with dark bands. Changes in the LGN and area 17 could be seen as early as 3 days post injection. Thus, neurons of the adult primate visual system remain extremely sensitive to the pattern of impulse activity arising at the primary afferent level. In the presence of axoplasmic transport, cessation of ganglion cell transmission can lead to enzymatic adjustment in neurons several synapses away.

183.4 TANGENTIAL ORGANIZATION OF THE SEROTONERGIC INNERVATION OF PRIMATE VISUAL CORTEX. B.E. Kosofsky, M.E. Molliver, M.S. Lewis*, and H.G.W. Lidov. Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The cerebral neocortex consists of a tangentially continuous sheet of grey matter in which the neurons with a particular morphology and density form horizontal layers that have characteristic afferent and efferent connections. The functional units of cortical signal processing are vertical columns in which the cells of each layer subserve a particular role in cortical circuitry. In an effort to determine which neuron sets are directly influenced by raphe-cortical serotonergic axons we have employed PAP immunocytochemistry with an antibody directed against 5-hydroxytryptamine (5-HT) to investigate the axonal morphology and ultrastructural specializations which characterize the serotonergic innervation of the visual cortex of Cynomolgus monkeys.

In primary visual cortex, 5-HT-positive axons display a sharply demarcated laminar distribution that varies in the density, morphology and predominant orientation of constituent fibers, and that is in register with the cytoarchitectonic layers. Within each cortical layer the density of axons is uniform throughout area 17, and we were unable to detect tangential periodicity in the distribution of fibers. A dense plexus of finely ramified 5-HT processes in lamina IVA, IVB and IVCα suggests the presence of a localized zone of terminal innervation in these layers. This zone is bordered above by the lower density plexus of layers II-III where fibers of oblique orientation predominate, and below by the sparsely innervated layer IVCβ where the fibers are predominantly radial in orientation. In contrast to the low density in IVCβ there is a dense tangential plexus of fibers in VA. This distribution of 5-HT fibers contrasts with that in the Squirrel monkey where the highest density of 5-HT axons extends throughout layer IVC and no fibers are found in layer V (Morrison, et al. PNAS 79 (1982) 2401-2405). Varicosities are distributed along 5-HT fibers with much greater spacing in the primate than in the rat. In area 17 of Cynomolgus, we have observed laminar differences in the density of varicosities (e.g., high in VA; low in IVB); the density does not vary in direct proportion to axon density. Ultrastructural examination reveals that 5-HT varicosities form typical synaptic contacts with asymmetric membrane specializations. While there are species differences in the specific sites of termination, we conclude that in area 17 serotonin input selectively and differentially influences vertically restricted neuronal elements in a tangentially continuous fashion. The laminar precision in each species together with the presence of specialized synaptic contacts provide further support for a high degree of anatomic specificity in the sites of serotonergic action in the neocortex. (Sup: GM7303, NS15199, RR5378)

183.5 GENICULOCORTICAL AFFERENTS IN THE MINK: EVIDENCE FOR ON/OFF AND OCULAR DOMINANCE PATCHES. S.K. McConnell and S. LeVay, Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

The mink's lateral geniculate nucleus contains a pair of A layers innervated by the contralateral eye and a pair of A1 layers innervated by the ipsilateral eye. Of each pair, one layer contains on-center cells and the other off-center cells (LeVay and McConnell, Nature 300:350, 1982). We have gone on to examine how these four sets of geniculate afferents terminate in area 17.

The distribution of left and right eye afferents was first examined by autoradiography following transneuronal transport of ^{3}H-proline injected into one eye. In the binocular regions of areas 17 and 18, label was distributed in a patchy fashion, suggesting the presence of ocular dominance columns. Contralateral eye columns were wider than ipsilateral columns (500 μm vs. 300 μm), consistent with the greater thickness of the contralaterally innervated layers of the LGN. The monocular region of area 17 was proportionately larger than that of the cat or monkey; this correlates with the mink's more extensive monocular field of view.

To study the distribution of geniculate afferents physiologically, we recorded from their terminal arborizations in layer IV of area 17 after silencing all cortical neurons in the vicinity with injections of 0.1% kainic acid. (This technique was developed by Helen Sherk.) In this situation, neural activity was almost entirely restricted to layer IV. Here multiple units were commonly recorded simultaneously; these showed fast spikes and a high spontaneous level of firing. When single units could be distinguished, they were monocular and had a center-surround organization. This activity presumably arises from geniculate afferents in layer IV. During tangential penetrations through layer IV, aggregate receptive fields advanced in an orderly fashion through the visual field, often showing changes in position after steps of as little as 25 μm. In these penetrations we encountered periodic shifts in ocular dominance consistent with the results from autoradiography. Although the centers of ocular dominance patches were monocular, transitions from one eye to the other were gradual. In addition, there were stretches during which responses were exclusively or predominantly on- or off-center. On and off patches were also found to alternate, with stretches of mixed activity between them. Reconstructions from parallel tangential penetrations indicated that these patches extended the full thickness of layer IV. They were about 100-300 μm in width, i.e. somewhat smaller than ocular dominance patches.

These results indicate that in the mink the segregation of responses according to both sign of contrast and eye of origin is maintained up to the level of layer IV of visual cortex.

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183.6 LAYER 6 CELLS PRIMARILY CONTACT SMOOTH AND SPARSELY SPINY NEURONS IN LAYER 4 OF CAT STRIATE CORTEX. B.A. McGuire, J.P. Hornung, C.D. Gilbert and T.N. Wiesel. (Department of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, MA 02115, U.S.A.)

The main afferent input to layer 4 of striate cortex is from the lateral geniculate nucleus (LGN), but the neurons in this layer also receive a substantial input from axon collaterals of layer 6 cells. We have studied the layer 6 to layer 4 pathway ultrastructurally to help determine how this input may influence the functional properties of cells in layer 4. Intracellular recordings were made from layer 6 neurons, their receptive fields were characterized, and the cells were then injected with horseradish peroxidase. After processing the tissue for light and electron microscopy, we reconstructed each cell and chose a portion of its axonal arbor in layer 4 for serial thin sectioning. Labeled axon terminals and their postsynaptic processes were identified by serial EM reconstruction. Compared to injected LGN terminals, which possessed repeated swellings making synaptic contacts en passant, the layer 6 terminals were smaller, each typically projecting off the parent axon collateral by a narrow (0.1-0.2 um) stalk and making a single asymmetric synaptic contact containing round vesicles. Sampling 138 synapses made by axon collaterals of 2 cells, we found that 71% contacted dendritic shafts and 29% contacted spines. This pattern is the reverse of that seen for the geniculocortical afferents, of which 80% end on spines and 15% on dendritic shafts[1]. We have thus far characterized 57 postsynaptic dendrites and subdivided them into 3 classes based on differences in spine frequency, density of synaptic inputs, and dendritic morphology: (1) beaded dendrites (10 cases), (2) smooth and sparsely spiny dendrites (40 cases), and (3) spiny dendrites (7 cases). Our finding that layer 6 cells form asymmetric synapses, and the presence of high affinity uptake of aspartate[2] suggest an excitatory effect on layer 4 cells. The great majority (50 out of 57) of the identified postsynaptic dendrites belonged to smooth and sparsely spiny stellate cells, which are thought to be inhibitory[3]. Thus, the overall effect of the layer 6 to layer 4 pathway is likely to be inhibitory, even if there was also some direct excitatory input to spiny stellate cells. In this pathway, one possible role for layer 6 cells with very long receptive fields could be to produce the property of end-inhibition via non-spiny stellate cells.

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2 Baughman, R.W. & Gilbert, C.D. J. Neurosci. 1: 427-439 (1981).
3 Ribak, C.E. J. Neurocytol. 7: 461-478 (1978).

183.7 TWO RESTRICTED LAMINAR LOCI IN MACAQUES MAY ALONE SUBSERVE THRESHOLD DETECTION OF MICROSTIMULATION IN STRIATE CORTEX. Edgar A. DeYoe and Robert W. Doty, Center for Brain Research, University of Rochester, Rochester, NY 14642

Electrical stimulation applied to the surface of striate cortex in man is known to elicit a simple, specific visual sensation, the phosphene. Since the physiological basis of this phenomenon is obscure, and since the macaque and human visual systems are so similar, we have investigated the physiological and behavioral effects of comparable stimulation applied within striate cortex of monkeys trained to respond when a detectable sensation(?) is thus electrically induced. Optimal, cathodal, constant current trains of 30, 0.2-msec. pulses, 100 Hz were presented via glass-insulated, Pt/Ir microelectrodes advanced step-wise perpendicularly through cortex. At any location transition from non-detection (<10%) to complete detection (>90%) occurred over a current range ±16.5% of threshold (50% detection). Electrode tip positions relative to the cortical laminae were determined with the aid of two electrophysiological indices: level of multiunit activity (taken as 2X the standard deviation of a 100-msec. sample at 16 KHz), which reached a maximum in layer 4C (terminology of Lund, J. Comp. Neurol. 147: 455, 1973), and diminished abruptly in layer 5; and the appearance of an early positive peak in the flash evoked potential at the transition between layers 4 and 5. Consolidating these data with histological verification, two minima (1-5uA) in threshold for stimulus detection were revealed, one in lower layer 3/upper 4, the other in layer 5. In other layers, significantly higher currents (up to the maximum 25 uA tested) were required. In 16 complete penetrations, the average lowest and highest thresholds differed from the mean within a given penetration by -35% and +75%, respectively. Taking the data of Stoney et al. (J.Neurophysiol.31:659,1968) on the relative excitability of pyramidal cells as a function of distance from the source of microstimulation, it can be calculated that only two bands of excited elements, at the junction of layers 3 and 4, and in layer 5 could account for the average pattern of detection thresholds in the present experiments; all higher thresholds merely reflecting current values required to affect these elements from a distance! The additional assumption of restricted "columns" of excited elements, perhaps related to the inhomogeneites revealed by cytochrome oxidase histochemistry (e.g. Humphrey and Hendrickson, J.Neurosci.3:345,1983), could account for significant absolute differences observed from one individual penetration to another. (Supported by NIH grant NS 16180.)

183.8 DIFFERENTIAL EFFECTS OF UNIFORM AND RANDOM-NOISE BACKGROUNDS ON THE ONGOING ACTIVITY AND EVOKED RESPONSES OF NEURONS IN FOVEAL A17 AND A18 OF THE MACAQUE. Y. Trotter*, S. Squatrito*, B.C. Motter* and G.F. Poggio, - Bard Laboratories of Neurophysiology, Dept. of Neuroscience, Johns Hopkins Sch. of Med., Baltimore, MD 21205.

We have investigated the effects of uniform (dark & luminous) and dynamic random-dot backgrounds on the ongoing activity and binocular responses of neurons in foveal striate and prestriate cortex of rhesus monkeys trained to fixate on a small target. Background fields subtending 5° square were centered over the receptive field of the neuron under observation. Stimulus bars of optimal size and orientation, moving bidirectionally across the field, were superimposed on the background which was continuously present both during and in between behaviorally controlled fixation trials. The position of the eyes was routinely measured.

Following onset of the monkey's attentive fixation of the target, the ongoing activity of more than 50% of the cells (N = 238) was differentially affected by the background. More frequently (44%), the neuron's maintained discharge rate was considerably higher in the presence of a textured background than a uniform one. In only 9% of the neurons, the uniform background specifically affected the ongoing activity. Moreover, for many cells (35%), the activation by visual noise was present also in the intertrial period of relaxed uncontrolled oculomotor behavior.

The effect of background on cortical activity may change when the animal shifts from the relaxed to the attending behavior. For noise background, we observed an increase in or the onset of high maintained activity at the time of the shift in 17% of foveal neurons, and changes to a higher or lower discharge rate were seen almost as frequently in other neurons to accompany the behavioral shifts in the presence of a uniform background.

An effect of the dynamic visual noise on the response to a moving bar was observed for 60% of neurons. Most frequent and dramatic was a significant reduction or the complete disappearance of the response in the ongoing activity (48%). In a few neurons (8%) the response was enhanced by the noise, being larger and briskier when evoked on the textured background than on the uniform one. Rarely (4%) the noise background revealed an inhibitory response to a bar which evoked an excitatory response when presented on a uniform background.

These findings emphasize the importance of the whole visual scene on the activity of visual cortical neurons and the need to consider static and dynamic aspects of both the visual scene and the attending behavior in any attempt to interpret processing of visual information by neurons.

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183.9 BINOCULAR INHIBITORY MECHANISMS IN STEREOSCOPIC PROCESSING. Jill C. Gardner, Research Laboratory of Electronics, Massachusetts Institute of Technology, Bldg. 36-864, Cambridge, Mass. 02139

In the combination of input from the two eyes, neurons in area 18 and the 17/18 border of the cat show widespread binocular inhibition. In normal animals, the large role played by inhibitory mechanisms in determining the disparity-sensitive response of visual cells was seen in binocular interactions to two types of depth-related stimulus motion. Stimuli moving in the same direction on the two retinae (in-phase) represented sideways motion at different distances from the animal while stimuli moving in opposite directions on the two retinae (antiphase) represented motion toward or away from the animal or motion in depth. Examining disparity-sensitive responses across these two movement conditions showed that binocular interactions to in-phase moving stimuli were characterized by strong direction selective inhibition in the preferred direction of motion and that they were significantly larger than interactions to antiphase stimulus motion ($p<.005$). When the facilitatory and inhibitory components of the binocular responses were analyzed separately, it was seen that differences between the two movement conditions were due exclusively to differences in levels of binocular inhibition ($p<.001$). No differences were seen between the distributions of binocular facilitation.

The significance of binocular inhibitory mechanisms in the development and function of stereoscopic processing was further illustrated in experiments examining the disparity-sensitivity of cells in cats in which normal binocular connectivity had been disrupted. Units in area 18 of animals reared with a neonatally-induced strabismus and units along the 17/18 border of cats with unilateral lesions of the visual cortex showed a reduction in sensitivity to binocular retinal disparity in conjunction with significant decreases in the strength of binocular inhibition ($p<.01$ and $p<.025$ respectively). In all preparations studied, the distributions of binocular excitation were matching, and were centered around the point of summation of the 2 eyes independent monocular responses. Inhibitory interactions were much more pronounced than facilitatory interactions and consistently reduced responses to below levels evoked by monocular stimulation.

These results indicate that in the determination of disparity-specific responses, it is binocular inhibition which is the crucial process. Although disparity signals may be generated by facilitatory responses, binocular inhibitory mechanisms appear to play a fundamental role in influencing the structure of stereoscopic systems. Since a unit's selectivity for stimulus disparity can be modified by experience, the data suggest that during the cat's early development, it is the inhibitory binocular connections which are the plastic ones.

183.10 SPATIAL AND TEMPORAL FREQUENCY SELECTIVITY OF NEURONS IN V1 AND V2 OF THE MACAQUE MONKEY. K.H. Foster*, J.P. Gaska*, M. Nagler*, and D.A. Pollen. Barrow Neurological Institute, Phoenix, AZ 85013.

The spatial and temporal frequency selectivity of over 230 neurons in V1 and V2 of the macaque monkey (Macaca fascicularis) was studied using sine-wave gratings of suprathreshold contrast drifting over the receptive field at the preferred orientation and direction. Neurons in V1 and V2 were selective for different but partially overlapping ranges of the spatial frequency spectrum. At a retinal eccentricity of 2^{o}-5^{o} from the fovea, the spatial frequency preferences for neurons in V1 ranged from 0.5-8.0 c/deg with a distribution of spatial frequency preferences and bandwidths essentialy similar to the results of De Valois et al. (Vision Res. 22, 545, 1982) based upon estimates of contrast sensitivity. However, spatial frequency preferences at this same retinal eccentricity in V2 ranged from <0.25 c/deg-2.8 c/deg and were, on average, about 2 octaves lower than in V1. Bandwidths in the two cortical areas were similar and ranged from 0.8-3.0 octaves.

Temporal frequency selectivity was low pass (down to 0.5 Hz) for 70% of neurons in V1 with little fall-off at high temporal frequencies until drift rates generally exceeded 4-5.6 Hz. Even the 30% of neurons in V1 with bandpass temporal tuning were most often very broadly tuned with a mean full bandwidth of 2.9 octaves. On the other hand, 70% of the neurons in V2 had bandpass temporal frequency characteristics with a mean full bandwidth of 1.8 octaves. Peak drift frequencies in V1 and V2 generally ranged from 1-8 Hz. Furthermore, at any given spatial frequency some examples of bandpass temporal frequency selectivity were found for neurons in V1 as well as in V2. The range of preferred temporal frequencies and their corresponding bandwidths suggest that there are at least two non-overlapping temporal filters at each spatial frequency.

These results in the macaque are surprisingly similar to those reported for the cat (Movshon et al., J. Physiol. 283, 101, 1978; Berardi et al., J. Physiol. 323, 603, 1982) in view of the differences in the geniculocortical projections within the two species. Moreover, the present results may provide a physiological substrate for psychophysical results in man which suggest several independent detector mechanisms centered at different temporal frequencies (Watson and Robson, Vision Res. 21, 1115, 1981), especially at low spatial frequencies (Bowker and Tulunay-Keesey, J. Opt. Soc. Am. 73, 427, 1983).

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183.11 SPATIAL PHASE DEPENDENCE OF CORTICAL VISUAL NEURON RESPONSES TO CONTRAST REVERSAL GRATINGS IN THE CAT.
S. Hochstein and H. Spitzer* Institute of Life Sciences, Hebrew University of Jerusalem, Jerusalem 91904 ISRAEL.

Cortical visual neurons have been classified as Simple or Complex on the basis of their performing linear or nonlinear spatial summation. This classification is analogeous to that of retinal ganglion and lateral geniculate neurons into the **X** and **Y** cell types, respectively. We now demonstrate dramatic differences betweeen the receptive field characteristics of Y-cells and those of cortical Complex cells. Specifically all retinal ganglion and lateral geniculate Y-cells in the cat lack a spatial phase dependence in their responses to high spatial frequency contrast reversal gratings, though at low spatial frequencies a linear aspect of their receptive fields introduces some spatial phase dependence. In the cortex, on the other hand, a distinct spatial phase dependence was found for nearly all cells studied, even those classified as Complex because of nonlinear spatial summation. The degree of spatial phase dependence is often a function of the spatial frequency of stimulation. A receptive field model is presented which may explain the mechanism underlying these stimulation dependent effects.

183.12 DIRECTION SELECTIVITY IN VISUAL CORTICAL NEURONS OF THE IMMATURE RABBIT. E. H. Murphy, A. M. Grigonis*, and G. J. Zingaro*. Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129.

Previous studies from this laboratory have demonstrated that many of the neurons of the primary visual cortex of the rabbit show orientation selectivity (OS), and virtually all (95%) of these cells also show direction selectivity (DS). Following early deprivation of visual experience, OS changes little, but the percentage of DS is significantly lower in strobe reared rabbits (40% DS), and in lid sutured rabbits (70% DS), compared with normals.

In the present study, in order to determine whether this reduced DS in deprived animals represents a failure in development or a breakdown of established organization, we have studied DS in visual cortical neurons of immature, normally reared pups. Some visually responsive cells are present at the time of eye opening (11 days), but OS is not observed until day 17. From the age of 17 days on, the percentage of cells with OS receptive fields increases rapidly, reaching adult levels by day 30, but the percentage of these cells which are also DS is approximately 70% throughout this time period. Thus, in normally reared animals aged less than 30 days, DS is significantly lower than that observed in the normal adult, significantly higher than that observed in the strobe reared adult, but approximately equal to that observed in the lid sutured adult.

The results suggest that the population of DS cells observed in the mature adult comprises several subsamples of cells which respond differently to experimental manipulation of sensory experience: some fail to develop DS, some lose DS, and some maintain DS despite abnormal early experience. The results also indicate that the normal development of DS in the rabbit visual cortex extends into the 2nd postnatal month, after the time that OS appears to have reached mature levels. Thus, DS develops later than OS and is more susceptible to the effects of early deprivation.

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183.13 INFORMATION FLOW THROUGH SINGLE CELLS OF CAT STRIATE CORTEX. B. Bridgeman and J. Artim*, Vision Research Laboratory, U. of Ca., Santa Cruz, CA 95064.

Most studies of single neurons in sensory systems have failed to present stimuli with information value to the experimental animal. We have compared responses of cat striate cortex neurons to informative and to non-informative stimuli. A cat learned to press a pedal in response to a flashed stimulus (a 20 msec pattern of random lines) repeated every 10 sec, receiving a soymilk reward if the press was within a 0.5-1.5 sec post-stimulus window. After overtraining on this task, hardware was surgically implanted to allow extracellular single unit recording from the awake and behaving animal. There were 3 trial types: 50% of the trials had only the initial informative flash, 25% had an additional physically identical but unrewarded flash 500 msec after the first flash, and the remaining 25% had an unrewarded flash 3-5 sec after the informative flash. Cats learned to respond only to the rewarded flashes. Neurons were divided into two categories: 27 that exhibited a primary response (30-70 msec post-stimulus) and 17 that did not. The distinction was arbitrary since all cells were exposed to the same flashed stimulus.

Neural firing rates varied with stimulus information content, momentary state of alertness, and behavioral response. Cells which exhibited a primary response also showed enhanced responses for the rewarded flashes compared to the randomly timed unrewarded flashes. The latency to peak response for the rewarded presentations was 10 msec less than for the randomly interspersed unrewarded stimuli. Additionally, the informative flashes showed a 21% enhancement in peak firing rate compared to the 500 msec delayed flashes. Background activity also changed with reward contingencies. Backward averaging synchronized with the behavioral response showed a decrease in unit firing ranging from 34% to 74% in the 100 msec before the pedal press. The results show that firing of striate cortex neurons is modified by task variables and by information content of stimuli, even when stimulus parameters remain constant.

(Supported by NIH EY04137.)

184.1 INCREASED TRANSLATIONAL PREINITIATION COMPLEX FORMATION EFFICIENCY WITH S40 RIBOSOMAL SUBUNITS ISOLATED FROM INDUCED DIFFERENTIATED NEUROBLASTOMA CELLS. K. Fan, Depts. of Pathology and Medical Research, VA Medical Center, Little Rock, AR 72206.

Cultured neuroblastoma cells can be induced to morphological and biochemical differentiation by chemical such as dibutyryl cyclic-AMP (db-cAMP), cAMP or serum-deprivation. In an attempt to study whether there is translational regulatory mechanism in operation during the induction stage of differentiation, experiments were conducted to study the efficiency of the preinitiation complex ($Met\text{-}tRNA_f \cdot eIF_2 \cdot GTP \cdot S40$ subunits) formation using S40 subunits isolated from db-cAMP induced differentiated mouse neuroblastoma 2a cells, as compared with the S40 subunits isolated from neuroblastoma cells not exposed to db-cAMP, i.e., undifferentiated cells. The $Met\text{-}tRNA_f$ was isolated from Sprague-Dawley rat livers and in-vitro charged with ^{35}S-methionine, mediated by E. coli Met-tRNA synthetase. The initiation factor (eIF_2) was also purified from the rat livers. During experiments, two independent steps were carried out: Step 1 was to form the initiation ternary complex ($^{35}S\text{-}Met\text{-}tRNA_f \cdot eIF_2 \cdot GTP$) in buffer (20 mM Tris-HCl, pH 7.2, 100 mM KCl, 1 mM dithiothreitol, 0.1 mM $MgCl_2$ containing 15 µg eIF_2, 4.5 mM GTP and approximately 50,000 CPM $^{35}S\text{-}Met\text{-}tRNA_f$ (3000 cpm $pmol^{-1}$). After 7 minutes at 37°C, 2 $O.D._{260}$ S40 ribosomal subunits isolated from differentiated and undifferentiated cells were added into the 0.2 ml reaction mixture (Step 2). After Mg^{++} concentration was adjusted to 2 mM, the reaction mixtures were further incubated at 37°C for 15 minutes, then applied onto a 10-30 percent sucrose gradient (50 mM Tris-HCl, pH 7.6, 4 mM $MgCl_2$, 50 mM KCl and 1 mM dithiothreitol). After centrifugation, the fractions contained the $^{35}S\text{-}Met\text{-}tRNA_f$ S40 complex were collected and the radioactivity determined. Alternately the reaction mixtures were washed onto Millipore HA type filters and the retainable radioactivity determined.

Findings indicated that 3-fold higher preinitiation complex formation efficiency was observable when the S40 subunits isolated from db-cAMP induced differentiated cells were used, as compared with S40 subunits isolated from undifferentiated cells.

The findings suggest that there is a translational regulatory mechanism in operation during differentiation stage of neuroblastoma; for the present study, probably via a cAMP-dependent phosphorylation of the S40 ribosomal subunits.

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184.2 MOLECULAR GENETICS OF THE MYELIN BASIC PROTEIN SYSTEM OF THE RAT/ A. H. Roach*, S. B. Prusiner[1] and L. E. Hood* (SPON: F. Strumwasser). Div. of Biology, Calif. Inst. of Technology, Pasadena, CA 91125, and [1]Dept. of Neurology, Sch. of Medicine, Univ. of California, San Francisco, CA 94143.

Myelin basic proteins (MBPs) are a family of highly basic (pI>10), abundant proteins found in myelin, related in sequence but differing in size. They are thought to play a structural role, and are an autoimmune antigen in the demyelinative disease multiple sclerosis. While many species appear to have a single MBP, rat and mouse have two major forms, differing only by the presence or absence of 40 amino acids at a site 13 residues from the C-terminus (reviewed in Carnegie, P. and Moore, W. in Proteins of the Nervous System, Bradshaw, R. and Schneider, D. (eds.) Raven Press, New York, 1980). Two additional minor species in mouse have also been reported (Barbarese, E. et al, Proc. Nat. Acad. Sci. USA 74, 3360 (1977)).

Synthetic oligonucleotides were used as a hybridization probe to select cDNA clones encoding MBPs from a library constructed using brain RNA from myelinating 18-day-old rats. Several clones were examined in detail with the aim of understanding the origin of the multiple forms of MBP in the rat.

cDNA clones of two classes were found, sharing homology by restriction site mapping and nucleic acid hybridization over much of their length but being nonhomologous in their 5'-untranslated regions. A 1.5kb stretch from one of these 2kb cDNAs has been sequenced and contains the entire translated region for the small (M.W. 14,000) rat MBP, as well as more than 1kb of untranslated sequence. Sequencing of a cDNA clone of the other class is being undertaken to elucidate precisely the pattern of relatedness of the transcribed sequences.

RNA blotting experiments show that MBP sequences are carried on polyadenylated RNAs of 2.1kb in length.

In order to determine the gene organization, analysis of genomic sequences was performed by Southern blotting using cloned MBP-specific probes. These and other experiments directed towards cloning the corresponding genomic sequences promise to explain the interesting relationship between the proteins.

184.3 INTRODUCTION OF TORPEDO ACETYLCHOLINE RECEPTOR GENES INTO MAMMALIAN CELLS. T. Claudio*, M.J. Palazzolo* and R. Axel* (SPON: J. Goodrich). Inst. of Cancer Res., Columbia Univ., New York, N.Y. 10032.

We are using gene cloning and gene transfer to study the structure and function of individual acetylcholine receptor subunits as well as the biosynthesis, assembly and physiology of the nicotinic acetylcholine receptor complex. The expression of exogenous acetylcholine receptor genes in recipient cells first requires that we clone the genes encoding each of the four subunits, construct appropriate vectors which facilitate their expression and introduce these genes into recipient cells where their function may be analyzed. To this end we have constructed a library of cDNA clones synthesized from the messenger RNA of the electric organ of Torpedo californica. We have isolated clones which contain the entire structural gene sequence of each of the four subunits (probes for two of the genes were generously provided by D. Noonan, D. Hershey, K. Mixter and N. Davidson). These clones were then inserted into a eucaryotic expression vector which places the genes under the control of the SV40 early promoter. This vector further contains an SV40 origin of replication allowing the replication of these recombinant molecules to high copy number in recipient cells which express T antigen. This gene transfer system allows for transient replication and transcription of donor genes and provides a rapid means of analysis of receptor gene expression. In a seperate series of experiments, we have constructed stable mouse cell lines containing the four exogenous Torpedo subunit genes by cotransformation. We are currently analyzing the expression of these genes. This system will allow us to analyze the various properties of wild type receptor as well as allow us to make specific mutations in the genes and examine their effects on receptor function.

184.4 STRUCTURAL ANALYSIS OF THE GENES CODING FOR THE CATECHOLAMINE BIOSYNTHETIC ENZYMES. E.E. Baetge, V.R. Albert, H.M. Moon*, D.H. Park, D.J. Reis and T.H. Joh, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

We have proposed that the catecholamine (CA) biosynthetic enzymes tyrosine hydroxylase (TH), dopamine-B-hydroxylase (DBH), and phenylethanolamine N-methyltransferase (PNMT) contain similarities in their primary protein structures, based on the following evidence: (a) antibodies specific for one CA enzyme in vivo, immunoprecipitated more than one enzyme from in vitro translation products, suggesting that the catecholamine enzymes possess common antigenic determinants, and implying similarities in protein structure. (b) Proteolytic fingerprinting and amino acid composition analysis of the purified enzymes revealed that the enzymes contain similar protein domains in their primary structure.

To further test this hypothesis, mRNAs for the CA enzymes DBH and PNMT were partially purified from bovine adrenal and used for the synthesis of complementary DNA. Recombinant DNA molecules were formed by annealing appropriately modified vector and cDNA, and these molecules used for bacterial transformation. Bacterial clones containing DBH and PNMT cDNA inserts were identified by positive hybrid selection. In addition to selecting mRNA coding for 31,000 dalton PNMT, a PNMT cDNA clone hybrid selected a small amount of mRNA coding for 72,000 dalton DBH. Similarly, DBH cDNA clones hybrid selected small amounts of mRNA coding for 31,000 dalton PNMT as well as mRNA coding for 72,000 dalton DBH. It was further demonstrated by Northern blot hybridization that a mRNA of 1100 nucleotides in length coded for PNMT and that a separate mRNA of 5500 nucleotides coded for DBH. Northern blot analysis with a PNMT cDNA probe under less stringent conditions revealed a significant cross-hybridization with a 5500 nucleotide mRNA, identical in size to DBH mRNA. Southern blot analysis of restricted bovine adrenal or rat brain DNA, using either DBH or PNMT cDNA probes, revealed hybridization to one or two restriction fragemtns per enzyme digest indicating that both enzymes exist as single copies in the genome. Comparison of DBH and PNMT Southern blots also showed hybridization of both cDNA probes to restriction fragments of common size, suggesting that the two genes are linked.

These results provide strong evidence that regions of homology exist in the genes coding for DBH and PNMT, and suggest that these CA enzymes may be coded for by genes which are members of a linked catecholamine enzyme gene family. (Supported by Grant HL 18974.)

184.5 EVIDENCE FOR THE EXISTENCE OF MULTIPLE mRNA SPECIES CODING FOR BOVINE DOPA DECARBOXYLASE. V.R. Albert, D.J. Reis, and T.H. Joh, Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021

DOPA decarboxylase (DDC, EC 4.1.1.28, aromatic L-amino acid decarboxylase) catalyzes the conversion of L-dopa to dopamine and 5-OH tryptophan to serotonin. The enzyme also decarboxylates other aromatic amino acids including tyrosine and tryptophan, but at a much slower rate. DDC is localized in neuronal cells, where its major function is the production of dopamine or serotonin, yet large amounts of this enzyme are also found in non-neuronal tissues such as liver and kidney, where no monoamines are produced. In the present studies, we present evidence to suggest that there are different mRNAs coding for DDC in neuronal and non-neuronal tissues.

DDC was purified to homogeneity from bovine adrenal medulla by ion-exchange chromatography, gel filtration and hydroxyapatite chromatography. Antibodies to the enzyme were raised in rabbits and determined to be specific by immunodiffusion, immunoelectrophoresis and Western blotting. Initial biochemical and immunochemical comparisons of the enzyme isolated from bovine kidney, liver, brain and adrenal showed no significant differences: the K_m of the enzymes for L-DOPA and tyrosine were essentially the same; immunochemical titration, immunodiffusion and immunoelectrophoresis experiments revealed identical cross-reactivities with the antibody directed against the adrenal enzyme. However, immunostaining of crude preparations of the enzyme from these tissues separated by SDS-slab gel electrophoresis (Western blot analysis) revealed very slight differences in molecular weight. The antibodies recognized a single 57,000 MW protein in bovine adrenal and brain, a single 56,000 MW protein in bovine liver, and a doublet of 57,000 and 60,000 daltons in bovine kidney. Even greater differences were found when mRNA translation products were compared. mRNA was isolated from bovine adrenal medulla, liver and kidney, and translated in a reticulocyte lysate cell-free translation system. Immunoprecipitation of translation products with DDC antibody demonstrated significant differences in the size of the newly synthesized DDC. The translation product from bovine adrenal has a MW of 55,000 daltons. Newly synthesized DDC from bovine liver appears to be only 53,000 daltons. Newly translated DDC from bovine kidney appears as a doublet of 54,000 and 53,000 daltons. These experiments indicate that DDC probably undergoes post-translational modification in vivo, possibly involving addition of carbohydrate, to increase its size by 2-4000 daltons. More importantly, the differences in size between the in vitro translation products suggests that there are separate mRNAs coding for the enzyme found in different tissues, and further raises the possiblity that several genes code for this enzyme. (Supported by Grant MH 24285.)

184.6 HORMONAL CONTROL OF PRO-OPIOMELANOCORTIN GENE EXPRESSION. James Eberwine*, and James L. Roberts. Columbia University Biochemistry Department 630 West 168th Street New York, NY 10032.

Glucocorticoids suppress secretion of the peptide hormone pro-opiomelanocortin (POMC) in cultured mouse tumor cells and dispersed rat pituitary cells. Recently it has been shown that POMC mRNA levels decrease in the anterior lobe of the rat pituitary, while remaining unchanged in the intermediate lobe, in response to the synthetic glucocorticoid, dexamethsone. We have investigated the effects of glucocorticoids on the rate of POMC gene transcription, utilizing a sensitive in vitro nuclear transcription assay. The results indicate, that in the rat anterior lobe there is a decrease of 2-fold in POMC transcription which occurs rapidly, within 45 minutes of intraperitoneal injection of dexamethasone or corticosterone. Over a time course of 6 hours after hormone injection, the transcription rates slowly increase to near normal levels after the initial decrease to the minimum value measured at 45 minutes. The intermediate lobe cells are unaffected by dexamethasone administration. These results are confirmed via Northern blot analysis of POMC HnRNA as performed over the same time course. One week after adrenalectomy, the anterior lobe rate of transcription increased 5-fold while there is only a 3-fold increase after two weeks of adrenalectomy. Again the intermediate lobe POMC transcription rate remains unaffected. These rates of transcription in conjunction with the established values of the steady state level of POMC mRNA and protein production and secretion allow us to assess the relative contribution of each of these processes to the control of POMC gene expression.

The hormonal influences of corticotropin releasing hormone, haloperidol and ergocryptine are currently being examined to determine the relative contribution of each of these factors to the rate of POMC gene transcription. (This work is supported by NIH grant AM 274874).

184.7 EXPRESSION OF THE PRO-OPIOMELANOCORTIN GENE IN THE PITUITARY AND HYPOTHALAMUS. J.-P. Gagner, P. Burbach*, J. Charron*, L. Jeannotte*, M. Nemer* and J. Drouin*. Lab. of Molecular Biology of Eukaryotes, Clinical Research Institute of Montreal, 110 Pine Ave W., Montreal, Quebec H2W 1R7, Canada.

Pro-opiomelanocortin (POMC) is the precursor to ACTH, β-lipotropin, β-endorphin and the melanotropins. We are studying the structure and expression of the POMC gene in three rat and porcine tissues where it is expressed: the anterior and intermediate lobes of the pituitary and the arcuate nucleus of the hypothalamus. We have isolated by molecular cloning from a rat genomic DNA library in bacteriophage λ1059 about 25 kilobases of DNA containing the entire POMC gene. The three exons of the gene and about 500 base pairs of upstream sequence have been sequenced; comparison of these sequences revealed a high homology with the human, bovine and porcine genes. Southern blot analysis suggest that there is only one POMC gene in the rat; this is born out in the fact that eight independent isolates of this gene contain the same genomic sequences. Northern blot analysis indicate that POMC mRNAs of similar size are found in pituitary lobes but that the arcuate nucleus mRNA is slightly bigger; this size difference can be accounted for by different lenghts of polyadenylate tails. Primer-extension studies suggest that the 5'-ends of POMC mRNAs from the two pituitary lobes are similar.

The processing of POMC differs in the anterior and intermediate lobes of the pituitary; the regulation of the POMC gene also seems to be different in these tissues. For example, glucocorticoids only decrease POMC mRNA levels in the anterior pituitary and do not affect these levels in the intermediate lobe. We have measured the effect of dexamethasone treatment of adrenalectomized rats on POMC transcription rates by incorporation of ^{32}P-labelled UTP into transcripts of isolated pituitary cell nuclei. The labelled RNAs encoding POMC and actin (an internal control) are selected and quantitated by hybridization to corresponding filter-bound genomic DNA fragments. The experiments suggest that glucocorticoids inhibit within a few hours the transcription rate of the POMC, but not of the actin gene, in the anterior pituitary, which is in accordance with their effect on mRNA levels; on the other hand, glucocorticoids have little effect on POMC transcription in the intermediate lobe. There is a concomittant reduction of the plasma concentration of ACTH, a POMC maturation product characteristic of the anterior hypophysis. The glucocorticoid-responsive sequencesare now being dissected by reintroduction into cultured cells of the in vitro-modified POMC gene.

(Supported by the Medical Research Council and the National Cancer Institute of Canada).

184.8 REFINEMENT OF IN SITU HYBRIDIZATION AS A TOOL TO STUDY GENE EXPRESSION IN THE BRAIN. Josiah N. Wilcox*, Connie Gee and James L. Roberts. Center for Reproductive Sciences, Columbia University College of Physicians and Surgeons, New York, NY 10032. (Spon: M. Blum)

In situ hybridization histochemistry is a procedure that has been developed for the visualization of specific mRNA's in individual cells. Fixed cryostat sectioned tissue is incubated with labelled cDNA probes under conditions allowing cDNA-mRNA hybrids to form. Unhybridized probe is washed off at the end of the incubation procedure and the tissue processed for autoradiography and counterstained to visualize cells. The distribution and density of silver grains after developing the slides then yields information regarding the presence of absence of mRNA's complementry to the cDNA probe used. This technique has been used to visualize specific mRNA's in various somatic tissues. Our laboratory has used in situ hybridization to demonstrate regulatory changes in growth hormone and proopiomelanocortin (POMC) mRNA's in rat pituitary. We have also demonstrated that there are cells in the peri-arcuate region of the rat hypothalamus that contain POMC mRNA.

This technique holds great promise for the study of gene expression in the brain in that it allows single cell resolution of the cDNA-mRNA hybridization, an important consideration given the heterogeneity of neural tissue. However, major problems associated with applying in situ hybridization to the brain have been unacceptably high backgrounds and variable success in visualizing positive cells. We have refined the in situ procedure as applied to the study of POMC gene expression in the brain reducing backgrounds and increasing the reliability of the signal. Using both 3H and 32P labelled cDNA probes we have been able to visualize the distribution of cells containing POMC mRNA in the arcuate nucleus of the rat hypothalamus. Eventually we hope to make this technique quantitative so that it can be used to study regulation of POMC gene expression in the brain. (This work was supported by NIH grant AM 27484).

184.9 CELL-FREE BIOSYNTHESIS OF RAT NEUROPHYSIN PRECURSOR POLYPEPTIDES FROM POLY(A)RNA ISOLATED FROM INDIVIDUAL HYPOTHALAMIC NUCLEI. T.G. Sherman* and J.F. McKelvy. Dept. of Neurobiology and Behavior SUNY, Stony Brook, NY 11794.

Previous experiments have shown that total rat hypothalamic poly(A)RNA was capable of directing the active cell-free translation of multiple high molecular weight forms of neurophysin protein (Sherman & McKelvy, Ann. N.Y. Acad. Sci., 394:82, 1982). *In vitro* translation studies which examine whether hypothalamic RNA may code for one or more neurophysin precursors beyond the vasopressin-neurophysin I (AVP-RNp I) and oxytocin-neurophysin II (OT-RNp II) common precursors were complicated by the fact that neurophysin proteins are synthesized within several different discrete hypothalamic nuclei. The supraoptic (SON), paraventricular (PVN) and suprachiasmatic (SCN) nuclei are three principal neurophysin-containing cell groups in the rat hypothalamus. In an effort to more closely examine the number and nature of the neurophysin precursors synthesized in each of these hypothalamic nuclei and in an endeavor to study the effects of certain physiological manipulations (suckling, salt-loading, diabetes insipidus) on AVP-RNp I and OT-RNp II precursors synthesized within each of these nuclei, a method was developed wherein poly(A)RNA could be isolated from a single hypothalamic nucleus (Palkovits punch) and translated in a rabbit reticulocyte lysate (RRL) cell-free translation system.

Using a 1.0 mm I.D. punch, the SON, PVN and SCN were dissected from frozen coronal brain sections (1.0 mm thick) and homogenized immediately in 100 ul of a buffer containing 6M guanidinium isothiocyanate and 10 ug carrier tRNA. The RNA was fractionated from contaminating DNA and protein by ultracentrifugation through a 5.7M CsCl cushion. The poly(A)-containing RNAs were selected with oligo(dT)-cellulose using a micro-batch procedure and were ethanol precipitated from potassium acetate in the presence of carrier. It was extimated that a single PVN punch, weighing 1.0 mg wet weight, yielded approximately 0.4-0.6 ug total RNA and 20-25 ng poly(A)RNA. When translated in a RRL cell-free system, 12-14% of the [35S]-cysteine-containing protein was immunoprecipitable with anti-rat neurophysin IgG, resulting in two bands migrating at 17,000 and 18,500 MW on an SDS-polyacrylamide gel.

Thus, it is now possible for the first time, to study the transcriptional regulation of peptide hormone mRNAs and the heterogeneity of protein translation products from discrete brain regions in a cell-free translation assay: a functional assay correlative to mRNA quantitation by specific oligodeoxynucleotide probe dot-blot analysis.

This study is supported by a grant from University Genetics Co.

184.10 STRUCTURE OF THE GENE ENCODING RAT PRE-PROSOMATOSTATIN. M.R. Montminy*, R.H. Goodman*, J.F. Habener*. Endocrine Div., Dept. of Med., New Engl. Med. Ctr. Hospital, Lab of Molec. Endocrinol., Mass. Gen. Hospital, Howard Hughes Med. Inst. Labs, Boston, MA 02114.

Somatostatin is a tetradecapeptide that regulates the release of pituitary, pancreatic, and gastrointestinal hormones. Rat somatostatin is synthesized in the form of a 116 amino acid precursor, pre-prosomatostatin. We have previously determined the amino acid sequence of rat pre-prosomatostatin from the nucleotide sequence of cloned cDNAs derived from a medullary thyroid carcinoma. We now report the use of this cDNA to isolate the gene encoding rat pre-prosomatostatin. Using plaque hybridization and reciprocal recombination (πVX) techniques, the rat somatostatin gene was isolated from a genomic library cloned in Charon 4A bacteriophages. The gene is approximately 1.3 kilobases in length, and is interrupted by a single intron of about 600 nucleotides located at base 200, near the middle of the pre-prosomatostatin coding sequence. The site of transcriptional initiation, identified by S-1 nuclease mapping, is located 102 bases upstream from the AUG codon that signals the start of translation. A variant promoter sequence (TTTAAAAA) is located 31 bases upstream from the transcriptional initiation (cap) site. The 5' region of the gene is flanked by two middle-repetitive sequences arranged in tandem. The structures of the recombinant bacteriophages are consistent with genomic Southern blotting data, indicating that these recombinants accurately represent their genomic counterparts. Restriction analysis of genomic DNA, hybridized with the cDNA obtained from the rat medullary thyroid carcinoma, is consistent with a single gene and a small number of additional bands which appear under low stringency hybridization conditions and might represent pseudogenes.

184.11 LOCALIZATION OF β-NGF mRNA IN TISSUE SECTIONS USING IN SITU HYBRIDIZATION. John E. Pintar* and Axel Ullrich* (SPON: Marie Gibson). Dept. Medicine and Anatomy, Mt. Sinai School of Med., New York, NY. 10029 and Genentech, Inc., South San Francisco, CA. 94080.

Nerve Growth Factor (NGF) is of critical importance for the development and maintenance of the peripheral nervous system and may have similar functions in the central nervous system as well. Although the amino acid sequence and biochemical properties of the bioactive β-subunit of NGF are relatively well understood, the physiological sites of NGF synthesis are unknown. We have begun to address this problem by using a recently isolated recombinant DNA probe to mouse β-NGF to localize cells containing β-NGF mRNA.

We have initially shown that in situ hybridization of male and female submaxillary gland tissue sections with radiolabelled NGF probes reflects the known difference in NGF mRNA between these tissues and, in addition, have demonstrated specific cell populations containing NGF mRNA. Submaxillary glands from male and female Swiss-Webster mice were excised, fixed in 4% formaldehyde and subsequently embedded in the same block. Single cryostat sections thus contained both male and female submaxillary gland tissue, which are easily distinguished histologically. Sections were then hybridized with ^{32}P-labelled NGF cDNA and subsequently exposed to X-ray film. Areas of the section containing male and female tissue showed dramatic differences in the amount of probe bound; film exposure was much greater over areas of the section containing male tissue. This difference was not seen if sections were hybridized with radiolabelled pBR DNA or with a radiolabeled probe to a portion of the proopiomelanocortin gene. To determine the cellular location of NGF mRNA in these tissues, slides were emulsion coated and exposed for autoradiography. Silver grains were concentrated in male secretory tubules and were essentially absent in the acini; this cellular distribution of grains correspons to the site of NGF storage in the submaxillary gland shown by immunocytochemistry.

These results suggest that in situ detection of NGF mRNA at the single cell level in embryonic and adult tissue sections should be feasible. Such experiments are presently in progress.

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184.12 MOLECULAR CLONING OF 7S NERVE GROWTH FACTOR: ISOLATION AND CHARACTERIZATION OF A GENE CODING FOR THE ALPHA SUBUNIT, Margaret Fahnestock* and Eric M. Shooter. (SPON: J. H. Pate Skene). Dept. of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Nerve growth factor (NGF), a polypeptide necessary for the growth and maintenance of sensory and some sympathetic neurons, is isolated from the male mouse submaxillary gland in a high molecular weight complex, 7S NGF, comprising the NGF dimer, a trypsin-like γ subunit, an acidic α subunit and zinc ions. The α subunit, whose function is unknown, has a molecular weight of 26,500. A biosynthetic precursor of the α subunit, of molecular weight 32,000, has been previously identified in incorporation experiments using mouse submaxillary gland slices followed by precipitation with specific anti-α antiserum.

An antibody to highly purified α subunit was prepared and used to characterize in vitro cell-free translation products of total male mouse submaxillary gland mRNA. Four polypeptides were precipitated ranging in molecular weight from 34,000 to 16,500. Furthermore, the α subunit antiserum was used to differentially purify submaxillary gland polysomes and to prepare from them a fraction of mRNA molecules enriched in α subunit sequences. This fraction was used as a probe to screen a male-specific cDNA library from the mouse submaxillary gland (obtained from James Scott and William J. Rutter).

One clone from this library was isolated and shown to contain cDNA coding for α subunit by its ability to hybridize and select mRNA which will direct the in vitro synthesis of the four antibody-precipitable proteins. Northern analysis indicated this clone hybridized to a single RNA of the expected size, and that this RNA, like the α subunit protein, was present in much higher amounts in male than in female glands.

DNA sequencing is being carried out in order to confirm the identity of the isolated clone.

184.13 EXPRESSION OF THE MOUSE NERVE GROWTH FACTOR GENE: THE PROTEIN PRECURSORS OF NGF. T. Darling*, P. Petrides*, R. Sherman-Gold*, S. Feinstein*,E. Shooter; M. Selby*, and W. Rutter.* Dept. Neurobiology, Stanford Univ. Sch. Med., Stanford CA 94305, VA Med. Cen., Washington, D.C. 20422; Dept. Bioch. and Biophys., Univ. California, San Francisco, CA 94143.

In the adult male mouse submaxillary gland the nerve growth factor (NGF) protein accumulates in a zinc ion stabilized multiprotein complex, 7S NGF, which is released into saliva by epinephrine stimulation. The other proteins are the gamma subunit, a trypsin-like enzyme, and an acidic alpha subunit. Each protein is synthesized as a higher molecular weight precursor. Both incorporation studies in submaxillary gland slices and translation of gland mRNA in reticulocyte lysates coupled with peptide mapping identifies preproNGF as an M_r 34,000 peptide. Processing results in the appearance of an M_r 31,000 proNGF peptide and of two lower molecular weight intermediates of M_r 22,000 and 19,000 respectively, as well as the M_r 13,300 NGF peptide chain. Precursor peptides labeled with ^{3}H-Valine in 60 minute incubations of tissue slices have been isolated with antibodies, separated by electrophoresis through polyacrylamide gels, in the presence of SDS, and detected by autoradiography. Each ^{3}H-Val labeled precursor peptide has been recovered in the appropriate section of dried gel, and subjected to proteolysis by trypsin. The radioactive tryptic peptides of each precursor have been analyzed by HPLC and shown to co-elute with authentic tryptic peptides of NGF, the latter having been identified by amino acid analysis. The sizes of the NGF precursor proteins are consistent with the nucleotide sequence of a cDNA derived from mouse mRNA (Scott et al, 1983, Nature 302:538), the positions of predictive proteolytic cleavage sites in the sequence and an open reading frame beginning 564 nucleotides upstream from the serine codon defining the N-terminus of NGF. The gamma subunit is postulated to specifically release the arg.gly dipeptide extension from the C-terminus of proNGF initiating the formation of the 7S NGF complex. (Supported by grants from NIH (NS 04270 and AM 21344) and NSF (BNS 791408).

184.14 SYNTHESIS AND CLONING OF COMPLEMENTARY DNA TO POLYADENYLATED RNA OF PERIPHERAL NERVE FROM NEONATAL RABBIT. H. D. Shine, P. R. Dobner* and L. Villa-Komaroff*. Departments of Neuroscience and Neuropathology, Children's Hospital and Harvard Medical School, Boston, MA 02115 and Department of Molecular Genetics and Microbiology, University of Massachusetts Medical Center, Worcester, MA 01605.

In order to study expression and regulation of genes involved in differentiation of Schwann cells we have produced a complementary DNA (cDNA) library to polyadenylated RNA (poly(A)$^+$ RNA) isolated from developing peripheral nerves of neonatal rabbits. RNA was isolated from 3 g of frozen nerves dissected from 100 one day-old rabbits by phenol extraction and enriched for poly(A)$^+$ RNA by oligo-(dT)-cellulose affinity chromatography to yield 50 μg of RNA. Approximately 2 μg of double-stranded cDNA in sizes ranging from approximately 300 to 1,700 base pairs was synthesized from 10 μg of poly(A)$^+$ RNA with reverse transcriptase, polymerase I and S-1 nuclease. The cDNA was dC-tailed with terminal transferase and annealed to pUC8 which was cut with Pst-I restriction enzyme and dG-tailed. E. coli (strain HB101) was transformed with the annealed vector at a transformation efficiency of 3.0 x 10^4 transformants/μg DNA. This library is enriched in Schwann cell-specific, developmentally active mRNA sequences since (i) most cells in the nerve tissue were fibroblasts and Schwann cells and (ii) the nerve was dissected at a stage of rapid Schwann cell development. We are now using several methods to identify Schwann cell-specific sequences in the library. Heterologous cDNA probes synthesized from poly (A)$^+$ RNA from cultured fibroblasts and non-neural tissues are being used to screen for colonies that contain sequences that are either common to Schwann cells and other cell types or specific to non-neural cells. Additionally, since pUC8 is a vector capable of expressing hybrid peptides coded by the cDNA insert cloned within a portion of the lacZ gene, peptides of interest may be identified by immunoassay with antibodies to Schwann cell-specific antigens.

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BIOLOGICAL RHYTHMS I

185.1 SURGICAL DISSECTION OF THE CIRCADIAN PACEMAKER AND BURST GENERATING FUNCTIONS IN THE BULLA AND APLYSIA EYE. G.D. Block and D.G. McMahon, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

The eyes of several opisthobranch molluscs express circadian rhythms in the frequency of optic nerve compound action potentials (CAPs). In Bulla (Block & Wallace, Science, 217: 1982) and in Aplysia (Strumwasser, Physiologist, 16: 1973) the pacemaking system resides among a small group of lower retinal neurons (LRNs--nomenclature of Strumwasser). In the present study we have attempted to localize further the circadian pacemaker by removing the somata of LRNs and then evaluating optic nerve impulse activity. This was accomplished by cutting, or in some cases ligating, the optic nerve at its insertion into the connective tissue capsule surrounding the eye. Optic nerve/retinal fragments produced in this fashion remained spontaneously active, generating regular bursts of CAPs which resembled the patterning observed in intact Bulla and Aplysia eyes at their peak CAP frequency within the circadian cycle. There was no evidence of a circadian modulation, however. Subsequent histological examination of these fragments revealed an intact optic nerve with a small portion of lower retinal neuropil. There was no evidence of LRN cell bodies.

Repeated attempts to record spike activity from the somata of LRNs in the retina separated from the optic nerve failed to show evidence of electrical excitability. While many cells within the severed retina exhibited substantial resting membrane potentials (40-80 mV), presumptive LRNs were uncharacteristically silent.

These results suggest to us that CAP burst generation and the circadian pacemaker system are functionally discrete. Previous experiments in Aplysia by Woolum & Strumwasser (Proc. Natl. Acad. Sci., 77: 1980) using ionizing radiation, and tissue reduction studies by Jacklet & Geronimo (Science, 174: 1971) likewise suggest that these two functions are separate. Our current results provide evidence that these two properties of LRNs are anatomically discrete as well.

Since fragments of retinal tissue containing intact LRNs generate CAP bursts modulated by a circadian pacemaker, while neuropil-only fragments support constant levels of CAP activity, a simple hypothesis can be formulated. The circadian pacemaker system is located in the LRN somata or immediately adjacent neurites and modulates CAP burst generators located in more remote regions of the neuron. If this hypothesis is correct, it should be possible to record circadian rhythms in membrane potential from non-spiking LRNs following removal of the optic nerve and associated neuropil. Membrane potential rhythms have been recorded from LRNs in intact eyes (McMahon & Block, this volume). Supported by NS15264.

185.2 LONG-TERM INTRACELLULAR RECORDING FROM LOWER RETINAL NEURONS IN THE BULLA EYE: RHYTHMS IN MEMBRANE POTENTIAL AND IMPULSE FREQUENCY FOLLOW CIRCADIAN RHYTHM. D.G. McMahon and G.D. Block. Dept. of Biol., University of Virginia, Charlottesville, VA 22901.

The eye of the marine mollusc Bulla gouldiana expresses a circadian rhythm in the frequency of compound action potentials (CAPs) recorded from the optic nerve. Block and Wallace (1982, Science, 217, 155-157) have shown that the entire circadian system, entrainment pathway, rhythm generation, and output pathway persists in retinal fragments containing as few as 30 lower retinal neurons (LRNs, nomenclature of Strumwasser). LRNs exhibit action potentials one for one with CAPs recorded in the optic nerve (Wallace and Block, 1982, Soc. Neurosci. Abstr., 8, 33) suggesting that they are a pacemaker output pathway, or part of the pacemaker itself.

We now report that long-term intracellular recordings from LRNs reveal rhythms in membrane potential and impulse frequency synchronized with the circadian rhythm in CAPs. Bulla eyes were dissected from the animal and maintained in FSW at 15°C. LRNs were impaled with glass capillary microelectrodes (40-80 MΩ) filled with 4M KCl. Following impalement, eyes were maintained in constant darkness. Stable recordings from LRNs yielded membrane potentials of -40 to -80mv and could be maintained for up to 20 hours. These records reveal two prominent features. First, LRNs exhibit a rhythm in impulse frequency corresponding to the CAP rhythm. Second, a rhythm in membrane potential underlies the rhythm in impulse frequency. During the subjective night LRNs are hyperpolarized and silent. They depolarize, initiating impulse activity near projected dawn and are most depolarized near peak impulse frequency. They repolarize as impulse frequency falls during the subjective day. The amplitude of the membrane potential rhythm is 7 to 13mv trough to peak. Importantly, the rise in membrane potential from its minimum preceeds initiation of impulse activity by about 1 hour.

These results suggest that the circadian rhythm in CAPs recorded from the Bulla eye reflects the membrane potential rhythm in the LRNs. These experiments do not distinguish whether LRNs generate circadian rhythmicity in the Bulla eye or are merely an output pathway. They do, however, demonstrate rhythmicity in single neurons of a circadian system, and are an important first step in the cellular-level analysis of a neuronal circadian pacemaker. Supported by NS15264.

185.3 THE PROPERTIES OF THE BURSATELLA CIRCADIAN SYSTEM DIFFER FROM THE PROPERTIES OF THE CONTRIBUTING PACEMAKERS. M. H. Roberts and G. D. Block, Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

We have reported previously that the two ocular circadian pacemakers of the marine mollusc Bulla gouldiana are mutually coupled and that their interaction can be studied in vitro (Roberts and Block, Soc. Neurosci. Abstr., vol. 8, p. 33, 1982). We found that experimentally induced pacemaker desynchrony is reduced if the pacemakers are allowed to interact for 48 hours. We recently evaluated ocular pacemaker coupling in another opisthobranch, Bursatella leachi plei. We were suspicious that the Bursatella ocular pacemakers might be coupled since, like Bulla, ocular phase information is transmitted from one eye to the other. Using our in vitro coupling assay we found that experimentally induced interocular phase differences of about 4 hours are reduced to one hour if the pacemakers are allowed to interact for 48 hours in darkness. Thus, like Bulla, the Bursatella ocular pacemakers appear to be coupled to one another.

Intriguingly, we find that the ability of the Bursatella ocular pacemakers to sustain a free-running rhythm depends upon ocular coupling. Out of 20 isolated eyes evaluated, 13 rhythms damped out and failed to produce a second peak of activity in vitro. The remaining 7 eyes displayed extremely short free-running periods (21.7 hrs) confirming earlier results obtained in our laboratory (Block and Roberts, J. Comp. Physiol. 142:403, 1982). In contrast, when the two eyes remained attached to the cerebral ganglion, the free-running period was about 1.5 hours longer (23.0 hrs, n=12) and none of the eyes failed to produce a second peak. Furthermore, many eyes (n=8) exhibited a third peak of activity in vitro. This difference is not due to influence from the cerebral ganglion since single eyes which remained attached to the nervous system behaved similarly to isolated eyes.

While additional control experiments need to be performed, these data indicate that two properties of the Bursatella circadian system (period and sustainability) differ from the properties of the contributing pacemakers. Supported by NS15264 to GDB.

185.4 CIRCADIAN PACEMAKER IN THE BULLA EYE CONTROLS LOCOMOTOR BEHAVIOR VIA A HUMORAL PATHWAY. P.R. Ralford* and G.D. Block. (Spon: J. Bennet, Jr.) Dept. of Biol., University of Virginia, Charlottesville, VA 22901.

The eyes of Bulla gouldiana contain circadian pacemakers which are involved in timing a nocturnal locomotor rhythm (Block and Davenport, J. Exp. Zool., 224, 1982). Eye removal leads to a permanent loss of free-running behavior in constant darkness and abolishment of the entrained nocturnal rhythm in animals exposed to light cycles. Many eyeless Bulla continue to exhibit a bout of diurnal activity for 1 - 2 hours after dawn. This period of activity seen sporadically in the records of intact Bulla, typically increases in regularity, intensity and duration in eyeless animals. It appears, however, to be a response to the onset of light, rather than initiated by the clock system.

We presently find that intact optic nerves are not necessary for ocular control of the locomotor rhythm. In 4 Bulla where the optic nerves were severed during free-runs in continual darkness, there was a return of rhythmicity within 3 weeks after surgery. The recovery period was highly variable, ranging from 5 - 20 days. This recovery of rhythmicity has never been observed in eyeless Bulla.

When Bulla were maintained on light cycles, bilateral section of the optic nerves led initially to either a shift to diurnal behavior (N=3), disorganized activity records (N=5) or, in one case, persistence of the nocturnal rhythm. Within three weeks the nocturnal rhythm was reestablished in 5 of the Bulla whose locomotor records were disrupted by surgery. Subsequent histological examination of these Bulla revealed no evidence of reconnection of the optic nerves: thus we conclude that humoral release is involved in ocular control of locomotor rhythmicity.

Humoral coupling of the Bulla eye to locomotor centers is surprising since in a related mollusc, Aplysia, this connection is neural. Section of the optic nerves produces permanent alterations in locomotor timing identical to eye removal (Lickey et al., Neurosci. Abstr., 1974). While these nerves in Aplysia are the critical pathways for locomotor control, it has been reported that the eye also exhibits a rhythmic release of several polypeptides which can influence central neurons (Strumwasser et al., Naito International Symposium, 1978). It is currently unknown whether similar polypeptides are released by the Bulla eye.

The presence of a humoral coupling pathway in Bulla provides an opportunity to perform transplantation experiments to further define the role of the ocular pacemakers in controlling locomotor behavior. Preliminary transplantation studies provide evidence that rhythmicity can be restored through the implantation of eyes. Supported by NS15264.

185.5 IDENDTIFICATION OF CIRCADIAN CLOCK FIBERS IN THE CNS OF APLYSIA L. Olson* and J.W.Jacklet (SPON: S.B. Tieman). Dept. of Biol. Sci., SUNY Albany, Albany, N.Y. 12222

Behavioral studies have shown that locomotion in Aplysia is influenced by a circadian clock in the eye. The output of the clock is through an identified population of retinal neurons, the secondary cells, which send axons out the optic nerve (ON) to the cerebral ganglion (CG). The synchronous firing of these cells is seen as a compound action potential (CAP) in the ON whose frequency is modulated with a circadian period. Understanding the cellular mechanisms involved in clock modulation of behavior requires knowledge of the distribution of clock information from the eye to the CNS. To this end we are conducting studies to localize "clock" fibers in the CNS. We find that clock information is sent to almost the entire CNS.

The ON contains afferents from secondary cells and photoreceptors, and a small number of efferent axons. HRP fills of the ON stain afferent axons and efferent cell bodies within the CG. This technique is being used for an ultrastructural analysis of optic fiber terminations in the CG. However, incubation of the eye in ^{3}H leucine with subsequent autoradiography of transported material best delineates the ON projection at the light level. Fibers ramify extensively throughout ipsi- and contra- CG neuropil. Large groups of fibers also project out of the CG: 1) the largest projection extends to ipsi- and contra- Pleural ganglia; 2) a smaller projection extends bilaterally to Pedal ganglia; 3) two much smaller projections are seen in the ipsilateral Anterior and Posterior Tentacle nerves of the CG; and 4) a projection leaves the Pleural ganglia along the Pleural-Abdominal connectives. It is likely that this projection extends to the Abdominal ganglion.

We are using two approaches to discriminate clock fibers from photoreceptor afferents in the ON: 1) recordings from Pleural and Pedal connectives reveal a CAP coincident with that seen in the ON. Conversly, an antidromic CAP is evoked in the ON after stimulation of these connectives, demonstrating that fibers from the secondary (clock) cells in the eye are bilaterally distributed to Cerebral, Pleural, Pedal and possibly Abdominal ganglia; 2) specific labeling of retinal secondary cells was accomplished with ^{3}H DOPA. Other studies have suggested that secondary cells, but not photoreceptors are dopaminergic. We find in fact that only the secondary cells specifically accumulate ^{3}H DOPA.

^{3}H DOPA transport studies in progress will confirm and extend the specific location of clock pathways in the Aplysia CNS.

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185.6 EVIDENCE FOR A CIRCADIAN CLOCK IN THE EYE CONTROLLING RETINAL SEROTONIN N-ACETYLTRANSFERASE AND PHOTORECEPTOR DISC SHEDDING. P. M. Iuvone*, J. C. Besharse*, and D. A. Dunis* (SPON: S. DeRossett). Depts. of Pharmacology, Anatomy, and Ophthalmology, Emory Univsersity School of Medicine, Atlanta, GA 30322.

Recent evidence indicates that in several vertebrate species the shedding and phagocytosis of rod photoreceptor disc membranes and the biosynthesis of melatonin in retina occur as circadian rhythms (see Besharse, Prog. Retinal Res. 1: 81, 1982). In order to gain insight into the localization of the circadian clock that regulates these rhythmic processes, we studied the regulation of retinal serotonin N-acetyltransferase (NAT), a key enzyme in the biosynthesis of melatonin, and the relationship of melatonin to disc shedding in eyes of Xenopus laevis. NAT activity, which was localized to neural retina, fluctuated in vivo as a circadian rhythm with peak activity in subjective darkness. To determine if the clock that regulates this rhythm is intrinsic to the eye, the activity of NAT was studied in an isolated, cultured, eye cup preparation. In eye cups cultured under a lighting schedule of 12h dark:12h light, NAT activity fluctuated as it did in vivo. Furthermore, the rhythm of NAT activity persisted for at least 80h in culture in constant darkness. Phase-reversal experiments were carried out in vitro to determine if the rhythm of NAT activity could be altered by a change in light cycle. Eye cups were first cultured for 48h on a 12hL:12hD cycle that was opposite that which had been used to entrain the rhythm in vivo, and were then cultured for 30h in constant darkness. Samples taken during constant darkness indicated that the rhythm of NAT activity had completely reversed. Thus, the circadian clock and the photoreceptor for its entrainment are intrinsic to the eye.

To determine if rhythmic disc shedding is related to rhythmic melatonin biosynthesis, the effect of melatonin on disc shedding was examined in eye cups. Light-evoked disc shedding was stimulated by addition of melatonin in concentrations as low as 50nM to medium containing insufficient bicarbonate to support disc shedding (non-permissive medium). The response occurred in eye cups preincubated in darkness with melatonin prior to light onset, but not when added at the time of light onset. The related indoleamines, serotonin and N-acetylserotonin, had no effect. Our findings suggest that the rhythm of melatonin biosynthesis in retina plays a role in the regulation of the rhythm of disc shedding, and that the circadian clock that influences these processes is intrinsic to the eye.

185.7 ENDOGENOUS CIRCADIAN RHYTHM IN THE ERG OF THE DIURNAL LIZARD ANOLIS CAROLINENSIS. D. H. Fowlkes, C. J. Karwoski and L. M. Proenza. Psychology Dept., University of Georgia, Athens, GA. 30602

Bilateral ERGs were recorded once per hour in constant dark (DD) for periods of 4-16 days from the diurnal lizard Anolis Carolinensis, which possesses an all-cone retina. Prior to testing, animals were maintained on the solar LD cycle for at least 3 weeks. Testing consisted of a 10 μsec flash of white light diffused by a translucent dome in which the animal was housed. Animals were allowed free range of movement within the dome, since physical restraint decreases ERG amplitude and abolishes ERG rhythms. Variations in ERG amplitude are not the result of changes in the amount of light striking the corneal surfaces, since 1) the animal's visual field is nearly homogeneously illuminated by the stimulus flashes, and 2) the eyelids of the animals were retracted for the duration of the experiment.

A relatively weak rhythm is discernable in a-wave amplitude, but the amplitudes of b-wave components (both b1 and b2) exhibit robust free-running rhythms that have a period of approximately 24 hr and that peak near projected midday with amplitudes 2-3 times greater than amplitudes during projected night. The ERG rhythms do not result from a modulation of pupil diameter, nor from any discernable morphological changes (e.g., retinomotor movements). Constant light (LL) disrupts the rhythms, as evidenced by phase delays and increased variability in period length.

Data were also obtained from the nocturnal lizard Gecko gecko, which possesses an all-rod retina, under conditions identical to those employed with Anolis. There is no evidence for an a-wave rhythm, and the b1 and b2 amplitude rhythms peak shortly after projected sunset. This phase difference in the rhythms exhibited by the 2 lizard species suggests that the ERG rhythms result from the modulation of visual mechanisms by processes endogenous to the animal, and not by processes exogenous to the animal.

In summary, (1) the Anolis ERG component amplitudes exhibit free-running rhythms in DD, (2) The rhythms are disrupted by LL, (3) The rhythms consist primarily of a modulation of post-receptoral (b-wave) processes, and (4) The modulating mechanisms are endogenous to the animal. Thus, ERG component amplitude rhythms observed in Anolis can properly be considered circadian rhythms resulting from endogenous modulation of retinal mechanisms.

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185.8 ENTRAINMENT OF CIRCADIAN RHYTHMS IN UTERO: ROLE OF THE MATERNAL SUPRACHIASMATIC NUCLEUS. F.C. Davis* and R.A.Gorski (SPON: A. Coquelin). Lab. of Neuroendocrinology, Brain Res. Inst. and Dept. of Anatomy, UCLA Sch. of Med., Los Angeles, CA 90024.

There is strong evidence in rodents that rhythms which are controlled by the suprachiasmatic nucleus (SCN) can be entrained in utero. To determine precisely when and by what mechanism such entrainment occurs, it is important to identify the relevant zeitgeber. As a starting point, we have found that entrainment of the circadian mechanism underlying the activity/rest rhythm of weanling hamsters is mediated by the maternal SCN and that this entrainment may begin before day 14 of gestation. Beginning on day 1 of gestation, female hamsters were maintained in running-wheel cages in dim constant light. On day 7 of gestation the mothers were given electrolytic midline lesions (2mA, 15 secs) directed at the SCN. When the pups of these mothers were 18 days old, they were separated and placed in individual running-wheel cages, still maintained in dim constant light. The phase angles of their activity onsets on postnatal day 18 were determined from records collected over the subsequent three weeks. A distribution of phase angles within a litter which is significantly different from uniform (Rayleigh test) is taken as evidence that the pups had been entrained sometime prior to weaning. Four to 10 pups were taken from each of 13 mothers, one with a sham lesion and 12 with various amounts of damage to the SCN. A lesion which destroyed at least 70% of a mother's SCN disrupted entrainment of her litter, i.e., the distribution of phase angles within litters from seven such mothers were not significantly different from uniform. In contrast, six litters from mothers with less than 70% SCN destruction showed phase angle distributions which were different from uniform indicating within-litter synchrony among pups. In addition, the scatter in phase angles within a litter was correlated with the degree of SCN destruction in the mother (r=0.915). Thus, entrainment of the pups within a litter, as measured by the synchrony of their activity rhythms at weaning, is disrupted by destruction of the mother's SCN on day 7 of gestation. To determine if entrainment occurs prenatally, three mothers were lesioned on day 14 of gestation, two days before birth. The percent SCN destroyed in these mothers was 100, 98, and 48, and in all cases the litters showed phase angle distributions which were significantly different from uniform. Therefore, SCN lesions to the mother which on day 7 of gestation disrupt entrainment do not do so when performed on day 14, suggesting that entrainment occurs before day 14. This is only one day after the termination of SCN neurogenesis suggesting that minimal SCN development is necessary for entrainment.

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185.9 THE ROLE OF THE SCN IN THE REGULATION OF THE ESTROUS CYCLE, THE ACTIVITY RHYTHM AND THE LH SURGE. J.Swann, K.Anderson* & F.Turek. Dept. Neurobiol. & Physiol., Northwestern U., Evanston, IL 60201.

The proestrus LH surge in rodents is regulated by an endogenous circadian oscillator. In ovariectomized (OVAX) estrogen-treated hamsters exposed to a light-dark (LD) cycle, constant light or constant darkness, the LH surge occurs daily about 4-5 hrs before the onset of activity. These results suggest that both the timing of the LH surge and the activity rhythm are regulated by the same circadian oscillator. Bilateral ablation of the suprachiasmatic nuclei (SCN), a putative circadian pacemaker, abolishes the circadian activity rhythm. Several studies have reported that both estrous cyclicity and the LH surge are also abolished following bilateral ablation of the SCN, but recent reports claim that estrous cyclicity and the LH surge are maintained in rats and hamsters with SCN lesions and that the medial preoptic nuclei (MPN) regulate the timing of the LH surge. We examined the role of the SCN in the regulation of the LH surge, the estrous cycle and the activity rhythm in individual golden hamsters. 18 adult, female hamsters were singly housed in running wheel cages and exposed to 16L:8D throughout the experiment. All females were examined daily for the presence of vaginal discharge. After 4 weeks on 16L:8D 14 of the hamsters received lesions aimed at the SCN; 4 females received sham lesions (SH). 4 weeks after surgery, all hamsters were OVAXed and 2 weeks later each animal was fitted with an intra-atrial cannula and implanted with a 4 mm silastic capsule filled with extradiol benzoate. 48 hrs after implantation, each animal was bled once an hour for 24 hrs and the serum was assayed for LH by RIA. Brains were prepared histologically to determine the extent of the lesion. The SH hamsters, lesioned hamsters with intact SCN, and 4 of the 5 hamsters with partial SCN lesions had entrained activity rhythms, a 4-day estrous cycle, and an LH surge 4-5 hrs before the onset of activity. The fifth hamster with a partial SCN lesion ceased cycling and failed to show an LH surge, but the circadian activity rhythm persisted. In 5 of 6 hamsters with bilateral lesions of the SCN (SCNL), the activity rhythm and the estrous cycle were completely disrupted and there was no evidence of an LH surge. The sixth SCNL hamster had an entrained activity rhythm and a normal LH surge, but it did not show an estrus smear. In all of the lesioned hamsters, the lesion extended rostrally into the MPN. However, there was no clear correlation between the extent of the damage to the MPN and the expression of estrous cyclicity or the presence of an LH surge. These results indicate that the SCN plays an important role in the regulation of the estrous cycle, the activity rhythm and the LH surge, and that bilateral lesions of the SCN which disrupt the activity rhythm also eliminate the LH surge and the estrous cycle in the golden hamster.

185.10 MICROINJECTION OF NEUROPEPTIDES INTO THE SUPRACHIASMATIC REGION OF THE HYPOTHALAMUS PHASE-SHIFT CIRCADIAN ACTIVITY RHYTHMS OF SYRIAN HAMSTERS (Mesocrietus auratus). H.E. Albers, C.F. Ferris*, S.E. Leeman and B.D. Goldman*. Worcester Found. for Exp. Biol., Shrewsbury, MA 01545 and Dept. of Physiol., Univ. of Mass. Med. Ctr., Worcester, MA 01605

Neurons within the suprachiasmatic nuclei(SCN) are essential for the generation and entrainment by light-dark cycles of many mammalian circadian rhythms. Immunohistochemical analysis of the SCN has indicated the presence of a variety of peptides within specific cell subpopulations. Vasopressin (VP)-like reactivity occurs in perikarya of the dorsomedial SCN, while avain pancreatic polypeptide (APP)-like reactivity is observed in neurons that project from the ventral lateral geniculate nucleus(vLGN) to the ventromedial SCN (Card & Moore, 1982). Studies were begun to examine the effects of these peptides on circadian rhythms following their microinjection into the suprachiasmatic region. Eleven intact and 4 blinded and castrated(BX) hamsters were implanted stereotaxically with chronic guide cannulae aimed at the SCN. Each hamster was then placed in a cage with an activity wheel to establish a stable free-running rhythm under constant light. At 10-14 day intervals the unanesthetized hamsters were removed from their cages and injected with VP(50pm) or APP(10pm), in 200 nanoliters of 0.9% NaCl via a 33 gauge needle. Injection sites were verified histologically. Injection of VP at various timepoints during the subjective night in intact and BX hamsters had little or no effect on the timing of the activity rhythm; phase-shifts ranged from -0.44 to +0.31 hr. Injections of VP during the subjective day produced more variable results with phase-shifts ranging from -0.85 to +0.55 hr. No relationship was observed between the circadian phase of VP injection and the direction and/or magnitude of the phase-shift. In contrast, injection of APP produced phase-shifts in activity whose direction and magnitude appeared to depend upon the phase of administration. During the subjective day APP consistently advanced the timing of activity (range= 0.45 to 1.08 hr), but during the late subjective night produced phase delays of up to 2.21 hr. The early subjective night appeared to be a transition period where APP resulted in either small phase advances or delays. The free-running circadian period was not altered by either VP (-0.03±0.03 hr;X±SEM) or APP (0.02±0.02). The present data demonstrating that APP but not VP can systematically alter the timing of circadian activity in a phase-dependent manner when injected into the suprachiasmatic region suggests that APP or an APP-like peptide may be involved in the transmission of photic information to SCN neurons.

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185.11 METABOLIC MAPPING OF CIRCADIAN OSCILLATION IN THE RAT BRAIN: A C14-2DG AUTORADIOGRAPHIC STUDY. Alan M. Rosenwasser, Gregory Trubowitsch† and Norman T. Adler, Department of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Previous studies by Schwartz and colleagues have demonstrated the existence of a circadian rhythm in the uptake of labeled 2-deoxyglucose by the suprachiasmatic nucleus (SCN) of the hypothalamus. This rhythm persists under constant darkness or after blinding, with an amplitude which is reduced relative to that seen under light-dark conditions. Inouye and Kawamura have shown by multiunit population recording that the SCN also demonstrates a circadian rhythm in electrical activity. Both the metabolic and electrical SCN activity rhythms have maximum activity during the light or "subjective day" phase of the circadian day. Further, the electrophysiological studies, but not the 2-DG studies, have shown that numerous other brain regions show circadian rhythms in their activity which are dependent on neural connections with the SCN. In the present study, we sought to replicate and extend the metabolic results using 2-DG autoradiography in conjunction with a newly developed computer-assisted image processing system. Animals were blinded by orbital enucleation 24 hours before they were given a 30μCi dose of C14-2DG and sacrificed 45 min. later. Nine different time points throughout the day were sampled in this manner. The results closely matched those of the earlier studies, and demonstrated an approximately 75% increase in 2DG uptake in the SCN during the subjective day relative to the night phase. We further attempted to identify intra-SCN heterogeneity in the pattern of 2DG uptake. This analysis showed that the left and right SCN displayed identical circadian variation, while the rostral and caudal poles of the SCN showed lower amplitude rhythms than did the middle third of the nucleus. During the subjective night, the SCN showed uniformly low 2-DG uptake, while during the subjective day the middle third of the nucleus showed the highest 2-DG uptake. However, the time of day effect was apparent at all rostral-caudal levels. We are currently extending these analyses by conducting a systematic survey of other neural structures in these same radiographs to search for non-SCN metabolic rhythms. This survey is guided by existing information concerning SCN afferent and efferent connections, and by the elctrophysiological reports of non-SCN rhythmic structures. We believe that if such structures exist, our methodology provides the best opportunity to identify them. Current conceptualizations of the mammalian circadian timing system lead to the expectation that the nervous system may contain multiple coupled but potentially autonomous oscillators.

185.12 ABLATION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) PREVENTS SHORT-DAY INDUCED GONADAL REGRESSION IN THE GOLDEN HAMSTER WITHOUT ELIMINATING THE CIRCADIAN RHYTHM OF LOCOMOTOR ACTIVITY. Gary E. Pickard and Fred W. Turek. Dept. of Psychology, University of California, Berkeley, CA. 94720 and Dept. of Neurobiology and Physiology, Northwestern University, Evanston, Ill. 60201.

Photoperiods of less than 12.5 hr of light/24 hr produce a well characterized neuroendocrine response in the golden hamster resulting in gonadal regression after 8-10 weeks. Although this phenomenon has been studied for many years, the complete neural circuitry underlying the response is unknown and the physiological mechanisms remain obscure. Two well characterized components of the photoperiodic neural circuit include the hypothalamic suprachiasmatic nucleus (SCN) and the pineal gland; ablation of either of these structures prevents short-day induced testicular regression in hamsters. Photic information (i.e. day length) is relayed directly from the retina to the SCN, a circadian oscillator which regulates many circadian parameters including the circadian rhythms of pineal melatonin synthesis and locomotor activity. However, the complete neural circuit from the SCN to the pineal remains unspecified. Recently, we have demonstrated that different SCN circadian effector circuits regulate the photoperiodic testicular response and the circadian rhythm of locomotor activity since lesions in the dorsal aspect of the SCN were found to disrupt short-day induced gonadal regression without eliminating the circadian rhythm of locomotor activity. In view of the recently established efferent projection from the SCN to the PVN which exits the SCN dorsally, we hypothesized that the PVN might act as an integral relay between the SCN and pineal. To demonstrate the possible role of the PVN in the photoperiodic response we examined both short-day induced gonadal regression and wheel-running activity in male golden hamsters with electrolytic lesions aimed at the PVN. After 8 weeks exposure to LD 6:18 (6 hr light:18 hr darkness) the testis width of sham lesioned animals (6.9± 0.6 mm;N=8) was significantly less ($P<0.001$) than long-day controls (11.6±0.6; N=7) housed in LD 14:10. The testis width of animals maintained for 8 weeks in LD 6:18 with bilateral lesions aimed at the PVN (9.9± 0.7; N=12) were not significantly different from long-day controls ($P>0.10$) but were significantly larger ($P<0.01$) than short-day animals. In at least 4 lesioned animals with large testes, the circadian rhythm of wheel-running acticity was completely uneffected; the phase angle of entrainment was similar to sham lesioned animals. Thus, the gonadal response to short-days was prevented without eliminating the circadian rhythm of activity. The results suggest that short-day induced testicular regression is mediated at least in part, by a neural circuit which includes the PVN. This research was supported in part by NIH grant NS 19223 to GEP and NIH grants HD 09885 and HD 12622 to FWT.

185.13 BILATERAL VENTROLATERAL CORTICAL LESIONS SLOW AND ACCENTUATE ULTRADIAN ACTIVITY RHYTHMS IN RATS. S. Finklestein*, M. Teicher, A. Campbell*, and R.J. Baldessarini. Mailman Research Center, McLean Hospital, Belmont, MA 02178, and Massachusetts General Hospital, Boston, MA 02114.

Motor activity rhythms were studied in male Sprague-Dawley rats with bilateral ventrolateral cerebral cortical suction lesions (4 X 12 mm). Activity was measured continuously during 2 days before, and 5-7 days after surgery, in 10 min. epochs, using computer-interfaced electronic monitors. Rats moved freely in home cages with food and water freely available, under a 12:12 hr light:dark cycle. Sham-operated controls (N=9) showed no changes in activity levels or rhythms after surgery. Cortically lesioned rats (N=8), however, showed a 92.8 ± 9.0% increase in post-operative mean activity (data are means ± SEM; $p<0.01$), as well as a striking accentuation and slowing of ultradian activity rhythms. In lesioned animals, Fourier-based spectral analysis showed a marked pre- to post-operative increase in the percentage of total variance in activity accounted for by "slow frequency" (3-8 cycles per day, CPD) ultradian rhythms from 14.1 ± 1.1 to 30.8 ± 3.3 ($p<0.003$), a decrease in that accounted for by "fast frequency" (16-24 CPD) ultradian rhythms from 12.4 ± 0.8 to 8.5 ± 1.0 ($p<0.02$), and little change in that accounted for by "medium frequency" (9-16 CPD) ultradian rhythms (16.4 ± 3.0 vs. 21.0 ± 2.4). No change was found in the circadian rhythm (1 CPD) of lesioned rats. The behavioral changes observed may be related to widespread but specific alterations in cortical and subcortical levels of monoamines and their metabolites also found a week after such lesions are made (Finklestein, et al., Brain Research, 1983). They may also parallel disturbances of sleep, appetite, and other ultradian-modulated behaviors found in humans with focal cortical injury (Finklestein, et al., Ann. Neurol., 12:463-468, 1982).

185.14 PHASE SHIFTING AND ENTRAINMENT OF THE HAMSTER CIRCADIAN SYSTEM: ROLE FOR ACETYLCHOLINE IN MEDIATING THE EFFECTS OF LIGHT. David J. Earnest* and Fred W. Turek (SPON: D. Ferster). Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

The light:dark environment is an important cue for the synchronization of many circadian rhythms. Entrainment of the circadian system to the periodicity of a light cycle (usually 24 hr) is thought to occur through a summation of the phase advancing and phase delaying effects of light. Depending on the phase of the rhythm perturbed by light, a circadian rhythm free-running in constant darkness (DD) can be phase advanced or delayed by a single pulse of light. While the quantitative aspects of these responses to light have been examined in great detail, little is known about the neural events underlying the phase shifting and entrainment of circadian rhythms by photic signals. Recent studies with rodents suggest that the neurotransmitter, acetylcholine, may be involved in mediating the effects of light on the circadian system. Therefore, we sought to determine if carbachol, a cholinergic agonist, could mimic the phase shifting and entraining actions of short light pulses. In the first study, adult male hamsters free-running in DD were injected via a cannula implanted in the right lateral ventricle with either saline or 0.01 M carbachol (vol. = 2μl) once every 12-20 days at various circadian times. Intraventricular administration of saline failed to alter the free-running pattern of activity in DD. In contrast, injections of carbachol evoked phase shifts in the activity rhythm that were similar to those induced by brief light pulses; carbachol administered early in the subjective night induced discrete phase delays of the activity rhythm (max. amplitude = 2 hr) while injections during the latter half of the subjective night advanced the phase of the rhythm (max. amplitude = 5 hr). However, carbachol injections continued to elicit phase shifts at circadian times (i.e., subjective day) during which light pulses have no effect on the activity rhythm. In the second study, adult male hamsters were transferred from LD 14:10 to DD and injected intraventricularly with either saline or 0.01 M carbachol (vol. = 1μl) once every 23.33 hr or 24 hr for 9 weeks. Irrespective of the period length of the injection cycle, all animals receiving saline exhibited a free-running pattern of activity in DD ($\tau > 24$ hr). In contrast, entrainment of the activity rhythm was observed in 6 out of 6 animals and 2 out of 6 animals subjected to carbachol injection cycles with period lengths of 23.33 hr and 24 hr, respectively. During entrainment to the 23.33 hr injection cycle, the onset of activity occurred 8-9 hours before the injection time; this pattern of activity resembles that exhibited by hamsters exposed to light cycles (1 hr light/cycle) with the same period length. These results suggest that acetylcholine may play a key role in the mechanism underlying the photoentrainment of circadian rhythms.

185.15 A CHEMICAL GATING THEORY OF CIRCADIAN RHYTHMS IN NOCTURNAL AND DIURNAL MAMMALS: PHASE RESPONSE CURVES AND ASCHOFF'S RULE. Gail A. Carpenter, Northeastern University, Boston, MA 02115 and Stephen Grossberg, Boston University, Boston, MA 02215.

A behaviorally, physiologically, and anatomically predictive model of how circadian rhythms are generated by each suprachiasmatic nucleus (SCN) of the mammalian hypothalamus is presented. This gated pacemaker model is defined in terms of competing on-cell off-cell populations whose positive feedback signals are gated by slowly accumulating chemical transmitter substances. These components have also been used to model other hypothalamic circuits, notably the eating circuit. The complementary reactions to light of diurnal and nocturnal mammals as well as their similar phase response curves are obtained at the SCN level. The "dead zone" of the phase response curve during the subjective day of a nocturnal rodent is also explained. Oscillations are suppressed by high intensities of steady light. An augmented model, which included the effect of metabolic feedback via the bloodstream, accounts both for the consistent obedience to Aschoff's rule by nocturnal mammals (i.e., increased light implies increased period) and for the various responses of the periods of diurnal mammals with increasing light. The same model has previously been used to explain split rhythms, long-term after-effects, and effects of hormonal manipulations (Neuroscience Abstract 8, 1982).

EPILEPSY: PHARMACOLOGY

186.1 INDIVIDUAL AND COMBINED EFFECTS OF CONVULSANT AND ANTICONVULSANT DRUGS ON REGIONAL BRAIN METABOLISM. M.A. Mirski* and J.A. Ferrendelli. Dept. of Pharmacology, Washington Univ. Med. Schl., St. Louis, MO 63110.

The effects of pentylenetetrazol (PTZ) alone, and in animals pretreated with ethosuximide (ESM) or phenytoin (PHT), on electroencephalographic activity and [^{14}C] 2-deoxy-D-glucose ([^{14}C] DG) uptake in brain were examined. Guinea pigs (250-350 g), divided into 6 groups of 3-6 animals, were continuously administered convulsant and anticonvulsant drugs at a rate of 4 mg/kg/min, alone or in combination, via a venous catheter beginning 15 min prior to the I.V. bolus delivery of [^{14}C] DG (30 μCi). Animals receiving infusion of anticonvulsant were additionally pretreated 30 min prior to infusion with an I.P. injection of the same drug, either ESM (300 mg/kg) or PHT (100 mg/kg). The dosage of PTZ administered was sufficient to induce electroencephalographic seizures. ESM prevented these seizures, although the EEG still exhibited some occasional spike activity. PHT lacked any anticonvulsant activity and apparently had a facilitory effect by shortening the onset of the PTZ-induced seizures.

Autoradiographs from animals treated with ESM or PHT alone were qualitatively similar to controls. In these, the mammillary nucleus, ventral posterolateral nucleus of the thalamus, and both the medial and lateral geniculate nuclei had the greatest autoradiographic density. PTZ treatment alone enhanced uptake of label in all grey matter areas, especially the cortex, hippocampus, thalamus, globus pallidus (GP), and substantia nigra (SN). Pretreatment of PTZ-infused animals with ESM returned the autoradiographic density of most brain regions to control levels. Enhancement of [^{14}C] DG uptake was demonstrated, however, selectively involving the mammillary nucleus, the mammillothalamic tract, the anterior nucleus of the thalamus, and the ventral tegmental nucleus in the midbrain. These structures were, in fact, increased in density relative to those from animals treated with PTZ alone. If the pretreatment dose of ESM was increased to revert the EEG to control activity, the enhancement in these structures was lost. Autoradiographs of PTZ-infused animals pretreated with PHT were similar to those of the animals given PTZ alone, with additional label uptake in the GP and SN.

These data suggest that the mammillary nucleus and its projections to the anterior nucleus of the thalamus and to the ventral tegmental nucleus may be important in the generation of seizure activity induced with PTZ and/or in the anticonvulsant action of ESM in this seizure model. Augmented electroencephalographic activity coupled with enhanced [^{14}C] DG incorporation into the GP and SN with PHT pretreatment of PTZ-infused animals supports the concept of the GP and SN involvement in seizure mechanisms.

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186.2 A LOSS OF INHIBITORY, GABA TERMINALS THAT SYNAPSE WITH AXON INITIAL SEGMENTS OF PYRAMIDAL CELLS IN EPILEPTIC FOCI. C.E. Ribak, Department of Anatomy, University of California, Irvine, CA 92717

Previous studies from this laboratory have shown that GABAergic, inhibitory axon terminals which form symmetric synapses with somata and dendrites of pyramidal neurons are preferentially lost at epileptic foci. Other GABAergic terminals in the neocortex form a dense plexus with the axon initial segments of pyramidal neurons, especially those in layers II and III. The cells that give rise to such an axonal plexus are called chandelier or axo-axonal cells. In the present study, the axon terminals of chandelier cells were analyzed in monkeys with cortical focal epilepsy produced by alumina gel to determine whether this type of GABAergic terminal is lost at epileptic foci. Epileptic and non-epileptic cortical tissue from monkeys used in a previous study were re-examined with the electron microscope. Axon initial segments of pyramidal neurons were identified by their characteristic origin from the base of these cells and by three ultrastructural features: fasciculations of microtubules, a multilayered electron dense subaxolemmal undercoating and numerous cisternal organelles. Some of the axon initial segments that were examined were traced for at least 40 μm in serial thin sections and beyond this point were observed to become myelinated. In single sections, 10-15 axon terminals were found to form symmetric synapses throughout the entire length of the axon initial segments from non-epileptic preparations. This finding is consistent with a previous description of the axon initial segments of pyramidal neurons in primate sensorimotor cortex (Sloper and Powell, 1979). Chandelier cell axon terminals that were reconstructed from serial sections were shown to align themselves parallel to axon initial segments and were observed to synapse with only these structures and not adjacent dendrites or spines. In epileptic cortex, the axon initial segments of pyramidal neurons were apposed by glial profiles that contained clusters of filaments typical of reactive astrocytes. Few if any axon terminals were observed to form symmetric synapses with these axon initial segments. Thus, the chandelier cell axons appeared to degenerate in epileptic cortex. The highly strategic site of GABAergic inhibitory synapses on axon initial segments suggests that they exert a strong influence on the output of pyramidal cells. The near absence of these chandelier cell axons in epileptic foci most likely contributes to the hyperexcitability of neurons in the epileptic focus.

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186.3 DISSOCIATION BETWEEN THE BEHAVIORAL AND ANTICONVULSANT ACTIONS OF INTRANIGRAL MUSCIMOL, D.S. Garant* and K. Gale. (SPON: R. Sheridan) Dept. of Pharmacology, Georgetown Univ. Schools of Medicine and Dentistry, Washington, D.C., 20007.

GABA agonist- or lesion-induced inhibition of nigral efferent activity, produces stereotyped behavioral hyperactivity and protects against a number of experimental seizure models (Iadarola and Gale, Science 218(1982)1237; Garant and Gale, Brain Res, in press). We are currently examining various nigral efferents to determine those responsible for mediating these effects.

A projection from SN to reticular formation terminates in the vicinity of the pedunculopontine nucleus (PPN), and has been demonstrated by Childs and Gale (Brain Res. 258(1983)109) to contain a GABAergic pathway. Effects of lesions or GABA agonists in SN may therefore be expressed by inhibition of this GABAergic projection. Indeed, the GABA agonist muscimol microinjected bilaterally into PPN completely blocks all components of stereotypy induced by bilateral intranigral muscimol (J.A. Childs and K. Gale, this volume). We therefore were interested in determining whether the maintenance of GABAergic tone in the vicinity of PPN would counteract the anticonvulsant action of nigral manipulations.

Muscimol was dissolved in saline and infused at a rate of 0.2 ul/min. Seizure activity was assessed using bicuculline (0.3 mg/kg iv) in 250-300 g rats, or by maximal electroshock in 90-120 g rats. 25 ng of muscimol was microinjected bilaterally into SN and either saline or 50 ng muscimol was injected bilaterally into PPN (coordinates for 300 g rats: AP, 5.8 mm caudal to bregma; 1.4 mm lateral to midline; 6.9 mm below dura; for 100 g rats: AP, 4.8 mm caudal to bregma; 1.3 mm lateral; 6.0 mm below dura). Rats receiving intranigral muscimol alone displayed stereotyped sniffing and gnawing and seizure protection. Muscimol injections into PPN abolished the stereotyped behavior induced by intranigral muscimol; however, the PPN injections failed to antagonize the anticonvulsant action of intranigral muscimol in either seizure test.

Thus the hyperactivity and the anticonvulsant activity produced by intranigral muscimol administration are separable phenomena. This suggests that the nigral efferents that mediate hyperactive, stereotyped behaviors and those that mediate anticonvulsant actions, while subject to similar neurotransmitter influences, are independent pathways.

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186.4 EVIDENCE THAT MORPHINE POTENTIATES SEIZURES INDUCED BY GABA ANTAGONISTS. Frederick Foote* and Karen Gale (SPON: D.Stoff) Dept. of Pharmacology, Georgetown U. Schools of Medicine and Dentistry, Washington. D.C. 20007

The effect of morphine sulfate was examined on several different rat seizure models: (1) Maximal electroshock (MES), (2) Bicuculline (0.3mg/kg i.v.), (3) Isoniazid (350mg/kg i.p.) and 4) Picrotoxin (2.0mg/kg i.v.). Morphine was administered to male Sprague-Dawley rats in doses of 10-50 mg/kg i.p., 30 min prior to MES, bicuculline or picrotoxin and 15 min before isoniazid. In a dose-dependent manner, morphine protected against seizures induced by MES and increased the incidence and severity of seizures induced by bicuculline. The dose-response characteristics of the two effects of morphine were similar and in the same range. For each effect, the ED_{50} of morphine was approximately 20 mg/kg and the morphine-induced alteration of seizure susceptibility was antagonized by naloxone (2-10 mg/kg s.c.). In addition, morphine (30 mg/kg i.p.) markedly potentiated seizures induced by isoniazid and by picrotoxin. In the case of isoniazid, morphine pretreatment caused major clonic and/or tonic seizures following a subthreshold dose of isoniazid.

These results demonstrate that opiate activity may influence the expression of seizures in contrasting ways dependent upon the mode of seizure generation. Morphine exerted an anticonvulsant effect on seizures induced by MES, a nonspecific mode of seizure induction. In contrast, the effects of morphine were uniformly proconvulsant with respect to seizures induced by interference with GABA-transmission, either as a consequence of GABA-synthesis inhibition (isoniazid) or as a consequence of GABA-receptor antagonism (bicuculline and picrotoxin). GABAergic systems may therefore be of particular significance for the elucidation of some of the complex and varied effects of morphine on seizure susceptibility.

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*F.Foote, ENS,MC,USNR, is on leave of absence from G.U. School of Medicine and the Armed Forces Health Professions Scholarship Program.

186.5 THE EFFECT OF DIAZEPAM, VALPROIC ACID, OR CARBAMAZEPINE ON L-METHIONINE-d,ℓ-SULFOXIMINE INDUCED CONVULSIONS AND BRAIN LEVELS OF S-ADENOSYLMETHIONINE AND S-ADENOSYLHOMOCYSTEINE. M.W. Gill*, C. Ryan*, B. Hassett*, J. Bellino*, and R.A. Schatz*. (SPON: J. Hall). Grad. Sch. Pharm. Allied Health Prof., Toxicol. Prog., Northeastern Univ., Boston, MA 02115.

The convulsant L-methionine-d,ℓ-sulfoximine (MSO) has been shown to accelerate flux through cerebral methylation pathways. These methylation reactions utilize S-adenosyl-L-methionine (AdoMet), as methyl donor and result in the formation of S-adenosyl-L homocysteine (AdoHcy), an endogenous inhibitor of methylation reactions. The ratio of AdoMet/AdoHcy has been used as an index of methylation, in vitro. (Increased ratios indicate accelerated methylation and decreased ratios indicate slowed methylation). The AdoMet/AdoHcy ratio is increased after MSO and this is further reflected by increases in the activity of several methyltransferase enzymes. The clinical anticonvulsants phenobarbital or phenytoin decrease the AdoMet/AdoHcy ratio, prevent MSO-induced alterations in methylation and protect against MSO seizures. We have extended these observations using valproate (VPA), diazepam (DZ), and carbamazepine (CBZ) to see if: 1) they protect against MSO seizures and 2) if the protective effect is modulated via alterations in the methylation pathway. Mice were injected i.p. with 25 or 50 mg/kg DZ, 250 or 500 mg/kg VPA, and 25 or 50 mg/kg CBZ. DZ significantly decreased both AdoMet and AdoHcy levels 180 or 360 min after drug administration. VPA decreased brain levels of AdoMet and AdoHcy at 180 min. CBZ significantly decreased AdoMet levels 360 min after drug administration. The effects of subacute (BID x 7 day) VPA (250 mg/kg) and CBZ (50 mg/kg) on AdoMet and AdoHcy levels were also determined. CBZ significantly increased AdoHcy levels and blocked the MSO-induced decrease in brain AdoHcy. The effect of the anticonvulsants on AdoMet/AdoHcy ratios were mixed. Acute and subacute CBZ treatment alone had no effect on this ratio, but did inhibit the MSO-induced increases in AdoMet/AdoHcy. Acute treatment with VPA did not affect these ratios, but subacute VPA, as well as acute DZ, actually increased the AdoMet/AdoHcy ratio. The protective effects of DZ and VPA against MSO-induced seizures either: 1) are not mediated via alterations in brain methylation or 2) the AdoMet/AdoHcy ratio does not necessarily reflect actual in vivo alterations in methylation (perhaps because of cellular compartmentalization of some components of the pathway). The protective effect of CBZ, like phenytoin and phenobarbital, may involve inhibition of the MSO-induced increase in methylation reactions. The biochemical mechanism responsible for DZ, VPA, and the protective effect of CBZ in blocking MSO induced convulsions remains to be elucidated. Supported by N.U. Provost Grant to RAS.

186.6 EFFECTS OF 6-HYDROXYDOPAMINE ON PINEALECTOMY-INDUCED SEIZURES IN THE RAT. C.A. Stockmeier, B.R. Larsen* and D.E. Blask. Department of Anatomy, University of Arizona, Tucson, AZ 85724.

Norepinephrine (NE) levels are depressed in the brain of the partially parathyroidectomized (PthX) rat induced to convulse by pinealectomy (PinX). To determine the role of NE in these seizures and whether changed NE levels are a cause or an effect of the convulsions we treated neonatal rats with 6-hydroxydopamine (6-OHDA) and observed them for various seizure parameters after PinX. Groups of neonatal male and female Long-Evans rats received s.c. injections of vehicle, 6-OHDA (100 mg/kg), desipramine (DMI) (25 mg/kg), or DMI plus 6-OHDA on each of the first three days after birth. At 21 days of age they were partially PthX under Innovar anesthesia. One week later members of each group were either PinX or sham-PinX under ether anesthesia, and caged one per cage and observed for 8 hr for evidence of wild runs, clonic and/or tonic seizures. 6-OHDA caused a 42% reduction in the mean latency to onset of the first seizure when compared with controls; in the DMI- and DMI/6-OHDA-treated rats the latency was not different from controls. Of the 6-OHDA-treated rats which convulsed 45% died during a seizure; none of the vehicle-, DMI- or DMI/6-OHDA-treated rats died during a seizure. Thus DMI appears to reverse the proconvulsant effects of 6-OHDA. With one exception, sham-PinX rats did not convulse. At the end of the observation period the telencephalons and brain stems of the sham-PinX rats were removed and frozen; NE and DA were extracted with 70% ethanol and assayed using high-performance liquid-chromatography with electrochemical detection. Neonatal 6-OHDA treatment reduced NE levels by 24% ($p<0.01$) in the telencephalon and increased NE levels by 66% ($p<0.0005$) in the brain stem when compared with vehicle-injected controls. Telencephalic levels of DA were elevated 20% ($p<0.01$) by neonatal 6-OHDA. The alterations in central catecholamine levels by 6-OHDA were prevented by pretreatment with DMI, suggesting that the proconvulsant effect of 6-OHDA may be due to altered NE function. Elevations in telencephalic levels of DA, which we observed, are usually not reported to be correlated with proconvulsant behavior. NE and/or DA appear to play a strong role in modulating PinX-induced seizures, however, a deficit in their function per se does not seem to be the fundamental cause of the seizures since sham-pinealectomized rats having altered catecholamine function did not convulse. Supported in part by PHS Grant #CA-27653.

186.7 ANTICONVULSIVE EFFECT OF YOHIMBINE AND PHENTOLAMINE ON SEIZURE INDUCED LIDOCAINE. K.C.Kim, M.D. Lab.Indiana University School of Medicine, Department of Anesthesia, Indianapolis, IN 46223.

Administration of epinephrine and norepinephrine altered the seizure threshold induced by pentylentetrazol. Changes in brain monoamines varied after seizures depending upon the individual drug. Depletion of norepinephrine in the brain tissue occurs often after drug induced seizures. Norepinephrine has an inhibitory effect on central nervous system activity(Swinyard,E.A., J Pharm Exp Ther., 144:52,1964). Yohimbine increases norepinephrine levels in the central nervous system by blocking the alpha-2 adrenergic receptor site. The purpose of this study was to investigate the effect of yohimbine and phentolamine on lidocaine-induced seizure. Female mice, CD-1 strain, weight 22±1 (M±SE) were used for this study. The drugs were injected intraperitoneally into the right lower quadrant of the abdomen of mice. Saline or yohimbine or phentolamine was injected 5 minutes before administration of lidocaine. Volume of injected drugs was 0.05 ml/10 Gram of weight and concentration of drug was adjusted to volume. The experiment was divided into three groups: saline, yohimbine, and phentolamine. Dosage of drug:yohimbine 0.1, 0.5,1,5, 10 mg/kg; pentolamine 1,5,10,30,50,100 mg/kg. Convulsion was defined by the following three symptoms: loss of righting reflex, tonic-chronic seizure, opisthotonus, and raising the tail. The CD_{50} was computed from the probit-log regression line using the method of Licifield and Wilcox. Parallelism and significance were tested by the same methods (Litchfield,J.T., J Pharm Exp Ther., 96:99, 1949). The CD_{50} for lidocaine in the control group was 66.2 mg/kg (table 1). Yohimbine 1 mg/kg and phentolamine 10 mg/kg increased CD_{50} by 40% and 37.7% respectively. The difference between yohimbine and phentolamine was not statistically significant. The effective anticonvulsive dosage range for yohimbine and phentolamine was 0.5-3 mg/kg and 5-30 mg/kg respectively. Yohimbine was 10 times more effective than phentolamine. Phentolamine has α_1 and α_2 antagonism, and yohimbine is α_2 antagonist. Therefore, anticonvulsive effect of phentolamine seems to be due to α_2 antagonism. Anticonvulsive mechanism of α_2 antagonism needs to be investigated.

Table 1

GROUP	TREATMENT	CD_{50}	95% CONFIDENCE LIMITS (MG/KG)
Control	Saline	66.2	60.7 - 72.4
Yohimbine	1 mg/kg	98.7	90-9 - 101.9
Pentolamine	10 mg/kg	91.2	91.1 - 100.6

186.8 INHIBITION OF BRAIN THROMBOXANE BIOSYNTHESIS DOES NOT REDUCE SEIZURE THRESHOLD. T.W. Lysz. Dept. of Pharmacology, UMDNJ-New Jersey Medical School, Newark, N.J. 07103.

It has recently been reported that the inhibition of prostaglandin (PG) biosynthesis by nonsteroidal anti-inflammatory agents lowers the convulsant dose-50 to tonic convulsions in mice challenged with pentylenetetrazol (PTZ) (Steinhauer and Hertting, Eur. J. Pharm. 69:(1981)199-204). These results suggest that a PG or other arachidonic acid metabolite has an anticonvulsant action. In an attempt to determine which PG may possess anti-epileptic activity, experiments were conducted to selectively inhibit the formation of thromboxane (TXB_2) in brain, one of the arachidonic acid metabolites elevated at the onset of clonic convulsions.

Adult female Swiss Albino mice were pretreated with 20 mg/kg OKY-1581, a selective thromboxane synthetase inhibitor, followed one hour later with 100 mg/kg PTZ. Two minutes after the onset of clonic convulsions, the mice were decapitated and the head dropped into liquid N_2. Analysis of endogenous whole brain PG levels by RIA revealed a 92% decrease in TXB_2 levels (44.1 ng/Gm wet wgt. in controls versus 3.6 ng/Gm in the OKY-1581 treated animals). PGF_2 alpha levels were unaffected by drug pretreatment (133 ng/Gm wet wgt. versus 146 ng/Gm wet wgt. in the OKY-1581 treated mice).

Despite the marked effect of OKY-1581 on TXB_2 brain levels, there was no effect on the tonic seizure threshold (77 mg/kg in OKY-1581 treated versus 75 mg/kg in controls). In contrast, 10 mg/kg ip indomethacin which also lowers TXB_2 as well as other PGs, reduced the tonic seizure threshold to 64 mg/kg.

These data suggest that inhibition of TXB_2 levels in the CNS does not affect the seizure threshold in mice and that some other product of arachidonic acid metabolism has an anticonvulsant action in mammalian brain. Supported by UMDNJ-Biomedical Research Support Grant (NIH 5 507 RR05393).

186.9 EEG AND NEUROPATHOLOGICAL CONSEQUENCES OF INTRAHIPPOCAMPAL QUINOLINIC ACID. G.S. Brush*, A. Vezzani*, A.C. Foster, E.D. French and R. Schwarcz (SPON: W.O. Whetsell Jr.). Maryland Psychiatric Research Center, Baltimore, MD 21228.

Quinolinic acid (QUIN), a hepatic tryptophan metabolite, is a structural analog of kainic acid, and has recently been demonstrated to occur in human and rat brain (Wolfensberger et al., this meeting). Upon intrahippocampal injection, QUIN produces a characteristic excitotoxic lesion similar to the local damage caused by kainic acid and the neuropathological features of Ammon's horn sclerosis. The present study was designed to examine the electroencephalographic (EEG) and neuropathological consequences of QUIN injections into the hippocampus of unanesthetized rats.

Unilateral injections of QUIN (30-300 nmol) were made into the dorsal hippocampus of freely-moving adult animals and EEG patterns continuously recorded from bilateral hippocampal and cortical electrodes. At all doses, QUIN-induced EEG changes consisted of fast activity (8-10 Hz), spiking in one or more leads or occasional seizures (characterized by such abnormal EEG-patterns in all leads). Some of these QUIN-related EEG-abnormalities were observed in all animals tested. However, reliable precipitation of ictal episodes, recurring at regular intervals, required QUIN-doses of ≥120 nmol. QUIN-induced seizures had an average latency of 25 min. This delay was not due to the slow accumulation of a convulsive QUIN-metabolite since virtually all radioactivity recovered 25 min after a test injection of ^{3}H-QUIN could be identified as unmetabolized QUIN.

Injection of 120 nmol QUIN invariably resulted in neuronal degeneration on the injected side, while the contralateral hippocampus, which showed identical EEG changes, remained undamaged. Thus, it appears that the physical presence of QUIN is necessary for neuropathological changes to occur.

Pre- and co-treatment with 12 nmol of (-)2-amino-7-phosphonoheptanoic acid, a synthetic excitatory amino acid antagonist with known anticonvulsant properties (Science, 216, 899, 1982), blocked the EEG changes and neurodegeneration caused by 120 nmol of QUIN. Similar blockade was also obtained by co-treatment of the endogenous tryptophan metabolite kynurenic acid (360 nmol) with QUIN (120 nmol; cf. Foster and Schwarcz, this meeting).

The data support and expand the earlier suggestion of Lapin (Epilepsia, 22, 257, 1981) that QUIN should be considered as a causative factor in seizure disorders. Moreover, our work indicates a possible role of kynurenic acid, a metabolically related substance, as an endogenous modulator of seizure phenomena.

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186.10 EFFECTS OF CORTICAL AND BRAIN STEM METRAZOL PERFUSIONS IN "ENCEPHALE ISOLE" CATS. F. Velasco, M. Velasco* and M.T. Pacheco*. Division of Neurophysiology. Sci. Res. Dept., Natl. Med. Ctr. IMSS. Mexico Cy. MEXICO.

Cats with implanted electrodes for recording EEG, EMG of facial and extraocular muscles and push-pull cannula guides aiming different cortical and brain stem structures, underwent the following procedures: C2-C3 spinal cord transection to immobilize them in the stereotaxic frame, tracheostomy to maintain artificial respiration and arterial cannulation for blood sampling and blood pressure monitoring. These procedures were performed under general anesthesia. Upon recovering, a solution containing 10 mg/ml of metrazol was perfused through a push-pull cannula, at a rate of 0.05 ml/min, in one of the following structures: Cerebral cortex: suprasylvian, motor or orbitofrontal. Mesencephalon: reticular formation (MRF), colliculi, periaqueductal gray, pes pedunculi. Pons: reticular formation (PRF), pontine nuclei, floor of 4th ventricle.

Cortical perfusions induced: focal EEG spikes without EMG seizures at 0.6 ± 0.2 ml. Intermittent bilateral EEG spikes without clinical seizures at 1.1 ± 0.4 ml, with EMG contralateral jerks at 1.3 ± 0.3 ml and generalized EEG and EMG tonic-clonic seizures more prominent in the contralateral muscles at 2.7 ± 0.9 ml.

MRF perfusions induced: a) EEG and EMG generalized tonic-clonic seizures at 0.6 ± 0.3 ml when cannula tip was placed in the dorsocaudal part of the nucleus. b) Bilateral EEG and EMG clonic discharges at 0.8 ± 0.2 ml when located in the rostral part of the nucleus. c) Bilateral EMG tonic-clonic discharges without EEG seizures at 0.4 ± 0.2 ml when located in the ventro caudal part of the nucleus.

PRF perfusions induced hypotonia and EEG spindle bursts. Outside these areas brain stem perfusions induced neither EEG nor EMG modifications but occasional nystagmus. Results suggest that primary generalized metrazol induced seizures are due to activation of MRF.

186.PO EFFECT OF PROSTAGLANDIN SYNTHETASE INHIBITOR PRETREATMENT ON PENTYLENETETRAZOL-INDUCED SEIZURES IN THE RAT. M.C. Wallenstein
Dept. Physiology, New York University, NY, NY 10010.

Prostaglandins (PGs) are present in mammalian nervous tissue and are released in increased amounts during both spontaneous and experimental seizures. However, whether PGs have a role in induction of seizures is still unclear. In the present study, the effects of pretreatment with PG synthetase inhibitors on pentylenetetrazol (PTZ)-induced seizures were investigated. Both the electrocortical and motor manifestations of these seizures were examined in free-moving rats with chronically-implanted supracortical electrodes. It was found that the seizures produced by 60 mg/kg PTZ were:

a) significantly delayed after pretreatment with either 150 mg/kg paracetamol, 50 mg/kg meclofenamic acid, 30 mg/kg ibuprofen or 10 mg/kg indomethacin.

b) blocked after pretreatment with either 450 mg/kg paracetamol, 150 mg/kg sulindac or 90 mg/kg ibuprofen.

These data were quantified through voltage integration and spectral analysis. The results suggest that PGs are involved in the mechanism underlying PTZ-induced seizures.

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(Supported by grant from New York State Research Council)

CONTROL OF LIMB MOVEMENTS

187.1 ANTAGONIST MUSCLE ACTIVITY DURING RAPID FLEXION OF THE FOREARM IN A FUNCTIONALLY DEAFFERENTED HUMAN SUBJECT. R. Forget* and Y. Lamarre. Centre de recherche en sciences neurologiques, Dépt. de Physiologie, Univ. de Montréal, Mtl., Québec, Canada, H3C 3T8.

Electromyographic activity (EMG) was recorded from the biceps (Ag) and triceps brachii during rapid flexion movements of the forearm (40 and 90 degrees) in ten normal subjects and one patient with a polyneuropathy affecting selectively the peripheral sensory myelinated fibers. In response to a sound cue the subject had to move from a departure zone to a fixed target zone displayed on a cathode ray monitor. All normal subjects showed a triphasic EMG pattern characterized by two bursts in the agonist (Ag_1 and Ag_2) and a burst in the antagonist occurring between Ag_1 and Ag_2.

In a previous study, there was no obvious burst in the antagonist of the deafferented patient (Soc. Neurosci. Abstr., 8: 732, 1982). These experiments have been repeated one year later. At this time, the patient's motor performance was much improved (day to day activities) despite the fact that she was still functionally totally deafferented. Along with a more accurate performance of the motor task, an antagonist burst was now present and was grossly appropriate to decelerate the arm in the region of the target end zone. The antagonist burst had a rather constant duration of 100 ms and ended precisely at the time of maximal deceleration (Dmax). The magnitude of the burst was significantly correlated with Dmax (P 0.01).

In this study all movements, including those which over- and undershot the target, were analyzed. Analysis of individual movements revealed that most were discontinuous and lacked the precision found in the normals. When all movements were averaged, the mean position at which Dmax occurred and the mean end position of the movement were the same as in normal subjects. However, the variance around the mean was significantly greater in the patient resulting in an equal amount of overshoot and undershoot. This finding indicates a lack of precise adjustment of the antagonist burst in the deafferented subject as compared to normal controls. Further analysis showed this deficit to be attributable to improper adjustment of both the size and the timing of the antagonist burst. In normal subjects, the timing of the antagonist burst varied as a function of peak velocity and the size of the antagonist burst was strongly correlated with the size of the first agonist burst. In the patient, these relationships were absent or very weak, particularly for larger movements (90°). It is concluded that a central "program" can generate a triphasic pattern of muscle activity and that peripheral feedback is required for precise adjustment of the size and timing of the antagonist burst. (Supported by the Canadian MRC).

187.2 INACCURATE LIMB POSITIONING AFTER UNEXPECTED PERTURBATIONS DURING MOVEMENT. Jerome N. Sanes Laboratory of Neurophysiology, NIMH, Bethesda, MD 20205.

It has at times been proposed that the brain controls muscles nearly independently of peripheral inputs and that the trajectory and end-point of a step movement can be executed and achieved, respectively, independently of processing of afferent inputs that occur during movement. This would imply that transient obstructions would not impair attainment of a planned final position. Nevertheless, recent data (Day and Marsden, J Physiol, 1982, 327; Sanes and Evarts, J Neurosci, 1983, 3) have demonstrated the disruptive effects of unexpected viscous loads and transient obstructions on final positioning of learned movements. The studies using viscous loads as a perturbing stimulus have shown that intact afferents were necessary to generate short latency muscle responses to compensate for unexpected changes in viscosity during performance of large movements. In the present work, these observations were extended by evaluating performance of large and small movements in intact subjects and patients with sensory loss when a viscous load was unexpectedly introduced or removed.

After training, subjects performed wrist flexion movements in a visual tracking task by moving a handle coupled to a torque motor. Discrete handle rotations of 3°, 10° and 30° were separately performed. Initially, handle movement was either unopposed or resisted by 1 of 3 viscous loads. For 50% of the trials visual guidance was removed and on 50% of these trials either 1 of 3 viscous loads was introduced or the initial viscosity was removed. Changes in visual guidance and viscosity occurred when movement began.

End-point error increased when visual guidance was removed and increased further when the initial viscosity conditions were altered. The greatest increases of error were observed for both the introduction and removal of the largest viscous load. Little change in error was observed for the smallest viscous loads. The overshooting of the intended position was relatively greater for small than for large movements. Short latency muscle responses were observed when viscous loads were unexpectedly changed. Patients with peripheral sensory neuropathy who do not detect changes in viscosity undershoot the learned end-point.

These findings demonstrate that achievement of an accurate learned position is disrupted by peripheral inputs much more for small than for large movements. Further, the short latency muscle responses resulting from unexpected changes in mechanical loads during limb movements do not always result in appropriate compensatory responses. The mechanisms responsible for inability to specify limb position may involve as yet unspecified interactions between the afferent input and central command in the motor neuronal pool.

187.3 TREMOR IN A "DEAFFERENTED" HUMAN SUBJECT, B.T. Shahani, Clinical Neurophysiology Laboratories, Neurology Department, Massachusetts General Hospital and Harvard Medical School, Boston Massachusetts 02114.

We have previously demonstrated severe involvement of all sensory fibres with normal preservation of the function of alpha motor axons in a patient with pansensory neuropathy. Detailed electrophysiological evaluation including "proprioceptive silent period" and unloading reflex studies (which were both absent) confirmed our original impression that the stretch reflex arc was not functioning in this virtually "deafferented" human subject. Surface electromyographic recordings from a pair of antagonistic muscles (extensors and flexors of wrist) and accelerometric recordings showed normal continuous electromyographic activity and 10-12 Hz oscillations (features of normal physiological tremor) when the patient held his arms outstretched. Whereas holding a 1-2 kg weight produced synchronization of motor unit potentials and increase in the amplitude (the rate of underlying tremor remaining unchanged) in a normal control subject, such "enhanced physiological" tremor was not seen in the patient under similar conditions. However during the task of holding the weight for 2 to 4 minutes, slow oscillations (1 to 3 Hz) were recorded due to movement at proximal joints. These findings in a "deafferented" patient (1) confirm the findings of Hagbarth and Young who on the basis of direct microneurographic recordings showed that servo-loop and muscle spindles are essential for the production of enhanced physiological tremor but are not necessary for the normal physiological tremor present during mild to moderate voluntary effort (2) there are some central oscillatory mechanisms which are not dependent on segmental reflex pathways.

187.4 PRE-MOVEMENT EMG SILENCE IN PARKINSON'S DISEASE. P. Eisenberg*, S. Sawyer Palmer, and J.A. Mortimer. Geriat. Res. Educ. Clin. Ctr., V.A. Med. Ctr., Minneapolis, MN 55417, and Department of Neurology, University of Minnesota, Minneapolis, MN 55455.

Silencing of tonic EMG activity immediately preceding the initial agonist burst is a common observation in normal subjects making rapid voluntary movements. The occurrence of pre-movement silence (PMS) is correlated with the peak acceleration of the subsequent movement (Mortimer and Eisenberg, 1982). The purpose of the present study was to determine whether PMS occurs in patients with Parkinson's disease instructed to make rapid ballistic movements and whether a similar relationship with movement acceleration is evident.

Eight patients were studied. Seven had significant bradykinesia as assessed by clinical evaluation. Their right forearms were strapped to an arm support that rotated in a horizontal plane. Surface EMG was recorded from biceps and triceps. Patients maintained an initial elbow position of 90 degrees against a 1 Nm load on triceps and were instructed to initiate maximum effort arm extensions following tone cues. Movements were arrested by a karate bag. Each patient performed 25-35 trials. A computer algorithm was used to identify periods of PMS that differed significantly from random variations in the baseline EMG.

PMS was seen in all 8 patients. Its frequency of occurrence (range: 16-50%, mean =30%) was lower than that seen in healthy young subjects under similar experimental conditions (range: 37-91%, mean = 58%). In only 1 of 8 patients was there a significant correlation between the occurrence of PMS and acceleration. By contrast, 7 of 9 normal subjects studied previously showed such a relationship. During 68% of the trials made by Parkinson patients, a sequence of "segmented bursts" led up to or replaced the single initial agonist burst seen in normals. The presence of such segmented bursts was associated with lower acceleration. The three Parkinson patients with the lowest mean accelerations had segmented bursts on 93-100% of their trials, whereas these bursts occurred on 3-88% (mean=51%) of trials in the other 5 patients.

In an earlier study, we hypothesized that the occurrence of PMS enables the motoneuron pool to become non-refractory and available for synchronous activation, producing higher movement acceleration. Although PMS was seen on many trials in Parkinson patients, little association was evident between its presence and movement acceleration. In Parkinson patients, the motor system appears to be constrained to a gradual and periodic recruitment of motor units over a longer time course than in normal subjects.

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187.5 CENTRAL AND PERIPHERAL FATIGUE OF INTERMITTENT SUBMAXIMAL VOLUNTARY CONTRACTIONS. B. Bigland-Ritchie, F. Bellemare* and J.J. Woods*, (SPON. J.T. Stitt). John B. Pierce Foundation and Quinnipiac College, New Haven, CT. 06519.

During fatigue of a sustained maximal voluntary contraction (MVC) we have previously concluded that, despite a decline in EMG, loss of force is not due to failure of either peripheral neuromuscular transmission, or of muscle activation by the central nervous system (central fatigue). Thus it must result mainly from muscle contractile failure. Similar methodology has now been applied to fatigue of sustained or intermittent submaximal contractions.

When single or multiple shocks are superimposed on ongoing voluntary contractions the force increment (Ts) declines as the voluntary force increases. If the muscle is fully activated by the CNS during a MVC then further stimulation results in no additional force; ie. Ts = 0 (Belanger and McComas). Submaximal contractions were repeated until the time that the target force (% initial MVC) could no longer be maintained (Tlim). Muscle contractile force (Tr) was monitored by periodically injecting single or multiple shocks between contractions. When these shocks were periodically superimposed on the ongoing voluntary contractions the decline in the consequent force increment (Ts) detects changes in muscle activation by the CNS. The evoked muscle potentials were monitored for failure of neuromuscular transmission. Initially, and at intervals, the force of brief MVCs was compared with the force during 50 Hz maximal nerve stimulation.

Preliminary results suggest that: a) endurance time (Tlim) can be predicted from the (% initial MVC x % contraction time per cycle), as shown previously by Bellemare and Grassino for similar contractions of the human diaphragm; b) the MVC declines progressively; c) at Tlim the decline in MVC (ΔMVC) $\simeq$ ΔF 50 Hz; d)the %ΔTr$\simeq$%ΔMVC; e) at Tlim, Ts$\simeq$0;f) little or no decline was seen in the evoked M waves. From this we suggest that fatigue is mainly due to muscle contractile failure. The substantial increase in M wave duration indicates a considerable slowing in muscle conduction velocity.

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Belanger, A.Y. and McComas, A.J. (1981) J. Appl. Physiol. 51: 1131-1135.

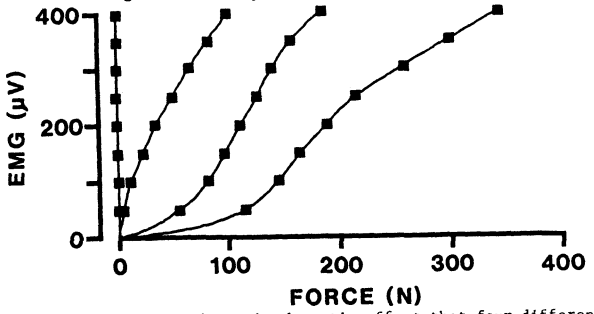
Bellemare, F. and Grassino, A. (1982) J. Appl. Physiol. 53: 1190-1195.

187.6 THE ROLE OF ANTAGONIST CO-CONTRACTION IN THE FORCE-EMG RELATION OF HUMAN BICEPS MUSCLES. L.A. Jones and I.W. Hunter. Department of Psychology & Biomedical Engineering Unit, McGill University, Montreal, Canada, H3A 1B1.

The exact form of the relation between the smoothed, rectified myoelectric signal (EMG) and isometric force, as measured about a joint, has been subject to much debate. Many investigators have reported a linear relation between the surface EMG and isometric force, while others have found this relation to be non-linear. Numerous variables ranging from fatigue to force dynamics have been shown to alter the form of this function.

An additional factor which may affect the force-EMG relation is the experimental method employed to generate isometric forces. Two distinct procedures have been used. The first method requires that subjects make ramp contractions by tracking a visual target until they reach a specified force level. In the alternative procedure forces of varying magnitudes are produced in steps with rest periods interspersed between each exertion. The former method frequently results in a non-linear force-EMG function, while the latter procedure often yields a linear relation.

In this experiment the effect of antagonist co-contraction was determined during step and ramp isometric contractions of the biceps brachii muscle. The force exerted at the wrist was measured and the surface EMG was recorded from the biceps and triceps brachii muscles. Ramp contractions generally produced a non-linear force-EMG relation, while step contractions resulted in linear functions in some subjects. The difference in the results obtained with the two procedures seemed to arise from co-contraction rather than from fatigue or force dynamics.



The above figure shows the dramatic effect that four different levels of co-contraction can exert on the force-EMG relation during ramp contractions. The non-linear relation found for the biceps muscle appeared to be linearized by co-contraction during the step procedure. These results indicate that it is critical to monitor the level of co-contraction whenever the force-EMG relation is determined.

187.7 MUSCLE-REFLEX COMPLIANCE: ELASTICITY AND PLASTICITY AT THE HUMAN ELBOW. Gerald L. Gottlieb and Gyan C. Agarwal . Rush Medical College, Department of Physiology, Chicago, Illinois 60612.

Following the experimental paradigm of Fel'dman (1), step torques were applied to the elbow joints of human subjects who were maintaining a specified joint angle against a constant load. These torques were random sequences of varying amplitudes in both unloading (of the steadily contracting biceps) and loading. This resulted in either shortening or lengthening of the active muscle while the subject was instructed not to react or "intervene voluntarily" after the perturbation.

The joint would equilibrate at a new angle within 200-400 ms after the perturbation, depending upon its direction and amplitude. We would plot the equilibrium angle versus the perturbing torque to generate a "characteristic curve" for the joint. Irrespective of the starting angle or torque, the characteristic curve was always S-shaped, decreasing in slope for larger amplitude perturbations, both in extension and contraction. Thus, the characteristic curve demonstrates the often described short-range stiffness properties of isolated muscles and human joints.

What was of most interest was that the characteristic curve always shifted with the operating point of the joint so that changing initial angle, torque or both together produced a new characteristic curve centered at the new values. We confirmed Fel'dman's observation that the average slope of the characteristic curve was insensitive to the initial angle and proportional to the initial torque. The S-shaped curve is incompatible with some of his conclusions concerning the linearity of the responses to multiple pulses.

The mobility of the characteristic curve represents a fundamental plasticity in the behavior of the joint under the experimental paradigm used. In this case such a nonlinearity may result in simplification of the control scheme because the joint always operates at the same place on the curve (the central inflection point), irrespective of shifts in torque or angle of either external or internal origin. This is one form of invariant property which can be exploited by the motor controller but the underlying mechanism is still uncertain.

(1) Asatryan, G. and Fel'dman, A.G. Biophysics 10: 1965, Fel'dman, A.G., Biophysics 11:. 1966.

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187.8 PASSIVE ELASTIC PROPERTIES OF MUSCLES MEASURED AT THE ELBOW IN MAN A. W. Wiegner* and R. L. Watts* (SPON: R. R. Young). Clin. Neurophysiology Lab., Massachusetts General Hospital, Boston, MA 02114

Recent theories of arm trajectory formation (Feldman; Bizzi and co-workers) emphasize the role of the motor control system in selecting, by means of appropriate activation, one of a set of length-tension relations for agonist and antagonist muscle pairs acting at a joint such that at equilibrium the arm is correctly positioned at the target. Still more basic is an appreciation of the role of passive elastic properties of these muscles in determining flexibility and range of motion in the relaxed limb. The present studies were undertaken to assess some passive elastic properties at the elbow joint in the relaxed arm.

Subjects were studied in a chair with the right arm supported on a table at shoulder height and the forearm, wrist, and hand strapped to the lever arm of a printed motor (Axem MC19S) mounted below the table. Lever arm position and torque were measured by a potentiometer on the motor shaft and strain gages on the lever arm, respectively. In order to insure that reflex activity would not obscure the passive elastic properties of the muscles acting about the elbow, a set of preliminary experiments were done with torque pulses of up to 7 N-m for 100 msec applied to the arm. Reflex responses were not seen when peak velocities at the joint were less than 100^{o}/sec. Subsequently, slow bidirectional ramps in torque (≤ 0.11 N-m/sec, producing velocities $< 20^{o}$/sec) were applied to the forearms of 10 normal subjects instructed to remain relaxed. Triceps EMG was monitored and any subjects demonstrating EMG activity or voluntary arm movement during the test were removed from the study. Data obtained during rising and falling torque ramps were combined to take into account the effects of limb hysteresis and 10 trials were averaged for each subject.

We observed: (1) Even in relaxed muscle there are underlying spring-like properties that, in the absense of friction and external torques, return the arm from a displaced position to neutral position (112 ± 10^{o} (SD) at the elbow). (2) Curves obtained by plotting displacement vs. applied torque were remarkably linear over a range of $\pm 30^{o}$ from neutral position, with linear regression r-values exceeding 0.98 in each subject. Elbow compliance ranged from 35 to 171 ^{o}/N-m, with a strong negative correlation ($r=-0.80$, $P < 0.01$) between upper arm mass and compliance at the elbow.

Our results suggest that normal values for passive elbow position and compliance may be obtained for comparison to limbs of those with pathological conditions such as spasticity, rigidity, or mechanical changes caused by contractures or arthritis at the elbow. This may assist in differentiating enhanced reflex activity from changes in physical properties of muscle and in monitoring the progress of therapy.

187.9 RESPONSES OF FORELIMB MUSCLES TO SMALL WRIST PERTURBATIONS. E. M. Schmidt and J. S. McIntosh. Laboratory of Neural Control, NIH, Bethesda, MD 20205.

To further investigate the role of sensory information in the control of movement and posture, monkeys were trained to perform wrist flexion - extension movements. The hand was coupled to a torque motor that simulated a spring load with zero force at the mid-wrist position. With this device different background loads and small amplitude perturbations of 50 ms duration were produced at different times in the task. The perturbations produced wrist rotations of approximately 1 degree. EMG electrodes were inserted into forearm flexor and extensor muscles. Electrode placement was verified by stimulating through the electrodes at the end of the recording session.

Unloading perturbations applied to an active muscle produced a reduction in EMG at a latency of approximately 15 ms followed by rebound excitation after the pulse. Loading perturbations applied to an active muscle produced a slight increase in EMG during the pulse with suppression of EMG following the pulse. Responses to perturbations varied as a function of time of application during the task. The largest response occurred near the end of a 1 sec. hold period while a minimal response occurred at the beginning of movement. These results could be due to the different levels of motor pool excitability present at different times during the task, to sensory receptor sensitivity variations, or variations in central processing of the sensory information. In order to investigate the effects of motor pool excitability on response to perturbations, the load was varied while the perturbations were applied at the same time in the task. The level of motor pool excitability, as evidenced by different levels of EMG activity, did affect the magnitude of the responses, but could not account for the much larger variations seen at different times in the task. Although these tests do not evaluate possible changes in receptor sensitivity, the present results suggest that sensory feedback may have little effect at the beginning of movements, as if the movement control system was operating open loop, but during the later phases of posture the control system operates closed loop relying heavily on sensory information.

Previously Schmidt, et al. (Soc. Neurosci., 8:150.3, 1982) have shown that small wrist unloading perturbations produce modifications of motor cortex neurons in as little as 12.3 ms. This time is too long for peripherally modified cortical activity to influence the beginning of the observed EMG unloading response but it is short enough to participate in the later phase of the EMG suppression produced by the perturbation.

187.10 SHORTENING RESPONSES IN NORMAL, SPASTIC, AND RIGID MUSCLE. B.J. Norton*, S.A. Sahrmann, (SPON: M.H. Clare) Appl. Kines Lab., PGM. in Physical Therapy. Sch. of Med., Washington Univ. St. Louis, MO 63110

Though first reported in 1918, responses elicited during passive muscle shortening have not been studied extensively. Preliminary studies suggest a possible role for SRs in normal motor control particularly as an element in muscle tone, and when absent or exaggerated, as an indicator of neuropathology. The purpose of this study was to compare the following characteristics of SRs and stretch reflexes (STRs) in normal subjects, stroke, and Parkinson patients: 1) frequency of occurrence, 2) onset latency, 3) response to fast vs. slow movements, and 4) response to continuous vs interrupted movements.

A computer controlled electrohydraulic system designed to produce passive joint movement was used to elicit EMG activity during both stretch and shortening of the biceps (Bi) and brachioradialis (Br) muscles. A total of 24 normal subjects, 22 stroke, and 26 Parkinson patients were studied. The values for both total EMG elicited by passive elbow movement and onset latencies were used for analysis. The significance of the frequency of occurrences was analyzed with Chi square ($p<.05$); the differences between SR and STR onset latencies, the responses to fast and slow movement, as well as continuous vs interrupted movements were assessed with the pared t-test ($p <.05$).

In each group, more subjects demonstrated SRs than did not for both Bi and Br, but only in the patient groups did the majority of subjects demonstrate STRs. The latency of the SR was twice that of the STR. Fast movement elicited a significantly larger response than slow movement for 1) both SR and STR in the hemiplegic patients (2 muscles), 2) STR in the Parkinson patients (2 muscles), and 3) STR in the normal subjects (1 muscle). Interrupted movements (3 second pause between flexion and extension) did not significantly change the STRs in normals or spastic patients when compared to continuous movements. Interrupted movements did decrease STRs and SRs in rigid patients and SRs in spastic patients.

Stretch reflexes have been studied extensively in order to define their role in regulation of muscle tone and in the control of movement. Shortening responses have been the subject of only a few studies, yet the frequency of their occurrence in normals suggests the need to study their possible role in posture and movement. The patterns of SR behavior vary between normals and patients with disorders of the nervous system. The alteration in SR behavior may be related to specific disease processes that affect suprasegmental motor systems and may provide useful physiological insights into their mechanisms.

187.11 EFFECT OF VOLITIONAL SET ON THE CORTICAL EVOKED POTENTIAL TO WRIST PERTURBATIONS IN HUMANS. W.J. Becker*, D.G. White* and R.G. Lee. Dept. of Clinical Neurosciences, Univ. of Calgary Fac. of Med. Calgary, Alberta, Canada T2N 1N4

The long latency component of the EMG response to muscle stretch (M2) is dependent on prior instruction or "volitional set" (Hammond, J. Physiol. 132:17, 1956). M2 is well developed when subjects are instructed to actively oppose a perturbation, whereas it is small or even absent when they are instructed to "let go" and allow the limb to be passively displaced. It is believed that M2 is mediated, at least in part, by a transcortical feedback loop. In monkeys, perturbations of the forelimb produce short latency responses from motor cortical neurons which are appropriately timed for them to be participating in such a loop (Evarts, Science 179:501, 1973). The objectives of the present study were: 1) to define the cortical evoked potentials to sudden stretches of the wrist flexor muscles, 2) to determine the manner in which these potentials are modified by volitional set, and 3) to relate these changes to alterations in the M2 component of the EMG response.

Recordings were obtained from six normal human subjects using a torque motor to apply sudden perturbations which stretch the wrist flexors. EMG activity was recorded with surface electrodes over the wrist flexors. Cortical evoked activity was recorded with scalp electrodes over the contralateral sensorimotor cortex. Subjects were instructed to either "let go" (passive mode) or to "oppose" the perturbation by producing a rapid voluntary flexion of the wrist (active mode). Four hundred responses were averaged for each condition. The cortical evoked potential to passive stretch consisted of an initial positive wave beginning 30-35 msec. following activation of the torque motor. This component was often double-peaked. It was followed by a negative wave with a peak at 75-90 msec. and then a late positive deflection. The evoked potential during the active condition had a similar configuration. However, computer subtraction of the "passive" from the "active" evoked potential revealed consistent differences during the 40-50 msec. and 85-110 msec. time periods following activation of the torque motor. The first of these differences could represent activity in cortical neuronal systems associated with the M2 component of the EMG response which began 55-65 msec. following the onset of perturbation. The second period during which the evoked potentials differed could be related to the voluntary EMG response which began beyond 100 msec. These results are compatible with the concept that a transcortical feedback loop contributes to the M2 component of the EMG response to muscle stretch.

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187.12 EMG AND MOVEMENT KINEMATIC CHANGES AS A FUNCTION OF LEARNING A MOTOR CONTROL TASK. S. P. Moore* and R. G. Marteniuk. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada, N2L 3G1.

Some neural and behavioral theories describe learning as fine-tuning, involving the elimination of unnecessary elements. This results in an efficient movement which, if desired, may be produced with little variability over repeated attempts. Most past studies which have utilized EMG to infer changes which occur at the neural level due to learning have used relatively short acquisition periods (40-100 trials). These short acquisition periods may account for the inconsistent and, at times, contradictory results of this literature. The purpose of the present study was to examine the acquisition of a fast time-constrained aiming movement over an extended period of practice. EMG and movement kinematics were used to assess changes due to practice.

Subjects were required to perform a 45° forearm extension in 200 msec for 100 trials on each of four days. Feedback given to subjects on each trial indicated errors in both the displacement and time domains. Temporal and amplitude measures of EMG activity of both triceps and biceps were derived from digital representations of the normalized rectified signals. Movement kinematics were obtained from a potentiometer mounted to the base of the manipulandum that subjects moved in the execution of their task.

The results indicated systematic changes in both EMG and task kinematics over the 400 trials of practice. The EMG changes noted were: a change from cocontraction early in practice to triphasic activity in late practice which resulted in a systematic decrease of average EMG activity over the 400 trials; and a general decrease in within-subject variability. Kinematic results also showed a consistent reduction in variability. Of interest was the fact that subjects with similar kinematic profiles produced markedly different EMG patterns.

In general, then, the results indicated continuing changes in both neural and behavioral indices of motor control over 400 trials of practice suggesting that previous studies may have been limited in making inferences regarding EMG changes and motor control acquisition. In addition, since no direct link between task kinematics and EMG profiles were found, future investigations must not only use large amounts of practice but also be concerned with individual differences in the acquisition of motor control.

187.13 CHANGES IN ELECTROMYOGRAPHIC PATTERNS DURING ACQUISITION OF A FINE MOTOR SKILL. L.D. Abraham, T. Kinugasa*, A.M. Baylor, J.C. Blankenship*, and D.W. Robertson*. Neuromuscular Lab, Univ. of Texas, Austin, TX 78712.

In order to assess neuromuscular characteristics of fine motor skill learning, electromyographic (EMG) recordings were made from muscles controlling ankle movement in normal adult human subjects performing a novel, bipedal task. The subjects were seated comfortably, and each foot was fixed to a pedal which restricted movement to ankle dorsi- and plantar flexion. Subjects were instructed to trace the outline of a geometric figure fixed to an oscilloscope screen with an electron beam controlled in the vertical dimension by the right ankle and in the horizontal dimension by the left ankle. For each subject, EMG recordings were made throughout a single session ($\leq$ 2 hours) during which performance improved to a criterion of excellence which demanded completion in less than 8 seconds with less than 1 degree of instantaneous spatial error.

Surface electrodes were used with 20 subjects to collect data from 5 muscles (tibialis anterior, peroneus tertius, peroneus longus, lateral gastrocnemius, and soleus) in the right leg. In 6 additional subjects, bipolar intramuscular fine wire electrodes were used to provide clearer records of isolated activity in the tibialis anterior, peroneus longus, lateral gastrocnemius, and soleus muscles. Three conditions of movement were isolated and analyzed separately: dynamic movement of the ipsilateral foot, static positioning during movement of the contralateral foot, and dynamic simultaneous movement of both feet. Analysis of learning-related changes in EMG patterns revealed that changes were specific to individual subjects. As has been typically found in studies of ballistic tasks, skill acquisition was characterized by a reduction in antagonist EMG activity (control by co-contraction). It was particularly interesting that changes could be observed during static positioning (when one might expect most learning to be evident contralaterally). However skill in this fine motor task was clearly related positively to speed. In some subjects this was evidenced by increases in agonist EMG activity at critical times in the movement, while for other subjects the increases in speed associated with skill acquisition were matched by shifts in activity levels in synergistic muscles.

This project was supported in part by BRSG funding from the University Research Institute, UT Austin.

187.14 CHANGES IN SINGLE MOTOR UNIT ACTIVITY DURING ACQUISITION OF A FINE MOTOR SKILL. A.M. Baylor, T. Kinugasa*, L.D. Abraham, and J.C. Blankenship* (SPON: M. Wolf). Neuromuscular Lab, Univ. of Texas, Austin, TX 78712.

Investigations of electromyographic (EMG) changes associated with motor skill acquisition have primarily employed ballistic tasks and surface recording techniques. In order to examine the contributions of single motor unit (SMU) recruitment to finely-controlled motor skills, we used bipolar intramuscular fine wire electrodes to obtain records of SMUs during acquisition of a novel, bipedal task. The subjects (6 normal adult humans) were seated comfortably with each foot fixed to a pedal which restricted movement of the ankle in the sagittal plane.

The task required tracing of a triangular shape on an oscilloscope screen, controlling the vertical motion of the cursor with the right ankle angle and the horizontal motion with the left ankle. The task was repeated in blocks of 25 trials until it was completed 3 times consecutively in fewer than 8 seconds with no spatial error greater than 1 degree of joint motion. The number of trials to criterion ranged from 96 to 200. EMG records were obtained, throughout the single session of skill acquisition, from 4 muscles in the right leg: tibialis anterior--a dorsiflexor, and 3 plantar flexors--lateral gastrocnemius, peroneus longus, and soleus.

Twenty individual SMUs were identified off-line by amplitude and waveform. Although integrated EMG activity is often found to decrease in studies of skill acquisition, in this task we often found increased levels of skill accompanied by recruitment of additional units. These increases were most common just prior to movement and early in each phase of the movement pattern. Since the task required only a small fraction of maximal voluntary activity in these muscles, this result is consistent with the observed increase in movement speed. The increased recruitment was particularly evident in agonist muscle activity; units in antagonist muscles commonly dropped out as skill level increased. These changes support the possibility that acquisition of skill in this fine motor task was accomplished by adopting a more ballistic movement pattern. In several cases, no SMU activity was found after skill acquisition in muscules which exhibited several active units during early trials. This was consistent with previous observations of refinements in synergistic muscle use during motor learning.

This project was supported in part by BRSG funding from the University Research Institute, UT Austin.

187.15 ALTERATIONS IN ELECTROMYOGRAPHIC PARAMETERS ACCOMPANYING LEARNING DURING A RAPID WRIST EXTENSOR TASK. Mark W. Cornwall* and Gary Kamen. Motor Control Laboratory, Indiana University, Department of Physical Education, Bloomington, IN 47405.

Individuals who practice responding to a visual stimulus generally improve their reaction time scores during 2-4 days of practice. A preliminary experiment was conducted to determine what electromyographic changes accompany this improvement in response latency using 4 subjects with no prior experience in laboratory reaction time tasks. Each subject was tested over four consecutive days. On each day, the subject responded to a visual stimulus with a rapid wrist extension movement of the right hand. Forty trials were given on each day, using 5 preparatory intervals which varied by 0.5 msec increments from 1.5 to 3.5 msec. Four catch trials were also given on each day. Surface silver-silver chloride electrodes were applied over the belly of the extensor carpi ulnaris. Identical placement of the electrodes over the same site was ensured using an indelible marker. EMG activity was amplified (x 1000), bandwidth limited (10hz-3 khz; -3db) and digitized at 2 khz (10 bit resolution). Results indicated that reaction time performance did indeed improve (258 msec on day 1 vs 251 msec on day 4), but by a margin somewhat smaller than that reported by previous investigators. Total integrated EMG (IEMG; integrated over 60 msec from the onset of electrical activity) varied inconsistently across the four days. However, number of zero crossings decreased during the practice period ($.05 < p < .1$) as did number of turning points ($p < .05$). Thus, these preliminary data indicate that repeated practice of a rapid response task can effect decreases in EMG frequency components without concomitant changes in IEMG.

187.16 CO-CONTRACTION OF ANTAGONIST MUSCLES DURING TRAINING OF WRIST MOVEMENTS IN THE MONKEY. J.W. Mink & W.T. Thach. Depts. of Anatomy & Neurobiology and Neurology & Neurosurgery, Washington University School of Medicine, St. Louis, MO 63110.

Rhesus monkeys were trained over a 6-month period to insert their hands into a wedge-shaped manipulandum, with fingers extended, and to track a moving visual target by flexing and extending at the wrist. Slow 10°/sec trajectories were performed from a midpoint 35° in flexion and extension directions with and without uniform torque loads (0-0.1 Nm). EMG activity was recorded with surface electrodes 2 cm apart over forearm flexors and extensors. Trained rapid alternating movements showed high amplitude EMG activity that was often strictly reciprocal indicating EMG electrode pickup only from the near muscle group and not its opposite. As in previous studies (Schieber & Thach, 1980) extensor EMG was always greater than flexor EMG under equivalent torque loads, presumably due to the greater passive elastic restoring forces of the flexor muscle group. Superimposed on the flexor and extensor activity providing the net wrist torque needed to make the trajectory was a pattern of co-contraction of both muscle groups that varied within and across trials, but was usually much greater than the net activity required for displacement. Co-contraction was present under both no load and load conditions, but was greatest in the first 2 or 3 trials following the introduction of a novel load.

This pattern of co-contraction both contrasts with and resembles that observed in monkeys trained over longer periods (2 years) who limited activity to forearm wrist flexors and extensors in strict reciprocal patterns, yet who demonstrated co-activated flexor and extensor spindle afferents. Is the presence of coactive flexor and extensor spindles produced simply by reducing a co-contracting drive that is common to all motorneurons such as to exclude alphas and retain gammas strictly according to the size principle? Or are gamma motorneurons more selectively controlled by the central nervous system? What are the CNS mechanisms that underlie this adaptive process that preserves precision yet minimizes muscle work? (Supported by NINCDS Grant NS-12777-09.)

LOCOMOTION II

188.1 INTERPRETING HUMAN LOCOMOTOR REACTION TIMES. L. K. Gorman*, N. V. Yarbrough* and M. C. Wetzel. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721.

Although electromyographic (EMG) segmentation has been reported in selected muscles after mechanical perturbations and in other motor tasks, latency measures, alone, have not revealed the causes. In traditional studies of reaction time (RT) the total effect is largely unknown of a typical "preparatory"-"response" signal sequence. The work summarized here tested a new way to interpret RT, based on the strength of a known causal event: a green light that was established as a discriminative stimulus (Sd) by operant conditioning. One measure of Sd strength was its successful production of a burst by the usually quiet rectus femoris (RF) muscle during treadmill walking, when latency was successively reduced across several weeks of training. Sd strength was also measured in relation to other, movement-produced stimulation that caused EMG responses (pre-existing or newly conditioned) during the periodic step cycle.

The Sd (green light) occurred at times randomly determined in relation to heel touchdown. On alternating cycles no light flashed. All 6 subjects met a criterion of 80% correct responses (after 50 or more light flashes) for the first, 400 msec, RT condition. Although the acceptable RT had to be 400 msec or less, a high tone sounded 500 msec after the light only if there was an RF burst (of preset integrated amplitude) that was at least 100 msec in duration. Interspersed randomly with the green light step cycles were cycles in which a red light flashed instead. It was followed by a high tone after 500 msec if no burst occurred. Errors were followed by a low tone 500 msec after the green or red light. Four of the 6 subjects met the next criterion, whose acceptable RT had to be 300 msec or less, and one met criterion at an RT requirement of 200 msec or less.

Evidence was gained for controlling stimuli other than the green light. RF bursts might occur within a few msec after the green light, or even before it, as could be seen when by chance a green light had flashed at about the same time in 2-3 previous steps. While brief or "negative" RTs were more frequent early in training, they were seen occasionally in later days as well.

In summary, latency of EMG to the green light was reduced by operant conditioning, and there was additional very rapid conditioning to rhythmically recurring events. In most previous RT studies, the delay between "preparatory" and "response" signal has been determined randomly, but within limits spanning only a few sec. Under such traditional semi-periodic conditions, RT to the "response" signal should be interpreted as "minimal" only if all other stimulation sources have been identified or ruled out.

188.2 LOCOMOTOR CONTROL IN SPINAL CORD INJURED HUMANS. M. R. Zomlefer, R. F. Gaines* and L. G. McCleary*. Rehabilitation Research and Development Center (153), VA Medical Center, 3801 Miranda Avenue, Palo Alto, CA 94304.

A number of previous studies have shown that many mammals can perform stepping locomotor activity with their hindlimbs after a complete spinal cord transection. The intent of this study was to determine whether spinal cord injured humans were capable of performing some form of patterned locomotor activity. Seventeen male volunteers (age 19-56 and between 10 weeks and 25 years after injury) were used in this study. Clinical exam demonstrated sensorimotor completeness in six of these subjects. The volunteers were suspended in a modified parachute assembly over a motor-driven treadmill; the maximum total time that the subject was held above the treadmill was 20 minutes. The harness design afforded the subjects an erect posture and subjects were allowed to maintain their stability by holding onto a handrail. A winch could lower the subject until his feet just touched the belt. Speeds were varied from 1.0 to 4.0 mph and, in addition, the treadmill was inclined from 5 to greater than 10 degrees in the sagittal plane. Electromyographic(EMG) activity was bilaterally recorded from selected leg muscles (quadriceps and hamstrings) using surface recording electrodes and a multi-channel telemetry system.

No spontaneous stepping movements were observed in any of the subjects tested so far. Nonetheless, six cases showed EMG patterns characteristic to those found in normal human locomotion when the investigators moved both legs to simulate mechanical locomotor behavior. In several cases, EMG amplitude increased with increasing belt speeds. This EMG activity could be the result of a spinal stepping generator, although other possiblities (e.g., alternating stretch reflexes with strong crossed components) may also be responsible. The presence of these EMG signals may be useful in the the future development and control of external functional stimulation to restore locomotion.

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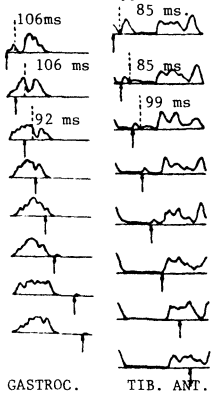
188.3 MODULATION OF THE RESPONSE TO NOCICEPTIVE STIMULUS DURING WALKING IN HUMANS. A.E. Patla* & M. Belanger* (SPON: G. Partlow). Dept. of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada

The response to nociceptive stimulus in mammals such as cats is found to be dependent upon the phase of the stepcycle when the stimulus is applied (e.g., see Forssberg et al., 1975,77,79). Although the responses of the lower limbs to nociceptive stimulus have been studied in humans (Hagbarth et al., 1963; Shahani et al. 1971; Faganel, 1973), how, if at all, this withdrawal reflex gets modified during locomotion was not investigated until recently. This study, an extension of the work by Patla & Belanger (1983), is designed to explore in detail the modulation of the response to nociceptive stimulus during the complete stepcycle.

While the subjects (n=4) were walking at their normal self-paced speed on a treadmill, a 2-3 msec electrical stimulus (3 X Threshold) was applied unexpectedly, via a Cu ring electrode on the second toe and a ground electrode on the dorsum of the foot a few cm apart. This site was most appropriate for eliciting a flexor reflex (Kugelberg, 1960). The delay on the stimulator, which was triggered by a heel strike switch, was varied to apply the stimulus during various phases of the stepcycle. Rectified and filtered (6 Hz) EMG signals from Tib. Ant., Gast., Bic. Fem. & Vas. Lat. were used to study the response.

Typical responses of the Gast. and Tib. Ant. are shown (starting from the top) for stimulus applied 100-600, 800 & 900 msec after heel strike (arrows indicate stimulus onset). When the stimulus is applied during the stance phase the following responses were observed: a) Inhibition (lat.~101 msec) of the Gast. followed by excitation. The inhibition is most prominent during the early part of the stance. b) Excitation (lat.~85 msec) of the Vas. Lat. c) Excitation of the Tib. Ant. (lat. ~85 msec) during the early part of the stance was suppressed later. The subsequent flexor activity was slightly enhanced. In contrast to this, when the stimulus is applied during the swing phase the following responses were observed: a) Excitation of the Bic. Fem. (lat~85 msec). The latency of the enhanced Tib. Ant. activity was difficult to determine because of background EMG activity. Thus the corrective responses to unexpected perturbations are adapted to ongoing locomotor activity permitting the subjects to maintain stability and forward progression.



Supported by NSERC Grant #A0070.

188.4 TOPICAL ANESTHESIA: CHANGES IN GAIT FOLLOWING DESENSITIZATION OF THE SKIN IN NORMAL SUBJECTS. S. Roy*, M. Sabbahi and C. J. De Luca (SPON: S. Wolf). NeuroMuscular Research Lab, Dept. of Orthopaedic Surgery, Children's Hospital Medical Center, Harvard Medical School, Boston, MA 02115 and Liberty Mutual Research Center, Hopkinton, MA 01748

Evidence for increased alpha-motoneuron excitability has been previously reported following desensitization of the skin by topical anesthesia (Sabbahi and De Luca, 1981, 1982). These changes in the discharge characteristics of the motoneuron pool have been associated with alterations in gait and other movement patterns for spastic patients. In the present study, we have investigated the effects of reduction of cutaneous receptor afferent discharge on gait parameters in normal subjects.

Vertical ground reaction forces were measured from a force plate during gait and while walking in place. Foot switches and infrared detectors recorded the foot contact histories and walking speed respectively. Tests were repeated four times consecutively to measure the movement variability.

Topical anesthesia was sprayed to all skin areas of one lower limb except for the skin overlying the tibialis anterior and the plantar surface of the foot. Measurements were repeated at intervals post anesthesia.

Vertical ground reaction force peaks corresponding to initial foot contact substantially increased for all subjects during walking in place after anesthesia. Most subjects demonstrated similar increases in ground reaction force at heel-strike during gait post anesthesia. Vertical ground reaction force peaks corresponding to weight shifting ,as single limb support begins, slightly increased after anesthesia. Similar results were recorded in the contralateral limb.

Our results indicate that the motor control of stereotyped movement patterns, such as those occurring in gait, are modified by the reduction of cutaneous receptor afferent discharges. Other observations made in this laboratory suggest that this observed effect may possibly be the result of increased excitability of the extensor motoneuron pool.

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188.5 RECORDING AND ANALYSIS OF THE GAIT-FORCES USING TELEMETRIC FORCE-TRANSDUCERS IN PATIENTS WITH DUCHENNE MUSCULAR DYSTROPHY. E. Toyoshima*[1], Y. Mano*[2], K. Ando*[3], D. Ishihara*[4], and S. Miyazaki*[5]. (SPON: R. F. Mayer[1]). [1]Dept. of Neurol., Univ. of Maryland Sch. of Med. & V.A. Med. Ctr., Baltimore, MD 21201. [2]Dept. of Neurol., Nara Pref. Med. Coll., Nara, JAPAN. [3]National Ctr. for Nerv., Ment. & Musc. Dis., Tokyo, JAPAN. [4]Natl. Sanatorium Higashi-Saitama Hosp., Dept. of Neurol., Hasuda, JAPAN. [5]Tokyo Med. & Dent. Univ., Inst. for Med. & Dent. Eng., Tokyo, JAPAN.

Gait analysis of patients with progressive muscular dystrophy of the Duchenne type (PMD) was serially studied during a 6-month period. Thirteen male patients, aged 8 to 14 years, were studied initially in the ambulatory phase (stages 1-3 of Swinyard, 1957) of the disease. During the period of observation, the disease progressed so that one patient required braces for walking, and 6 became unable to walk due to increased muscle weakness in lower limbs.

The patients' gait was studied while walking on a flat floor 5 meters in length. Foot-forces during walking were recorded continuously using a simple portable device, which consisted of 2 pairs of force-transducers, amplifier-transmitter units and receiver-processor units. The transducers (6x35-55x65-85 mm in size and 35 g in weight for each) were attached to both soles at the metatarsal region and the heels of a pair of shoes.

In the early stage of PMD, the gait foot-forces showed smaller values at the heel compared with those at the metatarsal region, reflecting pes equinus, which was almost not appreciable in the gait foot-prints and neuro-muscular testing. Variations in the gait pattern of foot-forces and right-left difference were observed in patients with similar amounts of muscle weakness and gait dysfunction. Gross fluctuations both in amplitude and duration of the foot-forces during the gait phase (both swing- and supporting-phase) were easily visualized in the superimposed records of the foot-forces at stationary steps. Serial recordings over 6 months revealed the following: further decrease of amplitude of the foot-forces at the heel, prolongation of the duration of the supporting phase and large fluctuations in foot-forces.

Changes in the foot-forces appear to reflect the muscle disability and progression of the disease of patients with PMD. The method and devices used in this study may be helpful in the objective, quantitative and serial assessment of pathological gaits in patients with neuro-muscular disorders. (Supported in part by the Institute for Health and Welfare, Japan, and a grant from the Veterans Administration, U.S.A.).

188.6 AFFERENT PERTURBATIONS DURING CONTROLLED LOCOMOTION IN THE TURTLE P.R. Lennard. Dept. of Biol.,Emory Univ., Atlanta, GA 30322.

The contribution of cutaneous and muscle afferent information to ongoing locomotor pattern production in the turtle was tested during "controlled swimming of a single hindlimb" elicited by electrical stimulation of the spinal cord. During each 10 sec. swimming sequence the electromyographic pattern of up to 12 hindlimb muscles was utilized as an assay of the locomotor output. Perturbations consisted of a single 0.1 msec., near threshold pulse delivered to a cuffed cutaneous or returnstroke muscle-nerve. Such transient electrical stimulation of both cutaneous and muscle-nerves was found to have similar phase dependent effects on the perturbed swim cycle but to have dramatically different influences on the long-term locomotor rhythm.

Analysis of phase-response curves indicates a sensitive period early in the swim cycle (measured from the onset of retraction). During this period, cuff stimulation resulted in a statistically significant shortening of the period of the perturbed cycle due to reduced burst duration in powerstroke muscles and reduced inter-burst durations for returnstroke muscles. A second sensitive period exists late in the cycle. Stimuli applied at this time resulted in an increase in the period of the perturbed cycle. This increase reflects a prolongation of ongoing activity in returnstroke muscles accompanied by a delay in the onset of activity in powerstroke muscles. Stimuli falling within the sensitive periods have no observable effect on the phase relationships between either synergistic or antagonistic muscles. That is, there is no evidence of disruption in the motor output pattern within the perturbed or subsequent locomotor cycles.

Analysis of post-perturbation effects on the swimming rhythm uncovered a major difference between muscle-nerve and cutaneous stimuli. Muscle-nerve stimuli during both sensitive periods lead to a permanent phase shift in the locomotor rhythm. In contrast, cutaneous-nerve stimuli resulted in a temporary phase shift. These data are not consistent with the modeling of a single central pattern generator for locomotion which combines both clock and intracycle pattern generating functions, but can be explained by either of two alternative models of locomotor production: 1) a coupled system composed of a master pacemaker clock or oscillator which entrains a pattern-generating slave oscillator; or 2) a single clock which controls the swimming rhythm and triggers on a cycle-by-cycle basis a non-oscillating pattern generating network.

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188.7 LOCOMOTOR ACTIVITY OF LARVAL AND ADULT FROG SPINAL CORD ELICITED BY EXCITATORY AMINO ACID AGONISTS. A.D. McClellan and P.B. Farel, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27514.

The spinal cords of vertebrates contain neural networks, sometimes called central pattern generators (CPG), which produce the basic features of motor patterns underlying rhythmic behaviors. In the tadpole, spinal CPGs produce the motor patterns underlying swimming movements. As the hindlimbs develop, other motor functions, such as jumping, kicking, stepping, and wiping, come under control of spinal motor networks. As an initial step in comparing the operation of larval and adult motor networks, we asked whether similar motor functions could be elicited from the spinal cords of tadpoles and frogs by the same excitatory amino acid agonists, particularly N-methyl-DL-aspartate (NMDA).

In bullfrog tadpoles (Rana catesbiana, st. V-XV) with a spinal cord transection at the level of the third segment, i.p. injection of NMDA (4.0-15.0 mg/kg) resulted in swimming movements which consisted of lateral undulations that propagated towards the tail and effectively propelled the animal forward. EMG activity during these movements alternated from side to side at the same segmental level and showed a rostro-caudal phase lag. Application of NMDA (0.05-0.1 mM) or D-glutamate (0.5-1.0 mM) to a bath containing an in vitro spinal cord preparation activated alternating bursting in ventral roots, but without a rostro-caudal phase lag. These differences in rostro-caudal phase lag are similar to results obtained in studies of spontaneous motor activity in the tadpole (Stehouwer and Farel, 1980).

In frogs (Rana pipiens) with an acute spinal cord transection between the third spinal roots and the obex, i.p. injection of NMDA (4.0-15.0 mg/kg) activated a general sequence of hindlimb motor functions: spontaneous wiping reflexes, coordinated stepping, and kicking or jumping. The forelimbs, when used during stepping, were coordinated with hindlimb movements. These motor responses were produced by characteristic patterns of EMG activity in hindlimb flexors and extensors. The motor patterns generated by in vitro spinal cords are presently under investigation.

The finding that the same excitatory agents activate swimming in the tadpole as well as rhythmic hindlimb motor activities of the adult hindlimb suggests that some overlap exists in neurons comprising the spinal CPG networks in the tadpole and frog.

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188.8 A SOLID-STATE CAMERA SYSTEM FOR COMPUTERIZED COLLECTION AND ANALYSIS OF HIGH-SPEED MOVEMENT PATTERNS. Chris M. Wieland * and Robert C. Eaton., (SPON: Anne C. Bekoff), Dept. Biol., E.P.O., Univ. of Colorado, Boulder, CO 80309

We have developed a system for the automatic collection and analysis of movement in animals. The system is applicable to a wide variety of experimental studies involving quantitative analysis of behavior patterns. Body or limb positions are recorded at rates up to 600 frames per sec and a digital representation of the image is stored for computer analysis. The system is based on a solid-state image sensor comprised of 10,000 photodiodes laid out in a 100 by 100 square matrix on a single silicon chip (Reticon Corp., Sunnyvale, CA).

This system significantly reduces the time and cost associated with the analysis of motion normally encountered when using conventional high-speed photography. Furthermore, its ability to capture data at high frame rates allows fine temporal resolution of fast movement not available when using video-based systems.

Our current application involves analysis of the C-type startle response in the goldfish. Simultaneous electrophysiological recordings may be obtained from animals while high-speed movement is recorded with the above system. A variety of measures of motor performance of this stereotyped behavior pattern may be generated using a minicomputer (Hewlett-Packard 9845B) programmed for image analysis techniques. Illustrated below are sequential stages in the computer analysis of a single frame showing the goldfish's position 20 msecs after initiation of the startle response behavior. The computer converts the original figure into a single line representing the midline of the fish. The X,Y coordinates of this line are then used for a descriptive analysis of motor performance and its correlation to underlying neural activity.

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LEARNING AND MEMORY: ANATOMY II

189.1 EFFECT OF BILATERAL CEREBELLAR LESIONS ON HEART-RATE AND NICTITATING MEMBRANE/EYELID CONDITIONING IN THE RABBIT. D.G.Lavond*, J.S. Lincoln*, D.A.McCormick* and R.F. Thompson (SPON: K.H. Pribram). Dept. Psych., Stanford Univ., Stanford, CA 94305.

We have reported previously that ipsilateral cerebellar lesions abolish a well-learned nictitating membrane (NM)/eyelid response without affecting the unconditioned response. These same rabbits easily learn with the eye contralateral to the lesion. We proposed that the cerebellum is critically involved in the neural circuit for learned responses. Several lines of evidence suggest that two processes are involved in learning of somatic responses with aversive UCS: the first stage is a temporary emotional or reactive phase (reflected by "conditioned fear", e.g., heart-rate conditioning). The second stage involves learning of the specific adaptive somatic response, which is abolished by cerebellar lesions. The present study was designed to determine the effect of cerebellar lesions on heart-rate conditioning, to determine the effect of bilateral lesions on NM/eyelid conditioning, and to determine the permanency of the abolished NM/eyelid response.

Stereotaxic lesions (N=4) and aspiration (N=1) of the cerebellum were made bilaterally in rabbits who were then allowed 1 week of recovery. Following one day of adaptation to the conditioning chamber the 5 experimental rabbits and 5 control subjects were trained for heart-rate conditioning by pairing a tone (1 KHz, 85 dB SPL, 5 sec duration) offset with a faceshock (2 mA, 500 msec). Both the control rabbits and those with bilateral cerebellar lesions learned cardiac deceleration ($F(3,30)=23.2$, $p<.01$ over blocks of training; $F<1$ between groups and for the interaction).

The following day all rabbits began training on the NM/eyelid response, 4 days of training on the left eye followed by 4 days on the right. Control rabbits learned the response in an average of 138 trials to a criterion of 9 correct responses in 10 consecutive trials. Rabbits with lesions of the cerebellum did not learn on either eye. One rabbit from the experimental group was allowed an additional 5 weeks of recovery and then given 8 more days of training on the NM/eyelid response without learning the habit. An additional rabbit was first trained on the NM/eyelid response on both sides before bilateral aspiration. He did not relearn the response when tested repeatedly over three months.

The present study has shown that bilateral lesions of the cerebellum have no effect upon heart-rate conditioning. NM/eyelid conditioning is permanently abolished. Furthermore, this abolition is not due to an asymetry of functioning caused by the unilateral lesion because bilateral lesions cause the same abolition of conditioned responding.

189.2 EFFECT OF KAINIC ACID LESIONS OF THE CEREBELLAR NUCLEI ON NICTITATING MEMBRANE/EYELID CONDITIONING AND AN HRP DEMONSTRATION OF CEREBELLAR AFFERENTS. J.S. Lincoln*, D.G. Lavond*, T.L. Hembree*, J.K. Thompson* and R.F. Thompson (SPON: M. Stanton). Dept. Psych., Stanford Univ., Stanford, CA 94305.

We have demonstrated recently that unilateral electrolytic or aspirative lesions of the lateral deep cerebellar nuclei prevent and abolish ipsilateral learning of the classically conditioned nictitating membrane (NM)/eyelid response in the rabbit. Learning on the eye contralateral to the lesion is not impaired. There is now an abundance of evidence from lesions (effective versus noneffective locations), recordings of neural units, stimulation, and pharmacology that the region including the medial dentate nucleus and the lateral interpositus nuclei are critically involved in the conditioned response circuit. In order to localize the effect better, we injected kainic acid into the deep cerebellar nuclei of well trained rabbits (kainic acid is presumed to cause less damage to fibers of passage). We hoped that kainic acid lesions would partially alleviate the problem of destroying fibers from the dentate nucleus caused by electrolytic lesions of the interpositus. Following 1 week of recovery, the rabbits were then retrained for 4 days on the ipsilateral eye, 4 days on the contralateral eye, and back 1 day on the eye ipsilateral to the lesion. The animals showed no retention or relearning ipsilateral to the injection but quickly learned on the contralateral eye. Histological analysis of the lesion suggests that the critical site is primarily the interpositus nucleus.

Injections of HRP or wheat germ agglutinized HRP were made into this same location in another group of rabbits. The results agree in terms of afferents previously reported for rabbit and/or for rat, cat and monkey. Retrogradely labeled cells were observed in the spinal and sensory trigeminal nuclei, the pontine nuclei, the reticular tegmental nucleus, the inferior olive, the red nucleus, the cuneate nucleus, the lateral reticular nucleus, the perihypoglossal nuclei, and neurons located throughout portions of the brainstem reticular formation. In our material a few cells were also labeled in the inferior colliculus as were some smaller cells in the superior olive. Interestingly, we also found bilateral labeling of cells in the locus cereleus and many bilaterally labeled cells within the periventricular gray. These data provide information concerning the convergence of putative circuitries for projection of the unconditioned and conditioned stimulus information to the deep cerebellar nuclei, a necessary condition if that is the locus of plasticity responsible for learning.

189.3 LATERAL HYPOTHALAMIC SELF-STIMULATION AFTER LESIONS IN THE ANTERIOR OR LATERAL HYPOTHALAMUS, C. Munoz*, U. Sprick* and J.P. Huston. Inst. of Psychology III, University of Düsseldorf, 4000 Düsseldorf, F.R.G.

The present experiments were performed as part of the investigation of the neural organization of self-stimulation (SS) in the lateral hypothalamus (LH) of rats. First, we examined LH-SS after destruction of LH neurons, but not fibers of passage, with neurotoxins. Bilateral injections of either kainic (1 µg, n=10) or ibotenic (1 µg, n=6) acid destroyed most of the cells in the region of the LH stimulating electrodes, but did not significantly influence SS between 12-24 hrs after the injections. Thus, LH neurons may not be essential for LH-SS.

Next we examined the effects of radiofrequency lesions in the anterior hypothalamic area (AHA) on LH-SS. Bilateral lesions (n=10), which destroyed AHA and damaged the medial preoptic area, ventromedial hypothalamus and anterior lateral hypothalamus, did not significantly attenuate SS between 5 hrs and 7 days post-surgery. However, more severe unilateral lesions (n=11), which caused additional damage to the lateral preoptic area, the diagonal band of Brocca, the thalamus and the posterior LH, led to lateralized deficits in LH-SS. SS in the hemisphere with the lesion was significantly reduced by 80%, but remained intact in the intact hemisphere. Thus, the AHA seems not to be essential for LH-SS. The lateralized effect on SS observed after the unilateral lesions may have been due to damage to structures which were spared in the bilateral lesion group, such as the lateral preoptic area.

189.4 ACQUISITION AND EXTINCTION OF A CONDITIONED TASTE AVERSION IN MICRENCEPHALIC RATS. Joanne Weinberg, Raef Haddad and Ruth Dumas*. Dept. of Anatomy, Univ. of British Columbia, Vancouver, B. C. V6T1W5, Canada; Dept. of Pathology, New York Med. Coll.-Coler Memorial Hosp., Roosevelt Island, N.Y. 10044; New York State Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314.

Micrencephaly can be produced in offspring of pregnant rats by giving a single injection of the neurotoxin methylazoxymethanol acetate (MAM Ac) during pregnancy. In a variety of behavioral tasks these offspring appear hyperactive and exhibit learning or performance deficits under both appetitive and aversive conditions. Recently, it has been suggested that altered emotional activity or arousal may contribute to some of these performance deficits. The present study examined acquisition and extinction of a conditioned taste aversion in micrencephalic rats using a paradigm designed to assess arousal, as well as performance.

Pregnant Long-Evans rats were injected intraperitoneally with 25 mg/kg of MAM Ac in saline (or an equivalent volume of saline alone) on day 15 of gestation (vaginal plug = gestation day 1). Male offspring from both MAM Ac and saline injected animals were tested at six months of age. Animals were given five exposures to a novel milk solution and then injected IP with either 0.4 M lithium chloride (LiCl), 7.5 ml/kg, or physiological saline. Following injection, all animals were food deprived for 72 hours and water deprived for the last 24 hours of the period and then re-exposed to the milk solution. Ad lib access to food and water was then restored and animals were given daily re-exposure to milk for nine consecutive days. Mam Ac and control animals exhibited similar intake of milk during the five exposures prior to injection, as well as on the first re-exposure to milk following injection. However LiCl treated MAM Ac animals showed an increased resistance to extinction. In addition, saline treated MAM Ac animals showed less suppression of intake than the saline treated control animals on the first ad lib re-exposure day. These data suggest changes in response inhibition and responsiveness to environmental cues in the micrencephalic animals which may reflect hippocampal as well as cortical dysfunction.

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189.5 MEDIAL GENICULATE BUT NOT AUDITORY CORTEX LESIONS BLOCK EMOTIONAL RESPONSES TO AUDITORY STIMULI
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We examined whether in rat behavioral and autonomic conditioned emotional responses elicited by acoustic stimuli are based on the neocortical processing of the sensory signal or on afferent information diverging from the auditory pathway at subcortical stations.

Male Sprague-Dawley rats (300-400g) were subjected to either bilateral subpial aspiration of the auditory cortex (ACX, n=19), as defined by neuroanatomical tracing studies (LeDoux et al, Neurosci. Abs., 1983), sham cortical operation (n=14), bilateral electrolytic lesion of the medial geniculate nucleus (MG, n=13), sham lesion of MG (n=7), or lesion of the lateral geniculate nucleus (LG, n=6). Two weeks after surgery, catheters were placed in the carotid artery for computer assisted recording of mean arterial pressure (MAP) and heart rate (HR) and the rats were subjected to aversive classical conditioning (30 trials) involving the pairing of a pure auditory tone (800 Hz, 80 db, 10 sec) conditioned emotional stimulus (CES) with an electric footshock (2.0 mA, 0.5 sec) unconditioned stimulus (US). The next day, conditioned responses were measured. Three days later, unconditioned responses to the US were measured in some animals. The extent of damage was evaluated using standard histological techniques.

Bilateral destruction of the auditory cortex had no effect on the rise in MAP (in mmHg: SHAM, 22±2; ACX, 20±3) and HR (in bpm: SHAM, 19±5; ACX, 14±4) or on the duration of immobilization or "freezing" (in sec: SHAM, 88±9; ACX, 74±9) elicited by the CES. In contrast, lesions of MG, but not LG, impaired the establishment of the MAP (SHAM, 22±3; LG, 21±4; MG, 4±1; $p<.01$), HR (SHAM, 19±3; LG, 30±4; MG, 11±3; $p<.05$) and immobilization (SHAM, 97±10; LG, 87±12; MG, 29±8; $p<.01$) responses. MG lesions did not affect unconditioned responses (MAP: SHAM, 36±5; MG, 36±2; HR: SHAM, 132±20; MG, 99±15).

These findings demonstrate that MG but not ACX lesions disrupt the establishment of autonomic and behavioral conditioned emotional responses to an acoustic stimulus. Since unconditioned responses are not affected by such lesions, the results cannot be due to a disruption of the efferent pathways through which the responses are expressed.

We conclude that MG is a crucial afferent station in the pathway through which the emotional significance of an acoustic stimulus is processed and that the next link in this emotive pathway must involve subcortical structures. Projections of MG to several areas involved in autonomic and somatomotor control (LeDoux et al, Neurosci. Abs., 1983) raises the possibility that these regions constitute subsequent links in the pathway through which autonomic and behavioral responses elicited by acoustic stimuli are regulated. Supported by PHS grant HL 18974.

189.6 A VISUAL PATHWAY THAT MEDIATES FEAR-CONDITIONED ENHANCEMENT OF ACOUSTIC STARTLE. M.D. Tischler* and M. Davis (Spon: M.H. Sheard). Dept. Psychiat., Yale Univ. Sch. Med., New Haven, CT 06508.

The way in which neural systems mediate associative learning represents an area of increasing interest to physiologists, pharmacologists and psychologists. Whether working in simple invertebrate systems or in the intact vertebrate, a necessary first step in this endeavor is to isolate the neural circuit where conditioning occurs. One approach to this problem is to work with a simple reflex that can be modified by prior associative learning. Fear enhanced startle in the rat fulfills these requirements. In this paradigm, startle magnitude is increased when the startle-eliciting stimulus is presented in the presence of a light (CS) that has previously been paired with a shock. Hence, conditioning is expressed through some neural circuit activated by the light which ultimately impinges on the startle circuit. Prior work using electrically elicited startle suggests that the light modulates startle in the ventral nucleus of the lateral lemniscus (Berg and Davis, 1983). The task of this work was to delineate a pathway that might mediate transmission from the eye to the ventral nucleus of the lateral lemniscus.

80 rats received 10 light-shock pairings on two successive days. 72 hrs after the final training session, subjects received lesions directed at the primary visual areas (deep and superficial layers of the superior colliculus, dorsal lateral geniculate nucleus, pretectal nuclei, visual cortex and thalamic reticular nucleus) or at the nuclei of the lateral lemniscus or reticularis pontis caudalis, proposed components of a primary acoustic startle circuit in the rat. Control animals were sham operated. One day later, all animals were tested for startle by presenting noise bursts in the presence or absence of the light. Potentiated startle (the difference between light-noise vs noise-alone trials) was significantly attenuated or eliminated by lesions directed at the dorsal nucleus of the lateral geniculate, deep layers of the superior colliculus, visual cortex, and the posteroventral region of the nucleus of the lateral lemniscus. Lesions directed at pretectal nuclei, superficial layers of the superior colliculus, thalamic reticular nucleus, nucleus reticularis pontis caudalis or dorsal nucleus of the lateral lemniscus did not attenuate potentiated startle. The results suggest that the conditioned stimulus pathway that mediates potentiated startle goes from the retina to the dorsal lateral geniculate nucleus to visual cortex to deep layers of superior colliculus and down to the postero-ventral region of the lateral lemniscus where acoustic startle is modulated.

189.7 MEMORY FOR STIMULUS-REWARD ASSOCIATIONS IN THE MONKEY IS MORE SEVERELY AFFECTED BY AMYGDALECTOMY THAN BY HIPPOCAMPECTOMY. R. R. Phillips*, B. L. Malamut* and M. Mishkin (SPON: D. L. Robinson). Lab. Neuropsychol., NIMH, Bethesda, MD 20205.

The amygdala appears to participate in stimulus-reward associative memory in two ways: coequally with the hippocampus in stimulus recognition; and alone (i.e. without participation of the hippocampus) in the attachment of affective value to the recognised stimulus. However, a critical piece of evidence in support of this conclusion was derived from an associative memory study in which monkeys had to learn a difficult conditional reaction (Spiegler and Mishkin, Behav. Brain Res., 3:303-317, 1981), a factor that may have contributed substantially to the impairment produced by amygdalectomy (Jones and Mishkin, Exp. Neurol., 36:362-377, 1972). To control for this possibility, we compared the effects of amygdaloid and hippocampal lesions on a nonconditional stimulus-reward association task (Gaffan, Learn. and Motiv., 10:419-444, 1979).

Six monkeys were trained preoperatively to choose the positive object of a trial-unique pair, each object in this pair having just been presented separately in acquisition, one with bait and the other without. Both the order of the positive and negative objects in acquisition and their spatial position in the choice test were determined pseudorandomly. Twenty trials were given daily with 10 second intervals between the successive events within a trial and 20 second intervals between trials. On reaching the criterion of 90 correct responses in 100 trials, three monkeys each were given bilateral amygdalectomy (group A) or hippocampectomy (group H), and after a two-week recovery period they were retrained to criterion. The number of objects presented in acquisition was then increased from the original list length of one pair, to list lengths of two and three pairs, each presented to criterion; and, finally, to five and ten pairs, each given for 300 trials. Group A performed more poorly than group H throughout, requiring more trials to attain criterion through list lengths of three pairs (881 trials for group A vs 160 trials for group H), and obtaining lower scores on list lengths of five and ten pairs (78% for group A vs 86% for group H).

Because of the nonconditionality of this task, the results provide unambiguous evidence in support of the proposal that the amygdala plays a more important role than the hippocampus in object-reward associative memory, presumably because of the amygdala's special role in the attachment of reward value to a stimulus.

189.8 OLFACTORY DISCRIMINATION AND REVERSAL LEARNING IN RATS WITH COMBINED HIPPOCAMPUS-AMYGDALA LESIONS. A. G. Kat*, H. Eichenbaum and N. J. Cohen*. Dept. Biology, Wellesley College, Wellesley, MA 02181

Humans and monkeys with combined damage to the hippocampus and amygdala (H-A) are normal in the acquisition of procedures despite a profound impairment for the outcomes of using procedures. To demonstrate this in rats, animals were trained on discrimination problems and reversals. It was expected that H-A rats and controls would similarly improve on successive discriminations during acquisition of the learning set procedure. Normals should take longer to reverse a discrimination, but if H-A rats forget specific material they would perceive the reversed stimuli as novel and might outperform controls.

Five Long-Evans rats with bilateral RF H-A lesions and 6 unoperated controls were trained to 90% correct in a 20 trial block on each of 3 go, no-go odor discrimination problems given on separate days. On the next day the valences of stimuli on the last problem were reversed. Then 3 more discriminations and their reversals were alternated across days.

Both controls and H-A rats rapidly learned the first 3 problems and both groups took many more trials to learn the first reversal and the following discrimination and reversal. Thereafter, both groups rapidly acquired all problems. On no individual problem did the control and H-A group scores differ significantly. However, there was a significant group by problem interaction attributable to patterns of group performance on the discrimination and reversal which followed the first reversal. On that discrimination problem the H-A group performed worse than the control group but improved on reversal. In contrast the controls showed the opposite pattern, i.e. took more trials to reach criterion on the reversal of the problem than on its discrimination.

Unlike the famous patient H.M. whose H-A was surgically removed, H-A rats can discriminate odors. Also their acquisition rate for odor discrimination and reversal is normal. The difference between H-A rats and normals in pattern of reversal learning provides some support for the prediction that reversal would less affect animals with H-A lesions than normals.

189.9 HIPPOCAMPAL AND NEOCORTICAL CONTRIBUTIONS TO SPATIAL LEARNING AND MEMORY. R. J. Sutherland and R. H. Dyck. Dept. of Psychol., Univ. of Lethbridge, Lethbridge, Alberta, Canada, T1K 3M4.

The following series of experiments addresses predictions by the spatial map, working memory, consolidation and cue salience theories of hippocampal (HPC) behavioural functions and examines the similarity of behavioural deficits resulting from damage to structures closely related to the HPC.

Several experiments were conducted using rats navigating to a hidden platform by means of distal visual or auditory cues in a 1.5 m swimming pool. 1. Rats with bilateral damage induced by intraHPC microinjections of 2µg/.5µl of colchicine could not swim directly to the platform and even though they found the platform on every trial (90 sec max., 8 trials/day), on probe trials when the platform location was changed their behaviour was unaffected - rats given several months of prelesion training were similarly impaired. 2. In an attempt to simplify the available navigational cues, the pool was surrounded by black curtains and covered by a one-way glass. Two conspicuous visual beacons were suspended over the water and both the beacons and the initial start point for the rats were in fixed positions relative to the hidden platform. Unlike normal rats, rats with HPC damage could not learn the location of the platform. In another experiment, auditory beacons were substituted, but again HPC damaged rats were impaired. 3. Bilateral septal lesions and bilateral aspiration of posterior neocortex (including areas 17, 18, 18a and retrosplenial cortex), but not mammillary body lesions or aspiration of areas 17 and 18 alone, produce a deficit in place navigation that is similar to the HPC deficit. 4. A comparison was made of performance by rats with bilateral amygdala lesions, bilateral HPC lesions or both in the Morris water task and in conditioned taste aversion learning. Only the HPC and both lesion groups were impaired in place navigation, whereas, only the amygdala and both lesion groups were impaired in learning to avoid a sucrose solution paired with lithium chloride-induced illness. 5. Daily exploration of a complex environment by normal rats and rats with HPC or medial frontal cortex damage was examined. Despite clear differences among groups in the steady state level ofseveral behavioural measures, both lesion groups demonstrated a normal dishabituation of exploration when the environment was rearranged in various ways, including when the position of proximal objects was rotated 180 degrees relative to the distal objects in the room.

The results are consistent with a view that the hippocampus is essential for solving problems that require knowledge of the position of a specific object or event in relation to the configuration of cues contained within map-like representations in association zones of the neocortex.

189.10 ON THE ROLE OF HIPPOCAMPAL CONNECTIONS IN THE PERFORMANCE OF PLACE AND CUE TASKS: COMPARISONS WITH DAMAGE TO HIPPOCAMPUS. L. E. Jarrard, H. Okaichi*, O. Steward and R. Goldschmidt*. Dept. of Psychol., Washington & Lee Univ., Lexington, VA 24450.

A selective impairment on a complex place but not a cue task was recently reported following extensive aspiration lesions of hippocampus and injections of kainic acid into the subiculum and lateral ventricles; following injections of kainic acid into the CA3 cell field performance did not differ from that of controls on either task (Jarrard, Neurosci. Abstr. 8:22, 1982). The present experiment extends this research by employing the same task together with selective damage to structures and/or pathways related to the hippocampus.

Using a within-subjects design, rats were trained before the operations to run on an 8-arm radial maze with a procedure that involves two kinds of learning (place and cue) and two memory functions [reference memory (RM) and working memory (WM)]. In the place version of the task the same 4 arms were baited from trial to trial and room cues (door, rack of cages, overhead lights) remained in the same spatial location. In the cue task 8 removable inserts of different materials were placed in the arms but the location was changed from trial to trial in a random order -- 4 of the 8 cues were consistently baited.

After learning the two tasks, the rats were divided into two control groups (operated and unoperated) and 4 lesion groups. Colchicine was used to damage cells in the dentate gyrus (DG), while lesions of the fimbria-fornix (FF), entorhinal cortex (EC), and mammillary bodies (MB) were made with radio-frequency. Following recovery, the animals were retrained to approach the last 4 arms (and 4 cues) that had been learned before the operations.

Analysis of the behavioral data indicated that performance of rats with FF and EC lesions was impaired overall, with performance on the place task being especially affected. Further analysis of the types of errors made showed that FF and EC animals made more WM errors than the other groups on both place and cue tasks, but more RM errors only on the place task. Even though rats in the DG Group suffered extensive loss of granule cells, there was only a temporary impairment on the place task. Performance of rats with lesions of the MB was like that of controls.

These findings, together with the results of the previous experiment, indicate that (1) unlike the WM impairment found following FF and EC lesions, there is no impairment of memory resulting from direct damage to hippocampus, and (2) cells in the hippocampus (DG and CA3) are not necessary for correct performance of the complex place and cue tasks used in these experiments.

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189.11 LONG-TERM DEFICITS IN ASSOCIATIVE AND SPATIAL RECOGNITION MEMORY AFTER EARLY HIPPOCAMPAL DAMAGE IN MONKEYS. L. Rehbein and H. Mahut (SPON: A. Skavenski). Dept. of Psychol., Northeastern Univer., Boston, MA 02115.

We reported earlier that 8 rhesus macaque monkeys with hippocampal removals sustained at 2 mos of age were impaired postoperatively, compared to 5 normal infants, on a spatial (L-R) discrimination reversal task and on an object discrimination retention task with 24- and 48-hr intervals between successive retention tests. The spatial deficit was no longer present, and the retention deficit became attenuated, on a second re-test, two years later. At 5 yrs of age, operated monkeys performed with normal ease on a task in which discriminations between members of 8 pairs of objects had to be learned concurrently and were not impaired, as a group, on the spatial delayed alternation task, though both tasks are sensitive to hippocampal damage in older monkeys. At the same time, however, they were significantly impaired on a trial-unique delayed non-matching to sample (DNMS) recognition memory task (Rehbein et al., Neurosci. Abstr., 6:8, 1980).

In view of the unimpaired performance on the two cumulative learning tasks but a co-existing deficit on a task in which object quality had to be remembered for not more than 2 min after a single presentation, we re-assessed spatial and visual capacities with the use of DNMS tasks. The same monkeys were tested, at 6 yrs of age, on 1. An object-reward association task in which they had to remember which of two equally familiar objects had been associated with food reward 10 to 130 sec earlier (Gaffan, 1979) and 2. A spatial recognition task in which they had to discern, on every trial, an unfamiliar from a familiar location of one of two identical plaques on an 18-well food tray. Operated monkeys were impaired with 70 and 130 sec delays on the first task, and with all delays, on the second. 3. A recognition task with increasing memory loads in which all previously presented items remained on the 18-well tray and food could be obtained by displacing only new, successively added items (identical plaques or different objects). Operated monkeys retained significantly fewer items than did normal monkeys. In 6 of the 8 operated monkeys there was moderate to extensive damage of inferotemporal cortex, but no correspondence could be found between performance and extent of neocortical damage in the present, and one previous (Mahut et al., J. Neurosci., 2:1214, 1982), experiment.

Thus, selective deficit on one of two classes of tasks points to the existence of two distinct neural systems: One, mnemonic, which depends on the integrity of the hippocampus, the other, a stimulus-response learning system (Hirsh, Behav. Biol., 12:421, 1974) which remains to be identified anatomically.

189.12 COMPARISON OF MEMORY IN RATS AFTER LESIONS IN MEDIAL SEPTAL AREA AND NUC. BASALIS MAGNOCELLULARIS. J. M. Ordy, Pennwalt Corp., Rochester, N.Y. 14623; G. Thomas, U. of Roch., Rochester, N.Y., 14624; W. Dunlap, Tulane U., New Orleans, LA. 70118; and J. Blosser, Pennwalt Corp., Rochester, N.Y. 14623.

Degenerating cholinergic neurons of nucleus basalis of Meynert (nbM) that project to cerebral cortex have been implicated in memory deficits of Alzheimer's disease. Studies with drugs and lesions have indicated an important role for cholinergic septo-hippocampal (s-h) neurons in short-term memory. In contrast to the compact neurons of discrete nbM in man and other primates, homologous cholinergic neurons in rats appear spatially more dispersed along the ventral pallium medial to the substantia innominata. They have been termed nucleus basalis magnocellularis (nbm). The aim of this study was to compare the effects on memory of discrete septal lesions and small bilateral lesions of nbm of rats. The short-term memory test was conducted in a T-maze adapted to present a spatial delayed-nonmatching-to-sample problem. The task included trial-specific memory at 10, 90, and 180 sec. delays. Start, choice, and goal latencies served as indices of drive and neuromuscular performance. Lesions were made electrolytically through a stereotaxically guided electrode, and they were verified microscopically from Nissl-stained sections cut on a Vibratome. Analysis of Variance evaluated pre- and post-operative differences in trial-specific memory and in motor performance. Operated controls did not differ significantly neither in memory nor performance. Rats with bilateral nbm lesions showed no significant post-operative changes in memory at 10-sec. delays, but they were significantly impaired at 90- and 180-sec. delays ($p<0.02$). Rats with medial septal lesions were significantly impaired in memory at all three delays ($p<0.01$). The pre-postoperative differences in motor performance were not significant among the three groups. The results indicated that nbm lesions in rats produced deficits in trial-specific memory, but they were not as severe as those produced by medial septal lesions. Thus, both cholinergic neurons of nbm and of medial septum appear to be involved in short-term memory. The findings are consistent with the reported correlations between recent memory deficits and degeneration of nbM neurons in Alzheimer's disease. Clarification of the effects of lesions on transmitter-specific basal forebrain circuits involved in memory in animal models is an important step in the development of therapeutic approaches to Alzheimer's disease.

189.13 LESIONS IN NUCLEUS BASALIS OF MEYNERT AND MEDIAL SEPTAL AREA OF RATS PRODUCE SIMILAR MEMORY IMPAIRMENTS IN THREE BEHAVIORAL TASKS. D. Hepler*, G. Wenk, and D. Olton. Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218; J. Lehmann and J. Coyle. Dept. of Psychiatry and Behavioral Science, Johns Hopkins School of Medicine, Baltimore, MD. 21205. (SPON: M. Larrabee).

The present investigation evaluates the importance of cholinergic activity in cortical brain regions for learning and memory. Rats received lesions in nucleus basalis of Meynert (nbM), the source of a major cholinergic projection to neocortex, or the medial septal area (MSA), the source of a major projection to hippocampus. Lesions were made with either radiofrequency current or ibotenic acid. Control rats received sham operations. Three behavioral tasks were used to assess changes in learning and memory: acquisition of a delayed-match-to-sample discrimination on a T-maze, retention of a discrimination learned preoperatively on a radial eight arm maze, and acquisition of active and passive avoidance in a two-way shuttle box. In the performance of all three tasks, rats with nbM and MSA lesions showed qualitatively similar changes, relative to the performance of controls ($p < 0.05$): performance was impaired in the delayed-match-to-sample task, the radial arm maze discrimination, and passive avoidance, but was facilitated in two-way active avoidance. Thus damage to cholinergic neurons in both MSA and nbM produced behavioral changes similar to those seen with damage to the hippocampus. Lesion size and location was assessed in nissl-stained histological material. Choline acetyltransferase (ChAT) assays were performed on samples taken from neocortex and hippocampus following the completion of behavioral testing. ChAT activity was decreased by 20% in the medial frontal cortex of rats with nbM lesions, and by 30% in the hippocampus of rats with MSA lesions. Rats with identical lesions sacrificed seven days after surgery had 50-70% ChAT decreases in both brain areas respectively. Thus a considerable recovery of ChAT occured by the end of behavioral testing. These results suggest that cholinergic activity in cortex may have a significant effect on learning and memory in the rat. (Supported by research grant NS18414 from the NINCDS).

189.14 PRODUCTION OF AMNESIA FOR REPRESENTATIONAL MEMORY BY SEPTAL AND CORTICAL LESIONS AND ITS RECOVERY IN RATS. G. J. Thomas and P.S. Spafford* Center for Brain Research, University of Rochester Medical Center, Rochester, New York 14642.

A theoretical distinction was made between dispositional memories and representational memories by supposing, first of all, that memory is a concept (not a datum) that is inferred from discriminative responses. Dispositional memories are indicated when learned discriminations are made between two or more cues that are present to the organism's sensorium at the time of behavioral choice. When successful discriminations are made with the critical cue not present at the time of choice to the animal's sensorium (i.e., the critical cue must be represented by a hypothetical memory trace in the organism's brain from past experience), an instance of representational memory is indicated. A delayed-nonmatching-to-sample task in a T-maze operationalizes this distinction. Considerable preoperative habituation and adaptation are necessary to ensure that the task is yielding relatively "pure" instances of representational memory.

After preoperative adaptation and training, all rats displayed efficient use of the information in sample runs to make correct disciminations on choice runs. The rats were then placed into four matched groups for surgery. Groups consisted of an operated-control group (no brain lesions), a group with posterodorsal septal lesions, a group with prelimbic (area 32) cortical lesions, and a group with both lesions. Lesions were produced electrolytically through a stereotaxically guided electrode. Postoperative testing was precisely the same as preoperative testing. Anatomical characteristics of lesions were determined from Nissl-stained sections cut from celloidin-embedded brains.

Septal lesions and lesions in prelimbic cortex resulted in amnesia for representational memories. The amnesia typically ameliorated as a function of continued reinforced postoperative testing. When lesions were placed in both structures in the same animals, amnesia for representational memories also occurred, and with continued testing, it ameliorated as indicated by group data. However, examination of behavioral scores and lesions in individual rats indicated that when both lesions were adequate, choices remained at chance levels. This observation suggests a permanent amnesia for representational memories.

That the lesion-induced amnesia was restricted to representational memories was indicated by the fact that the rats displayed no evidence of amnesia regarding what to do in the maze. They did not act like naive animals, as they should, if they had forgotten dispositional memories regarding maze performance.

It would appear that very nearly complete ablation of critical brain circuits is necessary to preclude recovery.

189.15 DIFFERENTIAL ENVIRONMENTS AND RADIAL MAZE PERFORMANCE: THE EFFECTS OF ANTERIOR CINGULATE CORTEX LESIONS. Eric Nisenbaum*, Janice Juraska, and Constance Henderson* (SPON: D.M. Schroeder). Dept. of Psychology, Indiana Univ., Bloomington, IN 47405.

Rats reared from weaning in a complex environment often are superior in maze learning in comparison to rats in an isolated environment (reviewed by Greenough in Neural Mechanisms of Learning and Memory). In two separate replications, male and female hooded rats raised in either a complex environment (EC) or an isolated environment (IC) were run on a 17-arm radial maze. EC rats made fewer errors in their first 17 arm choices, more correct arm choices until their first error and fewer total errors than IC rats in both replications. There were no sex differences or sex by environment interactions on any of these measures, in contrast to prior reports of male superiority on mazes (reviewed by Beatty, Horm. Beh., 12:117, 1979). The EC rats chose significantly more arms adjacent to their last arm choice than did IC rats; this adjacent arm strategy may have contributed to the superior performance of the EC rats.

In a second experiment, male EC and IC rats were exposed to the differential environments for a month, either lesioned or sham operated in the anterior cingulate cortex and run on a 17-arm radial maze. The EC rats in general made more correct arm choices to the first error, fewer total errors, and more adjacent arm choices. There also was a significant interaction between the environment and lesion conditions in total errors such that sham-operated EC rats made fewer errors than both their lesioned counterparts and lesioned and sham IC groups. The results imply that the anterior cingulate cortex, with its connections to the hippocampal formation, contributes to the superior performance of the EC rats, not in terms of the adjacent arm strategy but rather their working memory.

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189.16 BEHAVIORAL AND HIPPOCAMPAL DEFICITS DUE TO MILD PERINATAL ZINC DEFICIENCY. E.S. Halas, C.D. Hunt* and H.H. Sandstead*. Department of Psychology, University of North Dakota, and USDA, ARS, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202.

A recent study found that adult rats who were severely zinc deficient (ZD) during lactation suffered impaired learning and working memory. Rats who were undernourished (PF) during lactation were deficient in learning but not working memory. Reference memory was not impaired. In the present study, a total of 162 adult male rats were used. Every litter was reduced to 9 pups 24 hrs after delivery. Every litter contributed at least 1 male to Groups A, B, and C. There were 13 AL, 11 PF, and 11 ZD litters. Fifty-four adult male rats, who suffered mild zinc deficiency (ZD) during gestation and lactation, were divided equally into three groups. Group A were sacrificed at 100 days of age and their hippocampi, cerebellum, and cortex were histologically examined under light and electron microscopy. Group B, starting at 100 days old, were tested for working memory, reference memory, and learning in a 17-arm radial maze. Group C were individually housed in cages until they were 300 days old. Fifty-four adult male rats, who suffered mild undernutrition (PF) during gestation and lactation, were also divided into Groups A, B, and C and given the same experimental procedures as the ZD rats. A control group of 54 adequately nourished (AL) rats were also divided into Groups A, B, and C. The rats in groups B and C were sacrificed at 300 days of age and their hippocampi, cerebellum, and cortex were histologically examined. Because of interactions between age, nutrition, and learning, it was hypothesized that there would be greater anatomical differences between the ZD, PF, and AL rats in Group C than the AL, PF, and ZD rats in Group A and also Group B. Comparing the ZD, PF and AL rats in Group B with their counterparts in Group C will show if learning can compensate for nutritional deficiencies. The anatomical deficits, if any, will be correlated with the behavioral deficits. ZD rats were deficient in both learning and working memory while PF rats suffered only a mild learning deficit. The anatomical data is currently being analyzed. Prior work in our laboratory found both anatomical and behavior deficits due to mild zinc deficiency and/or undernutrition.

189.17 ACQUISITION AND RETENTION OF A RADIAL 8-ARM MAZE TASK BY RATS SUBJECTED TO TRANSIENT FOREBRAIN ISCHEMIA. T. P. Cecere*, H.P. Davis, W. A. Pulsinelli, and B. T. Volpe*. Dept. Psychol., St. John's Univ. and Dept. Neurol., Cornell Univ. Med. Coll., NY, NY 10021.

Neuropathological and behavioral assessments of a possible animal model of hypoxic ischemic injury in humans following stroke or sudden cardio-pulmonary arrest are reported. The acquisition of the "reference" and "working" memory components of a radial 8-arm maze task were impaired in Wistar rats subjected to 30 min of forebrain ischemia by the method of 4- vessel occlusion (Pulsinelli & Brierley, Stroke, 10, 267, 1979) as compared to control rats ($p<0.05$). While rats made ischemic were slower to learn the "reference" aspect of the task (5 of 8 arms baited), they demonstrated performance identical to that of control rats after 60 trials suggesting the importance of repetition in acquisition of this aspect of the task. In contrast, working memory performance by 4-vessel occlusion rats during acquisition trials did not approach the level attained by control rats. Rats trained prior to stroke demonstrated normal retention of the reference component during postoperative trials as compared to controls ($p>0.20$). Postoperative performance by 4-vessel occlusion rats on the working memory aspect of the 8-arm maze task is being assessed and will be reported. Alterations in locomotor activity could not account for performance deficits since control and experimental rats demonstrated equivalent choice time per maze arm. Neuropathologic analysis of 4-vessel occlusion rats showed bilateral destruction of the CA-1 region of hippocampus and bilateral focal infarcts of dorsal caudate nucleus.

189.18 THE EFFECTS OF LOCAL COOLING OF INFEROTEMPORAL CORTEX ON THE PERFORMANCE OF VISUAL TASKS. J.A. Horel, Dept. of Anatomy, Upstate Medical Center, Syracuse, New York 13210.

Three monkeys (Macaca fascicularis) were trained on an automated delayed match to sample (DMS). The animals were restrained in a chair facing three rear projection screens. Seven hundred forty colored photographs of objects were used as stimuli and were rear projected onto the screens. The sample appeared at the center screen and the match and non-match at the two side screens. Delays between presentation of the sample and the occurrence of the match were 0, 15, 30 or 45 sec., presented in random order. Correct performance was rewarded with a squirt of juice to the mouth. Cooling devices were then implanted bilaterally over the inferotemporal gyri for making localized reversible functional lesions while the animal performed the DMS. These were 10 x 3 mm oblong loops of stainless steel tubing. They were assembled into an array of four, each one 3 mm apart and placed outside the dura over IT so that each one would cool about 1/4 of IT. We call the most anterior #1 and the most posterior #4. Cooled methanol was pumped through the tubing and temperature was monitored by thermocouples placed on the tubes. On experimental trials this was set at 0°C. Blocks of 20 trials were run in which one of the four pair of probes was cooled bilaterally or control blocks were run with no cooling. These blocks were randomly intermixed. One hundred twenty trials were run per day, one block for each of the four probes and two control blocks. The animals were run for 4 days. There was a significant deficit produced by cooling probes 1, 2 and 3 but not 4. There was no significant interaction with delay. In contrast to previous findings of the same cooling in the temporal pole, (Horel et al, Neuroscience Abstracts, 1982) the curves were flat, showing no drop off at the longer delays. At the anterior probes, average performance was between 70-81% correct; on probe 4, performance was 86-96% correct and 80-98% correct on controls. There was improvement in performance over the 4 days of testing. The animals were then trained on a horizontal vertical stripe discrimination and tested for retention during cooling. No deficit was found from any of the probes. However, a deficit was found when the anterior probes were cooled during learning of object discriminations, more from probe 2 than 1 or 3 and not at all from probe 4. Thus, we were able to reproduce some, but not all of the effects of tissue removal lesions with small reversible cold lesions. Visual learning was more readily disrupted than was retention.

(Supported by NINCDS Grant NS 18291)

189.19 EFFECTS OF COOLING ORBITAL AND INFERIOR LATERAL PREFRONTAL CORTEX ON LEARNING AND RETENTION OF VISUAL DISCRIMINATIONS AND THEIR REVERSALS. Mary Lou Voytko. Dept. of Anatomy, Upstate Medical Center, Syracuse, NY 13210.

Cooling the temporal pole disrupts the ability of monkeys to learn visual discriminations (Voytko et al., Neuroscience Abstracts, 1982). The temporal pole has reciprocal connections with the orbital and inferior lateral surface of the frontal lobe. In this study, we explore the ability of monkeys to learn visual discriminations and perform the reversal discriminations while cooling these frontal areas.

Implanted loops of stainless steel tubing covered the orbital and inferior lateral prefrontal cortex. During the experimental trials, the temperature of the loop was set at $0^{o}C$. The subjects faced 3 rear projection screens. A white light, projected to the center screen started a trial, and a response to it extinguished the light and exposed the discriminative stimuli on the two side screens. Randomly paired photographs of objects were used as stimuli and the correct stimulus alternated sides randomly.

The ability of the animals to learn a visual discrimination while cooling the orbital and inferior lateral cortex, was severely disrupted. Once the task was learned however, the animals showed excellent retention of the discrimination the next day when the cold was again applied. On the third test day, the animals were trained without the cold on the reversal of the previously learned task. In the first 20 trials, the animals performed below chance levels, but soon thereafter performed at a high level. They showed good retention of the reversal discrimination the next day, but on the fourth test day, with the cold applied, the animals' retention of the reversal discrimination was below control levels. This procedure was repeated for 3 more pairs of photographs.

The below chance performance levels in the initial trials of acquiring the reversal discrimination shows that at least some of the task that was learned under cold was retained when tested without the cold. Thus, cooling the orbital and inferior lateral prefrontal cortex severely affects the ability to learn visual discriminations, but does not affect their retention under the cold, if they also are learned under cold. Cooling these frontal areas disrupts the retention of reversal visual discriminations learned before the cold is applied. (Supported by NINCDS NS18291)

189.20 COMPARISON OF PERFORMANCE ON A PIAGETIAN OBJECT PERMANENCE TASK IN HUMAN INFANTS AND RHESUS MONKEYS: EVIDENCE FOR INVOLVEMENT OF PREFRONTAL CORTEX. A. Diamond and P. Goldman-Rakic, Sect. of Neuroanatomy, Yale Sch. of Med., New Haven, Conn. 06510.

One of the classic tests of cognitive change during the first year of life is Piaget's $A\bar{B}$ Stage IV Object Permanence Test. $A\bar{B}$ is very similar to Delayed Response (DR), the task most firmly linked to the prefrontal cortex in nonhuman primates. In both tasks the subject watches as a reward is hidden at one of two identical wells, a delay follows, then the subject is allowed to reach. The performance of human infants below 12 months on $A\bar{B}$ matches that of prefrontally operated monkeys on DR, suggesting that developmental changes in prefrontal cortex may be related to the improvement on $A\bar{B}$ seen between 7-8 months and one year in children.

To explore the link between the prefrontal cortex and $A\bar{B}$ more directly, the present study investigated $A\bar{B}$ in normal infants and, for the first time, in operated and unoperated rhesus monkeys. It is important that the same task was used because $A\bar{B}$ and DR are not identical. (In DR, side of hiding is varied randomly across trials; in $A\bar{B}$, side of hiding remains constant until the subject is correct, then it is reversed.) Subjects were 25 full-term infants, tested every 2 weeks throughout the second half of the first year, and 5 rhesus monkeys (Macaca Mulatta, age 2-4 years): 2 with bilateral lesions of dorsolateral prefrontal cortex including the principal sulcus, 2 unoperated, and 1 with a bilateral parietal ablation. Monkeys were tested in the WGTA with care taken to make the procedures as identical to those used with children as possible. Reversal trials were administered following correct reaches on 2 consecutive trials.

When infants began to search for hidden objects at 7-8 months of age, they made a characteristic set of errors on $A\bar{B}$, even with delays of only 1 or 2 seconds. By 12 months they did not err with delays as long as 10 seconds. Like the younger infants, prefrontally operated monkeys failed $A\bar{B}$ at delays of 2 seconds; while unoperated, and parietally operated, monkeys performed perfectly. Moreover, the pattern of errors displayed by monkeys with prefrontal lesions was identical to that seen in infants. When side of hiding was repeated after a correct reach, both human infants and prefrontally operated monkeys performed correctly. When side of hiding was reversed, however, infants and prefrontal monkeys were impaired and repeated the same error over consecutive trials.

These findings are an important demonstration of a link between the prefrontal cortex and a test sensitive to developmental changes in the cognitive ability of the human infant. (Supported by NSF BNS-8013-447, HD-10094, MH-00298, MH-38456, and grants from the Danforth and Sloan Foundations.)

LEARNING AND MEMORY: PHYSIOLOGY

190.1 FURTHER STUDIES OF THE INVOLVEMENT OF LOCUS COERULEUS IN PLASTICITY OF AVIAN LATERAL GENICULATE NEURONS DURING LEARNING. C.M. Gibbs, J.L. Broyles* and D.H. Cohen. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

We previously reported (Neurosci. Abstr. 7: 752) that during visually conditioned heart rate change in the pigeon a population of neurons in the avian equivalent of the dorsal lateral geniculate (LGN_e) shows enhancement of its CS-evoked activity. This population consists of those LGN_e cells showing decreased (Type II) discharge to foot-shock, the US. In contrast, cells responding with increased (Type I) discharge or which are unresponsive show attenuation, as do cells during nonassociative training. More recently, we described findings suggesting that locus coeruleus (LC) mediates the Type II response and thus may be necessary for plasticity during learning (Neurosci. Abstr. 8: 666). We now describe studies showing that: (a) cells in both LC proper and subcoeruleus (SC) may be antidromically activated by LGN_e stimulation; (b) stimulation of these structures affects the discharge of most LGN_e cells; and (c) pairing such stimulation with whole-field illumination (CS) may serve as an effective US for inducing plastic change in LGN_e neurons.

Acute electrophysiological experiments demonstrated that LGN_e stimulation antidromically activates some cells in both LC and SC, confirming earlier anatomical data. These cells have slowly conducting axons (0.5-1.2m/sec), consistent with mammalian findings. Moreover, we extended our earlier findings regarding the US-responsiveness of LC neurons by showing that 62% of the SC cells respond with increased discharge to foot-shock. Thus, the cells of origin of the coeruleus projection to the LGN_e appear to be distributed throughout the complex. In another series of studies we found that electrical stimulation of LC and SC affects the discharge of most LGN_e cells. These stimulation effects are quite similar to those produced by foot-shock (Neurosci. Abstr. 8: 666).

Given this, we initiated studies to determine whether coeruleus stimulation may serve as an effective US for inducing changes in the light-evoked activity of LGN_e cells. Our preliminary studies involved single cell recordings during a conditioning paradigm in which whole-field illumination is repeatedly paired with coeruleus stimulation. The results to date are strikingly consistent with our earlier findings using foot-shock as a US; the CS-evoked discharge of LGN_e cells with Type II discharge to LC stimulation showed rapid modification during training, while cells with Type I discharge to stimulation showed response attenuation. Thus, electrical activation of the coeruleus complex constitutes a sufficient US for training-induced modification of LGN_e cells. This provides an opportunity to develop a thalamic slice as an in vitro "analog model" for associative learning at a vertebrate synapse. (Supported by NSF grants BNS8016396 (DHC) and NIMH Fellowship 086001 (CMG).)

190.2 DISRUPTION OF PERIPHERALLY INDUCED SPINAL FIXATION IN RATS BY A STRONG ELECTRICAL STIMULUS. J. E. Steinmetz*, D. Molea*, and M. M. Patterson (SPON: L. Edds). College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

Peripherally induced spinal fixation is a long term persistence of hindlimb flexion that follows appropriate periods of thigh skin stimulation. The present study examined potential similarities between fixation and hypothesized memory consolidation processes underlying information storage in the brains of intact organisms. It was hypothesized that if fixation resembles intact memory consolidation, the fixation process should be susceptible to retrograde disruption by a strong electrical shock in a manner similar to the retrograde disruption of intact memory often seen with application of electroconvulsive shock (ECS).

Forty-eight anesthetized (pentobarbital, 50 mg/kg, ip) rats received 30 min of thigh skin stimulation (2 mA, 100 pps, 7 msec) after spinal cord section. Flexion present 5 min and 40 min subsequent to stimulation offset was recorded by suspending weights from the flexed limb. In addition to this common treatment, rats were randomly assigned to six groups (ns = 8) that differed as to when a strong electrical stimulus (10 mA, 500 msec) was applied to the spinal cord at L-1. Spinal shock was given either before stimulation, 15 min after stimulation onset, 10 sec after stimulation offset, 20 min after stimulation offset, or 35 min after stimulation offset. A sixth group served as a control and received no spinal shock. Results of this study showed a significant disruption of stimulation induced flexion only when spinal shock was delivered 10 sec after stimulation offset. Furthermore, loss of flexion was apparently permanent since flexion was not observed in this group 40 min after stimulation. Spinal shock applied at other times failed to affect the poststimulation retention of flexion.

The retrograde effect of spinal shock suggests that spinal alterations are not completely "consolidated" at the offset of stimulation. Moreover, spinal shock seems to disrupt the fixation process in a manner that resembles ECS disruption of intact memory processes. These data support a "consolidation theory" explanation of peripherally induced spinal fixation.

190.3 CLASSICAL CONDITIONING OF THE FLEXION REFLEX: ACUTE VS. CHRONIC SPINAL CAT. S. Onifer* and R.G. Durkovic. (Spon: S. Nord) Dept. Physiol., Upstate Med. Ctr., Syracuse, NY 13210

Classical conditioning of the flexion reflex was investigated in acute, 2 week and 3 month chronic spinal cats. Experimental techniques were the same as those detailed in Physiol. & Behav. 14:297 (1975). Briefly, the left hind limb of cats with T-10 spinal transections made 3 months, 2 weeks or just before decerebration was anchored with a femur pin and foot clamp. The distal tendon of the tibialis anterior (TA) muscle was cut and connected to a transducer for monitoring reflex tension. The classical conditioning paradigm involved 30 pairings of the CS (10 Hz stimulation of the saphenous nerve for 1.5 sec) with the US (30 Hz stimulation of the cutaneous superficial peroneal n. for 0.5 sec) using a 1.0 sec interstimulus interval. Intertrial intervals were 1 minute in duration. Sensitization control animals received the same number of CS and US presentations over the same time span, but the US was presented 30 sec after each CS presentation (exclusively unpaired).

For all animals the response measured was the maximum tension from the TA muscle during the first second of each CS presentation. Reflex responses during acquisition were compared to baseline control values (mean of five CS alone trials) obtained just before acquisition.

In acute spinal animals the conditioning paradigm resulted in a rapid increase in flexion reflex amplitude in response to the CS, while the responses from sensitization control animals remained near baseline levels. A significant difference between conditioning and sensitization 30 trial group means was obtained ($p<.01$). In 2 week chronic spinal animals no facilitation was observed as the reflex response to the CS declined over trials in both conditioning and sensitization control animals at equal rates. In 3 month chronic spinal animals the magnitude of the reflex in conditioning animals remained near baseline levels over trials while that of sensitization control animals declined. Differences between 30 trial means of 3 month chronic conditioning animals were significantly greater than those of the sensitization animals ($p<.05$).

The results show that the ability to demonstrate classically conditioned responses from the spinal cord can depend upon time after transection. This may help to explain the failure to observe conditioning in chronic spinal animals in earlier studies of other investigators.

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190.4 RETENTION OF CLASSICALLY CONDITIONED FLEXION REFLEX FACILITATION IN SPINAL CAT. R.G. Durkovic, Dept. of Physiology, Upstate Medical Ctr., Syracuse, NY 13210.

To investigate the retention (duration) of classically conditioned flexion reflex facilitation in acute spinal cat, conditioned stimulus (CS) trials were presented for a 2 1/2 hour period following conditioning or sensitization paradigms. Using decerebrate, T-10 spinal cats, conditioning involved 30 pairings of the CS (10 Hz stimulation of the saphenous nerve for 1.5 sec) with the US (30 Hz stimulation of the cutaneous superficial peroneal n. for 0.5 sec), using a 1 sec interstimulus interval. Random intertrial intervals averaged 3 min. Sensitization animals received the same number of CS and US presentations, over the same time span, but the US was presented in the middle of the interval between CS presentations. Flexion reflex responses to the CS and US were measured by a tension transducer attached to the distal tendon of the tibialis anterior muscle. CS and US cutaneous nerve recordings were monitored to assure consistent inputs to the spinal cord over the course of the experiment. Aα and Aδ cutaneous fibers of these nerves were activated during stimulation.

For all animals the response measured was the maximum tension from the TA muscle during the first second of each CS presentation. Reflex responses during acquisition were compared to baseline control values (mean of three CS alone trials) obtained just before acquisition.

During paired CS-US presentations, the reflex response to the CS increased rapidly over trials compared to preacquisition levels. In contrast, the responses to the CS in sensitization animals declined in magnitude.

For retention analysis 30 CS alone trials were presented at 5 minute intervals following acquisition. Over the 2 1/2 hour period significant differences between conditioning and sensitization groups were maintained even to the last trial. The results show that a classical conditioning paradigm applied to the spinal cord induces reflex changes of long duration, a result consistent with the characteristics of a learned response.

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190.5 EYEBALL RETRACTION LATENCY IN CONSCIOUS RABBIT MEASURED WITH A NEW TECHNIQUE. K.J. Quinn, P.R. Kennedy, C. Weiss and J.F. Disterhoft. Department of Cell Biology and Anatomy, Northwestern University Medical School, Chicago, IL 60611.

Classical conditioning of rabbit nictitating membrane (NM) extension has been widely used in recent years as a model system for investigations into the neural basis of mammalian associative learning. NM extension is a passive consequence of eyeball retraction (Cegavske, et al, 1976). Thus eye retraction is the primary behavioral event being conditioned. We have been doing single neuron recording and lesion studies of the final output motoneurons in accessory abducens nucleus. For this work it was necessary to have a direct, readily quantifiable measure of the primary behavioral response. We have developed a new, sensitive technique for direct measurement of eye retraction and used it to measure the latency of eye retraction and to demonstrate classical conditioning of the response.

The detector circuit is made up of an infrared LED, a photodiode, and the appropriate amplification components. The interface attached to the eyeball consists of a contact lens connected by thin tubing to a film strip. The film strip has a linear light intensity grating exposed on it. The contact lens is placed on the rabbit's eye and the film strip in a track between the photodiode and LED. Thus, the detection circuit monitors variation in intensity of the LED transmitted light caused by movement of the film strip grating. The detection circuit output voltage is linear with film travel. The device is well tolerated and the baseline is sufficiently stable to reliably record eye retraction movements of .05 mm in the conscious rabbit.

Average eye retraction latency to stimulation with .1 ms, 200 Hz pulse trains of the abducens nerve (where almost all retractor bulbi axons run) in 5 rabbits was 4.8 ms (S.D. = 0.6 ms). Average retraction latency to periocular stimulation with .1 ms, 200 Hz, 10 ma pulse trains was 9.5 ms (S.D =2.0 ms). Thus the central conduction time of the response - the time from periocular shock onset to a wave of VI nerve axonal firing - was 4.7 ms, on average. Berthier and Moore (1983) showed accessory abducens unit latencies of 4 ms to periocular shock. We found peak antidromic field potentials in accessory abducens to VI nerve stimulation of .8 ms (Quinn, et al, 1982). The central conduction value of 4.7 ms we measured with our eye retraction transducer matches the 4.8 ms predicted value nicely.

We have successfully used this technique to monitor classical conditioning of eyeball retraction using a white noise CS and an airpuff US aimed at the eyelids. The rabbits acquired eye retraction CRs in one to three 80 trial sessions.

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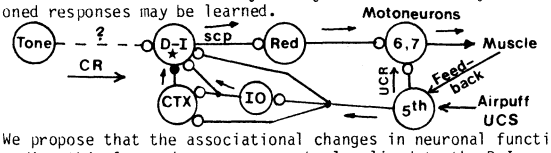
190.6 EVENT-RELATED POTENTIALS REFLECT STIMULUS SIGNIFICANCE DURING DISCRIMINATIVE NM CONDITIONING IN THE RABBIT. D.J. Weisz, G. McCarthy*, C.C. Wood, and D.T. Thompson*. Departments of Psychology and Neurology, Yale University, New Haven, CT 06520, and VA Medical Center, West Haven, CT 06516.

Studies in both humans and animals have demonstrated that event-related potentials (ERPs) are sensitive to the significance or signal value of a stimulus within a task. Knowledge of the neural generators of task sensitive ERP components may contribute to an understanding of what brain systems and structures are involved in learning and attention. We investigated ERPs elicited during two-tone discriminative conditioning of the nictitating membrane (NM) response in the rabbit. There were two experimental phases: in the initial tones alone phase nonreinforced tones were presented, while in the second phase discrimination training occurred. The stimuli in both phases were 1000 Hz and 7000 Hz (85 dB) tones presented in random order. During discrimination training one of the tones served as CS+ and the other as CS-, with tone significance balanced across animals. Tone duration was 850 msec with the CS+ tone co-terminating with a 100 msec air puff to the eye. Intertrial intervals were 20 to 40 seconds. Electrodes were chronically implanted and targeted for cortical, hippocampal, and subcortical sites. Average ERPs for both tones in both experimental phases were sampled at 500 Hz for a 1024 msec epoch beginning 100 msec prior to tone onset.

During the tones alone phase, comparable ERPs were evoked by high and low frequency tones. During the first day of discriminative conditioning marked differences appeared between ERPs elicited by the CS+ and CS- tones with the CS+ eliciting larger potentials than those elicited by the CS-. With further training ERPs to CS- presentation diminished greatly in amplitude. Differences in ERPs elicited by CS+ and CS- trials were most pronounced for potentials with approximate latencies of 80-100 msec from tone onset. Potentials showing such stimulus significance effects were recorded from many cortical and subcortical sites, although steep spatial gradients were only seen in the electrodes targeted for the hippocampus. A later slow potential (latency of 250-300 msec) was less frequently seen, but did appear sensitive to stimulus significance with larger potentials elicited by CS+ tones.

190.7 POSSIBLE NEURONAL SUBSTRATE OF CLASSICAL CONDITIONING WITHIN THE MAMMALIAN CNS: DENTATE AND INTERPOSITUS NUCLEI. David A. McCormick† and Richard F. Thompson° †Dept. Neurology, °Dept. Psychology, Stanford Univeristy, Stanford, CA 94305

Classical conditioning of the eyeblink response in the rabbit was achieved by pairing an auditory conditioned stimulus with a corneal airpuff unconditioned stimulus and used as a paradigm to investigate the neuronal structures underlying this basic form of associative learning in the mammal. Previous investigators have shown that no neural tissue above the level of the thalamus is necessary for the learning and/or retention of this response. We have found that: 1. Lesions of the lateral cerebellum, medial dentate-lateral interpositus (D-I) nuclei, or the superior cerebellar peduncle (SCP) all permanently abolish the learned eyeblink response without effecting the reflexive eyeblink response or the ability of the animal to learn with the contralateral eyelids; 2. Lesions of any region of the cerebellar cortex (flocculus not tested), lateral dentate nucleus, and fastigial nuclei do not permanently abolish the learned eyeblink response; 3. Lesions of the rostromedial inferior olivary complex, which receives a direct projection from the spinal fifth sensory nuclei and projects to the critical region of the D-I nuclei, prevent the maintained performance of the conditioned response; 4. Recordings from the D-I nuclei have revealed increases in neuronal activity which parallels the learning of the eyeblink response (r=.90); 5. Stimulation, either before or after learning, of the D-I nuclei can cause an eyeblink response to occur; 6. The D-I nuclei display neuronal responses to auditory stimuli and to the somatosensory components of the UCS; 7. Neuronal recordings from throughout the brainstem have revealed that the red nucleus, pontine nuclei, and inferior olive, respond in relation to the performance of the learned eyeblink response. Therefore we propose the following neuronal circuit as a model system by which classically conditioned responses may be learned.



We propose that the associational changes in neuronal function encoding this learned response may be localized to the D-I nuclei (star in diagram). Alternatively, the changes may occur afferent to the D-I nuclei with the D-I nuclei forming an essential efferent for generation of the response. Supported by NIH fellowship # 1-F31-MH08673-01 to DAM and NSF grant # BNS-8106648 and ONR contract # N00014-83-K-0238 to RFT.

190.8 EFFECTS OF CONTRALATERAL RED NUCLEAR LESIONS ON RETENTION OF THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE/EYELID RESPONSE. D. A. Haley*, D. G. Lavond* and R. F. Thompson (SPON: Roy King). Department of Psychology, Stanford University, Stanford, CA 94305.

Recent studies have shown that lesions of the ipsilateral dentate-interpositus nuclear region of the cerebellum abolish the conditioned response of rabbits well-trained in short-delay classical conditioning of the nictitating membrane (NM)/eyelid response (McCormick, D. A., et. al., PNAS, 79:2731, 1982; Clark, G. A., et. al., Neurosci. Absts., 8: 22, 1982). The current study investigates the role of the red nucleus, a structure receiving major efferent projections from the cerebellar deep nuclei, in retention of the classically conditioned NM/eyelid response. New Zealand white rabbits were implanted with monopolar lesion electrodes and allowed 5 days of postoperative recovery. Animals were then trained on the eye contralateral to the chronically implanted electrodes using standard procedures for classical conditioning of the NM/eyelid response (117 trials per day). Animals reaching a criterion of 8 conditioned responses on 9 consecutive trials were overtrained for one additional day and then electrolytically lesioned. We here report that lesions of the contralateral red nucleus also abolish the conditioned NM/eyelid response. These results are in agreement with previous reports on the effects of rubral lesions on the conditioned forelimb flexion response in the cat (Smith, A. M., Physiol. Behav., 5:1121, 1970). In addition, such lesions of the red nucleus produce varying degrees of disruption of the unconditioned response and various motor impairments. In at least some animals, the unconditioned response shows recovery over postoperative training, but the conditioned response does not. In contrast, the eye ipsilateral to the rubral lesion can be trained to perform conditioned NM/eyelid responses. These results, along with converging pharmacological evidence (Madden, J. IV, Haley, D. A., Barchas, J. D. and Thompson, R. F., Neurosci. Absts., 1983), suggest that the contralateral red nucleus is necessary for the performance of the conditioned NM/eyelid response.

190.9 BIDIRECTIONAL ASYMMETRICAL SELECTION FOR BEHAVIORAL AROUSAL TO NOVEL ENVIRONMENT: NAPLES HIGH (NHE) AND LOW EXCITABLE (NLE) RAT STRAINS. A.G.Sadile, A.Cerbone*, G.Manzi*, A.Grimaldi*. Inst.Human Physiol., 1st Med.Sch., Univ.Naples, Italy.

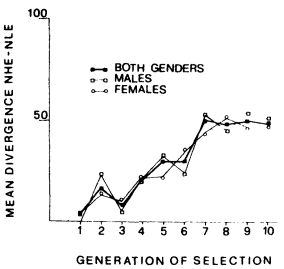
By continuous genetic pressure, two strains of rats have been obtained from a random-bred Sprague-Dawley population, the Naples High (NSD-HE or NHE) and Low Excitable (NSD-LE or NLE), since 1976 over 15 generations.

The behavioral trait selected hereto was reactive horizontal and vertical activity (rearings+corner crossings) during two 10min "forced" exposures to a modified Lat's box, at 24hr interval, at the beginning of dark phase of a 12:12LD cycle (Sadile,A.G. et al., Abh.Akad.Wiss.DDR, 5:303, 1978). Population's extremes within high and low scoring samples were mated, with great care paid to keep low the inbreeding coefficient a) to increase potentiality of selection; and b) to keep high "fitness" throughout generations.

After six generations, there appeared no population overlap between high (NHE) and low scoring (NLE) rats, with NHE > NLE by a factor of two. Ever since, the NHE's stabilized at high level ("ceiling effect"), whereas the NLE's have still to reach the "floor". While differing in reactivity to novel environment, the two strains show similar daily and day-night variations in spontaneous activity (Sadile, Tobler and Borbely, in preparation).

A series of electrophysiological, neurochemical and neuroanatomical correlative studies suggest a "dis-integration" at the hippocampal dentate area interface in both strains.

The NHE and NLE rats are proposed as new genetic models to study the role of non-associative processes in behavioral plasticity.



190.10 THE EFFECT OF CAROTID OCCLUSION ON THE PASSIVE AVOIDANCE RESPONSE IN THE F-344 RAT. G. A. King. Dept. of Pharmacology, Ayerst Res. Labs., P.O. Box 6115, Montreal, Que., Canada H3C 3J1.

Preliminary results obtained in a new rodent model of cerebral ischemia are described. Young male Fisher (F-344) rats were implanted under pentobarbital anesthesia with loose-fitting ligatures around both carotid arteries. Several days post-operatively the ligatures were tightened for varying durations - without the need for anesthesia - in order to block carotid blood flow. The ligatures were removed at the end of the occlusion period. Animals which survived the procedure were trained in a one trial step-through passive avoidance paradigm either 24 or 72 hr post-occlusion. Prior to sacrifice, the carotid arteries were inspected to insure that blood flow has been reestablished post-occlusion. Rats were sacrificed by intracardiac perfusion of fixative, under deep pentobarbital anesthesia and brains processed for histology.

In the F-344 rats an increase in the duration of carotid occlusion resulted in an increase in mortality. Within the first 72-hr post-occlusion period, 0, 39 and 66% of rats died after 30, 60 and 90 min of occlusion, respectively. Furthermore, rats occluded for > 60 min were significantly impaired in the retention of a passive avoidance response, compared to sham controls, if training was given 24 hr post-occlusion and retention tested 24 hr after training. No impairment was observed if training was given 72 hr post-occlusion or if retention was tested 1 hr after training. Therefore, carotid occlusion may cause a temporary deficit in long-term memory.

These experiments demonstrate that bilateral carotid occlusion in the F-344 rat can cause cerebral ischemia which results in increased mortality and failure of memory encoding processes in surviving animals.

190.11 AREA POSTREMA LESIONS ABOLISH LOW-INTENSITY GAMMA RADIATION INDUCED TASTE AVERSIONS IN RATS. K.-P. Ossenkopp and L. Giugno*. Dept. of Psychology, Univ. of Western Ontario, London, Ontario, Canada, N6A 5C2.

Ionizing radiation is very effective in producing conditioned taste aversions (CTA) when paired with novel taste stimuli in rats. In a recent study Ossenkopp (Behav. Brain Res., 1983, 7, 297) demonstrated that area postrema (AP) lesions in rats attenuate the magnitude of gamma-radiation induced CTA when 200 rad of radiation was paired with a novel saccharin taste stimulus. In the present experiment we examined the possibility that AP lesions might abolish the CTA induced by low levels of gamma radiation. Previous research (Cairnie & Leach, Pharm., Biochem. Behav., 1982, 17, 305) had shown that as little as 20 rad of radiation could produce a CTA if multiple pairings of the CS and US occurred. In the present study six groups of male rats were used. Three groups of rats received lesions of the AP and the other three groups received sham lesions. Following a recovery period all rats were adapted to a 23.5 hr/day water deprivation schedule. A sodium saccharin solution (0.1%) was offered to all rats during the regular drinking period (½ hr) on two days per week, with water available on the other days. Presentation of the saccharin was followed by exposure to 0-, 20- or 40-rad of gamma radiation, with one lesioned and one sham lesioned group exposed at each dose level. Four pairings of saccharin with the respective dose of radiation were then followed by adaptation to a 23 hr/day water deprivation schedule. All animals then received a two-bottle choice test (water vs. saccharin) for 1 hr/day over the next six days. Saccharin intake levels on conditioning days showed that sham lesioned rats given 20- or 40-rad radiation, developed a strong CTA to the saccharin ($p<.01$) that was dependent on the radiation dose. The sham lesioned rats exposed to 0-rad and all the AP lesioned rats displayed comparable increases in saccharin intake levels over conditioning days. Similarly, the sham lesioned rats receiving 20 and 40 rad radiation displayed very low levels of saccharin preference on the two-bottle choice test ($p<.01$), whereas the 0-rad and all the AP lesioned rats displayed strong comparable preferences for saccharin. Thus, AP lesions completely abolished the CTA normally induced by multiple pairings of saccharin with 20 or 40 rad gamma radiation.

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190.12 THE EFFECTS OF KINDLING ON THE ACQUISITION OF CONDITIONED TASTE AVERSIONS (CTA) IN RATS. F. G. Freeman and P. J. Mikulka*. Dept. of Psychology, Old Dominion Univ., Norfolk, Va. 23508.

The amygdala appears to be an important area for the development of CTA. This study examined the effects of amygdala kindled seizures on the acquisition of taste and odor aversions and was prompted by the prior work with ECS an CTA. The subjects were Long Evans rats that were kindled in the amygdala until a minimum of 10 stage V seizures had been produced.

In Experiment 1 all subjects were given 15 min. access to a 10% sucrose solution followed 30 min. later by a 2.5 mEq injection of LiCl. Subjects in Group A were kindled 15 min. after the sucrose solution access. Group B subjects were kindled 15 min. after the LiCl injection and the Group C subjects were not kindled. After two days of recovery all subjects were allowed 15 min. access to the sucrose solution. Analysis of these intakes indicated that the Group C subjects drank little sucrose (2.2ml) while the Group A and B subjects drank significantly more (11.1 and 10.8 mls, respectively) $F(2,16)= 7.5$, $p < .01$). Thus, kindling blocked the acquisition of CTA whether it occurred within the CS-US interval or after the occurrence of the US.

Experiment 2 was designed to examine the effects of cue overshadowing/potentiation of a CTA. All subjects received access to $odor_1$ cued solution or a taste-$odor_2$ solution paired with a LiCl injection on separate days. After 15 min. access to the solution the subjects were given a LiCl injection 30 min. later. Half of the subjects were kindled 15 min. after access to each solution, while the other half were not kindled. Over a three day test period the subjects were given a "single cue" solution ($odor_1$, $odor_2$ or taste) on each of the three days. The order of testing was counterbalanced. The results indicated that the kindled subjects drank significantly more of each cue than the control subjects, $F(1,14) = 77.1$, $p < .01$. Again kindling occurring in the CS-US interval disrupted the acquisition/retention of a CTA.

Experiment 3 looked for a time gradient of kindling produced disruption after the US is administered. Using the same design as Experiment 1 the subjects were kindled 15 or 60 min. after the LICl injections. Control subjects were not injected. Tests clearly indicated that only the 15 min. group showed a disruption of the CTA; the 60 min. and control subjects were not different, $F(2,13) = 11.6$, $p < .01$.

190.13 POSTTRIAL MICROWAVE EFFECTS ON LEARNING AND MEMORY IN MICE. J. A. Beel*, L. J. Fisher, R. E. Gerren, and M. W. Luttges. Department of Aerospace Engineering Sciences, University of Colorado, Boulder, CO 80309.

Biological tissue exposed to low-level microwave exposure is thought to experience overall heating, heating gradients and di-electrically-induced polarization shifts. These effects are believed to be the basis on which a variety of behavioral alterations occur in response to microwave treatments. While the usefulness of microwave irradiation for studies of neural enzymes is reasonably well established, such usefulness as a tool for studying the neural substrates of behavior has not been exploited. In the present studies we have shown that brief, low level microwave treatments delivered posttrail yield significant effects on learning and memory in mice. Following both active and passive avoidance training, irradiated mice received either 15 or 30 min. of continuous exposure to pulsed 3GHz with an average power of 18-22 mW/cm^2. In half the cases irradiation yielded fixed fields and in the other cases the fields were continuously disrupted and reoriented. Control mice received sham exposures. These exposure levels resulted in $< 0.5^{o}$ C differences in core temperatures. Significant enhancement of learning and memory following 15 min. exposure occurred both with five consecutive days of multiple trial, active avoidance training and with single trial, passive avoidance training. These performance changes were evident in tests given one week after training and microwave exposure. Repeated daily exposures of 30 min. duration produced enhanced performance during the first three days of training and performance deterioration thereafter. Thus, a doubled total irradiation appears to produce detrimental consequences for learning and memory reminiscent of pharmacological dose-response curves. These posttrial effects are not likely to be related to the geometry of microwave fields biasing the polarization of specific neural structures. Heat or heat gradient effects are the more probable causes of the performance changes. This type of non-invasive modification of learning and memory mechanisms deserves further study.

190.14 DIFFERENTIAL EFFECTS OF CONCUSSIVE HEAD INJURY ON BEHAVIOR IN CATS. C. E. Dixon*, G. F. Heath*, Y. Katayama*, R. J. Hamm*, and R. Hayes. Dept. of Psychology and Dept. of Neurosurgery, Virginia Commonwealth Univ., Richmond, VA 23284.

Cerebral concussion is a clinical syndrome characterized by immediate suppression of widespread reflexive functions and loss of consciousness due to mild head injury without gross structural changes and is always followed by post-traumatic amnesia. Recent clinical studies have indicated that disturbances in memory functions following concussive head injury include long-term storage and retrieval. It is established that similar reflex suppression and consciousness disturbances can be produced in laboratory animals by various models of experimental concussive head injury. However, virtually no attempt has been made to clarify whether disturbances in memory functions are elicited in these experimental animals. Thus, in contrast to various symptoms of concussion, mechanisms underlying disturbances in memory functions remain unclear. This study examined the effects of concussive head injury on the retention of a well-consolidated classically conditioned eyeblink response in the cat using fluid-percussion head injury (Sullivan et al. J. Neurosurg, 45, 520, 1976), a model with which reflex suppression and loss of consciousness can reliably be elicited.

Prior to conditioning, all animals (N=4) had a stimulating electrode chronically placed in the medial edge of the superior palpebra. Classical conditioning of the eyeblink consisted of pairing a tone with an electrical shock via the electrode until a criterion of $\geq$ 80% correct responses on intermittent tone-alone trials was met. After five days each animal was tested on its pre-injury retention of the eyeblink response. The animals were retrained to criterion, then given a concussive head injury, produced by a single, brief (25 msec), hydraulically-induced pressure transient (1.9-2.1 atmospheres) conducted into the intracranial cavity. This level of injury consistently produced immediate and transient abolition of various reflexive activities (e.g., pinna, blink, and flexion reflexes) and concurrent disturbances in consciousness as inferred by motor indices of arousal and orientation. After five days each animal was tested, retrained, and five days later retested again. A comparison of the means of the percent correct for the three retention tests yielded no differences indicating that clinically reported disruptions of memory retrieval are not produced by the same mechanisms for reflex suppression and loss of consciousness. Further work is being done to study the effects of concussive head injury on other memory functions, including consolidation and short-term memory.

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190.15 EFFECT OF PREVIOUS LEARNING UPON UPTAKE OF ^{14}C 2-DEOXYGLUCOSE EVOKED BY VISUAL STIMULATION. A. Isseroff and Y. Madar*.Dept. of Isotope Research, Weizmann Institute of Science, Rehovot, Israel.

We report that specific training can produce localized changes in cerebral functional activity evoked by a particular stimulus when it is presented outside the training situation. The effects of training are distinct from those of nonspecific stimulation provided by environmental enrichment.

Between the ages of 30 and 90 days, 5 hooded rats were trained in a "Y" maze in increasingly difficult visual discriminations, until they could distinguish a vertical bar from one presented at 6° off vertical. During the same period, 6 rats were raised in an enriched environment, and 5 control subjects were raised in ordinary group cages. Autoradiography of ^{14}C 2-Deoxyglucose (2-DG) was used to assay functional activity (Sokoloff et al. J. Neurochem. 28: 897 1977) evoked by visual stimulation. With one eye occluded, rats were placed in a restrainer and exposed to moving bars shown at different orientations on two television screens. All animals were unfamiliar with the test conditions and apparatus. 2-DG was injected I.P. and animals were stimulated for 45 min. They were then overdosed with anesthetic and perfused with 3.3% buffered formalin. Autoradiograms of 30 µm cryostat sections were analyzed with the aid of a microcomputer based system (Lancet and Isseroff, Neurosci. Abs. 8:1003, 1982).

Uptake of 2-DG was estimated by dividing the optical density of visual structures by that of reference areas. Data were analyzed for laterality (stimulated vs. unstimulated hemisphere) and differences in uptake between groups. As expected, significant (α = .05 2-tailed for all tests) lateralization of uptake was observed in most areas of the visual system in all groups. In the enriched group only, significant laterality was also observed at more anterior points in visual and parietal association cortex. Compared to naive rats, the trained ones, but not the enrichment group, showed significantly increased uptake in the stimulated hemisphere in area 18L near the occiput, at the border of areas 17 and 18L more anteriorly, and in the ventral superior colliculus, and bilateral increases in retrosplenial cingulate cortex. No differences between groups were observed in areas 17 or 18M, auditory or somatosensory cortex, hippocampus or subcortical visual areas save the ventral superior colliculus.

We believe that the differences found in trained rats partially reflect functional activity related to retrieval from memory, recognition and apperception of familiar objects and to attention.

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190.16 **A MATHEMATICAL MODEL FOR MULTIPLE MEMORY DOMAINS.**
P. A. Anninos and M. Kokkinidis*. Dept. of Physics, University of Crete, Iraklion Crete, Greece.

Previous studies with neural nets constructed of discrete populations of formal neurons have assumed that all neurons have the same probability of connection with any other neuron in the net(Anninos et al, J. of Theor. Biol.,26:121,1970). However, in this new study we incorporate the behavior of the neural systems in which the neural connections can be set up by means of chemical markers carried by the individual cells. A case of such specific chemical markers are the neurotransmitters which effect the intercellular communication in the nervous system. The effects of the chemical and structural nature of neurotransmitters has been studied in a previous work(Gieren,A and Kokkinidis M, Nat, 68:482,1981). However in this present study we do not include the properties of markers, but we consider only neural nets consisting of neurons with different chemical affinities. With this general approach we studied again the dynamics of isolated neural nets as well as the dynamics of isolated neural nets with sustained inputs. Results obtained with this approach show simple and multiple hysteresis phenomena. Such hysteresis loops may be considered to represent the basis for short-term Memory.

LEARNING AND MEMORY: HIPPOCAMPAL PHYSIOLOGY

191.1 POSTTRIAL RETICULAR FACILITATION OF DENTATE MULTIUNIT CONDITIONING IS FOLLOWED BY AN INCREASED LONG-TERM POTENTIATION. S. Laroche, O. E. Bergis* and V. Bloch*. Dépt. de Psychophysiologie, CNRS, 91190 Gif-sur-Yvette, France.

Posttrial electrical stimulation of the mesencephalic reticular formation (MRF) is known to facilitate learning in a variety of behavioral tasks. The hippocampal circuitry was used as a model to study the neural basis of this facilitative effect. Previous studies showed that posttrial MRF stimulation (1) facilitates the development of associative changes in hippocampal (CA3) and dentate multiunit activity during classical conditioning in rats and (2) results in a long-term enhancement of hippocampal responsiveness to the conditioned stimulus. It was hypothetized that posttrial MRF stimulation might facilitate the perseveration of neural processes triggered in those networks by incoming information and that this perseverative process might help long-lasting modifications to occur at the synaptic level. This hypothesis was mainly based on the fact that post-event MRF stimulation enhances the magnitude and duration of long-term potentiation (LTP) at the perforant path to dentate granular cells synapses.

If a synaptic modification like that associated with LTP is involved in the elaboration or maintenance of associative learning at the cellular level, it might be more easily detected as an actual change in LTP itself rather than as an enhanced single evoked potential. In the present investigation, dentate multiunit activity was recorded in rats submitted to a classical conditioning procedure involving six tone-footshock pairings during six days. One group of animals (MRF group) received a mild (mean intensity 4.5 µA) stimulation of the MRF, 10 sec. after each trial. LTP was induced 2 days after the end of conditioning by 10 high-frequency stimulations (400 Hz, 20 msec.) of perforant path fibers with 5-min. intervals. The analysis of dentate evoked potentials (population spike as well as population EPSP components) elicited after each high-frequency stimulation showed that, in the non MRF group, the amount of LTP was increased after the learning experience. Moreover, this effect was greatly enhanced in the MRF group. Appropriate control groups assured that it was the association of learning experience-MRF and not the total amount of MRF stimulation which had the effect on subsequent LTP.

It is suggested that, during associative learning, a perseverative process is taking place along the neuronal circuits under study which might be enhanced by MRF activation. This perseverative process could initiate long-lasting synaptic modifications revealed by LTP enhancement.

191.2 HIPPOCAMPUS AND TRACE CONDITIONING OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE. P. R. Solomon, E. R. Vander Schaaf*, A. C. Nobre*, D. J. Weisz, and R. F. Thompson. Department of Psychology, Williams College, Williamstown, MA 01267.

Previous research has shown a substantial neuronal change in hippocampus during acquisition of the conditioned NMR. Yet the structure's precise role in conditioning remains unclear since hippocampal ablations do not affect acquisition of the conditioned NMR. A number of investigators have suggested that the hippocampus may be essential only when the events to be associated (e.g., the CS and UCS) are separated in time. To test this possibility, we have examined the role of hippocampus in a trace conditioning paradigm during which there is a 250 msec tone CS, followed by a 500 msec trace interval during which no stimuli are present, followed by a 100 msec air puff UCS.

Our first study examined the effects of bilateral hippocampal ablations on acquisition of the trace CR. We found that normal animals acquired the CR in about 500 trials. Hippocampal animals, in contrast, did not acquire the CR over the 900 trials that we conditioned them. When switched to a standard delay paradigm (250 msec ISI), hippocampal animals readily acquired the CR.

A second study examined multiple unit activity in area CA1 of hippocampus during conditioned NMR acquisition. Three groups of animals were tested: Animals conditioned in the same trace conditioning paradigm as used in the lesion study (Group T-500), animals that received explicitly unpaired presentations of the CS (tone) and UCS (air puff; Group UP), and animals that underwent conditioning with a 2,000 msec trace interval between CS offset and UCS onset (Group T-2000). Previous work has shown that this trace interval does not support behavioral conditioning. Animals in Group T-500 acquired the behavioral response within an average of 500 trials. Early in training, and well before any CRs occurred, there was a substantial increase in neuronal activity in hippocampus that began during the CS and persisted through the trace interval. There was also an increase in the UCS period. Here, the neuronal activity both preceded the behavioral response and formed a temporal model of its amplitude-time course. Later in conditioning, as CRs emerged, there was no longer neuronal bursting throughout the CS+Trace period. Rather, the activity shifted to later in the trace interval and formed a model of the amplitude-time course of the behavioral CR. Activity in the UCS period was similar to that seen earlier in conditioning. Animals in Groups UP and T-2000 showed no behavioral conditioning and no increased neuronal activity. These findings strongly suggest that the hippocampus is essential for conditioning in situations in which the CS and UCS do not occur contiguously in time. Supported by NIMH Grant MH36539-01

191.3 FUNCTIONAL RELATIONSHIP BETWEEN HIPPOCAMPAL UNIT ACTIVITY AND RABBIT NM RESPONSE CONDITIONING DURING DISCRIMINATION-REVERSAL LEARNING. W.B. Orr and T.W. Berger. Psychobiology Program, Departments of Psychology and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.

This laboratory has previously shown that hippocampal pyramidal cell activity increases significantly during acquisition of a two-tone discrimination-reversal task using the rabbit nictitating membrane (NM) conditioning paradigm (Berger, Soc. Neurosci. Abstr., 8, 1982). Moreover, latency and amplitude characteristics of poststimulus time-histogram measures of pyramidal cell activity correlate highly with the topography of conditioned responses (CR) to the CS+ during both discrimination and reversal. No significant change in unit activity is recorded on CS- trials when no CR is elicited. In a test of the functional significance of this unit activity, the effects of aspiration lesions of the hippocampus on discrimination-reversal NM response conditioning were measured.

Three groups of subjects were surgically prepared: animals with hippocampal lesions (HLs), with neocortical lesions (CLs), and operated controls (OCs). The effects of hippocampal lesions on the rate of discrimination-reversal acquisition have already been reported (Berger and Orr, Behav. Brain Res., 8, 1983). No difference was found between groups in the number of trials to discrimination criteria. However, during reversal training HLs required significantly more trials to reach criteria than either control group. No difference between OCs and CLs for reversal training was observed. Here we report that conditioned responses made by HLs have a topography which differs significantly from that of OCs and CLs during reversal learning but not during discrimination conditioning. Five different parameters were used to describe response topography: i) latency to onset, ii) latency to response peak, iii) peak response amplitude, and iv) integrated NM response in both the CS-UCS interval (CS area) and v) the UCS period (UCS Area). Analyses of each parameter showed that the responding of HLs to the two conditioned stimuli is significantly different from the responding of OCs and CLs. Additionally, when the CS area of responses to both CS+ and CS- trials are plotted as learning functions only the curves for HL animals show a significant cubic component to their shape. In contrast, the CS area curves for OC and CL animals are linear. These results indicate that disruption of hippocampal pathways significantly alters the topography of conditioned NM responses during reversal. These data also suggest that the process(es) by which HL animals eventually acquire reversal is different than control animals.

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191.4 HIGH RESOLUTION MAPPING OF THE 'SPATIAL' FIELDS OF HIPPOCAMPAL NEURONS IN THE FREELY MOVING RAT. R.U. Muller*, J.L. Kubie and J.B. Ranck, Jr. Dept. of Physiology, SUNY, Downstate Med. Ctr., Brooklyn, N.Y. 11203.

O'Keefe and Dostrovsky (Brain Res. V34, 171-175, 1971) first reported that many neurons in the hippocampus of freely moving rats fire fastest when the animal is in a particular region of its environment, and that the firing is largely independent of the rat's behavior within this region, called the "spatial field". Subsequently, many groups have confirmed the existence of spatially firing hippocampal neurons and further described their properties. Nevertheless, because this work has been qualitative, fundamentals such as the size and shape of spatial fields are still not adequately characterized.

We report here that we have developed and used a high resolution, quantitative method for mapping spatial fields. An unrestrained, 20 hour food deprived rat is encouraged to run freely throughout a cylindrical (76 cm diam; 61 cm high) enclosure in search of randomly scattered food pellets, while we record from a well isolated complex-spike cell. We use a video camera 8 feet above the enclosure as the input to a threshold device which tracks the position of a bulb mounted on the rat's head. Every 1/60th of a second (TV frame rate) we send 16 bits (2 bytes) of information to a small computer. 12 bits signal position (6 for X and Y) and 4 bits signal the number of discriminated action potentials during the frame. The TV frame is thus divided into a 64x64 grid of rectangular 'pixels', each 3.4 cm wide by 2.8 cm high. We amass 57600 samples during a 16 minute recording session.

We analyze the data by creating three 64x64 arrays to represent the number of samples the rat spent in each pixel area, the number of spikes (AP) which occurred in each pixel, and the firing rate in each pixel. We use these arrays to print 6-color maps of where the animal spent its time and how fast the cell fired in each position (AP/sec). We have collected a total of about 30 hours of data from 20 cells in 6 rats. Our behavioral method is adequate since the rats visit most every pixel during each recording session, although they tend to spend more time near the wall. Almost all units whose average firing rate is higher than 0.25 AP/sec show one or sometimes two regions of intense firing. The area of such "spatial fields" ranges between about 5 to 30% of the cylinder's area. These fields have obvious firing rate contours, diminishing from 20 AP/sec or more in the hottest parts to virtually zero outside the field. Fields which do not encroach on the boundary tend to be radially symmetrical, while those near the edge are crescent shaped rather than truncations of circles. The fields are stable with time; nearly identical shapes and positions are seen with sessions done several days apart. (Supported by NIH grant NS 14497).

191.5 MANIPULATIONS OF THE GEOMETRY OF ENVIRONMENTAL ENCLOSURES CONTROL THE SPATIAL FIRING PATTERNS OF RAT HIPPOCAMPAL NEURONS. J.L. Kubie, R.U. Muller* and J.B. Ranck, Jr., Dept. of Physiology, SUNY, Downstate Medical Center, Brooklyn, N.Y. 11203.

Most hippocampal complex-spike cells in freely moving rats fire fastest when the rat is in a particular part of its environment. In the previous abstract, we described a TV system which permits us to generate firing rate maps which clearly show the position, shape, size and contours of such place fields.

We reasoned that if these neurons are part of a cognitive mapping system, the significance of their spatial fields would be most clearly revealed by manipulating the geometry of the environment in which the recording is done. Using simply shaped chambers with opaque walls, we obtained spatial firing rate maps for individual neurons when the rat was successively placed into each of four enclosures. Two of these were cylinders (76 cm diam, 61 cm high; 152 cm diam, 122 cm high), while the others were rectangular boxes (46x61 cm, 61 cm high; 92x122 cm, 122 cm high).

The principal finding is that a single complex-spike cell has similarly positioned spatial fields in enclosures of a given shape; the field centers are located at the same relative place, and the field is larger in the larger of the pair of enclosures. By contrast, knowing the field locations in one shape does not predict the field locations in the other shape. Frequently, a cell which had obviously similar spatial fields in, say, the rectangles had no fields in the cylinders. For a minority of cells, we found unrelated fields in a pair of similar enclosures. A second major result is that the shape of a field near the boundary of an enclosure appears to be determined by the shape of the enclosure itself. 'Edge' fields in the rectangles are often linear, while those in cylinders are usually crescent-like. We stress that this effect is not merely due to the impossibility of crossing the boundary; the part of the field facing the inside of the enclosure also runs parallel to the boundary.

These finding show that it is possible to detect meaningful relationships among spatial fields in different environments, and that the shape of the environment is a crucial determinant of the shape and position of the spatial field. They also suggest that the rat's hippocampus may participate in globally representing the current environment.

(Supported by NIH grant NS 14497)

191.6 SINGLE UNIT ACTIVITY IN THE HIPPOCAMPUS OF RATS: BEHAVIORAL CORRELATES IN A NONSPATIAL DELAYED-MATCH-TO-SAMPLE TASK. R. L. Findling*, M. Shapiro, and D. S. Olton. Department of Psychology, the Johns Hopkins University, Baltimore, MD 21218.

Hippocampal function has been linked to the types of memory processes required for normal performance in delayed conditional discriminations. The present experiment recorded from single units in the hippocampus while rats solved a nonspatial delayed-match-to-sample discrimination. At the beginning of each trial, the rat was presented a distinctive visual stimulus as the sample stimulus to be remembered for that trial. The stimulus was removed for the delay interval of 10 seconds. The rat was then given a choice between this sample stimulus and a second one. Responses to the sample stimulus presented at the begining of the trial were reinforced. A bundle of tungsten microelectrodes was implanted just above the hippocampus and slowly lowered through the CA1 pyramidal cell layer. When activity from a unit was electrophysiologically stable and had a sufficient signal-to-noise ratio to be isolated from the background, a series of up to 80 trials was given in the delayed conditional discrimination. Recordings have been obtained from 30 complex spike units. Of these, two-thirds had rates of activity that were strongly associated with the discriminative stimuli. For each of these units, the rate of activity was markedly increased in the presence of one stimulus, and not in the presence of the other. Most of the remaining units had place fields that were associated with the apparatus itself, rather than with the extramaze stimuli surrounding it. These data indicate that when nonspatial stimuli are made salient and relevant as in this discrimination problem, hippocampal units respond to them. The results of this study are interpreted in the context of three other sets of experiments: the effects of hippocampal lesions in rats on performance in conditional discriminations; the activity of single units in rats in other types of tasks; the activity of single units in other brain areas in these types of tasks in primates. Supported by Research Grant MH24213 from the National Institute of Mental Health.

191.7 SPONTANEOUS SYNAPTIC ENHANCEMENT IN HIPPOCAMPI OF RATS EXPOSED TO A SPATIALLY COMPLEX ENVIRONMENT. P.E. Sharp, B.L. McNaughton, and C.A. Barnes. Dept. of Psych., Univ. of Colorado, Boulder CO 80309.

High frequency electrical stimulation results in a long-term enhancement (LTE) of the perforant path-dentate granule cell synaptic connection. Several characteristics make this phenomenon a reasonable candidate for a biological mechanism of learning. This hypothesis requires that LTE should occur during normal acquisition of information, as well as from artificial stimulation. Since the hippocampus is thought to be involved in processing spatial information (O'Keefe and Nadel, 1978), exposure to a spatially complex environment was used in this study in an attempt to generate such natural LTE.

Five albino rats were chronically implanted with stimulating electrodes in the perforant path and recording electrodes in the dentate gyrus. Prior to recording, the animals were housed for at least one month in single cages in a dimly lit room. Since electrically induced LTE decays over several weeks, this spatial impoverishment was included to permit any naturally present LTE to decay, thus facilitating the detection of any subsequent enrichment effect. Daily recording of the granule cell response to single perforant path stimuli was then initiated. During the first ten days of recording the animals remained in the impoverished environment. Three of the animals were then each transferred to individual rooms (150 sq ft approx) filled with boxes, ramps, and other stimulus objects as their permanent living quarters.

In the enrichment group, both the population spike and EPSP exhibited a gradual increase over a period of several days following transfer to the new environment, reaching significant peak increases of about 48% and 16% respectively. These effects were not due to behavioral changes during the recording sessions.

Further pilot work suggests that prolonged exposure to the spatially complex living quarters results in the eventual decay of the granule cell response back to baseline levels. This fits in well with the notion (e.g., Marr, 1971; Squire, 1983) that the hippocampus serves only as a temporary storage place for memory traces. Subsequent transfer to a second complex environment, however, results in renewed growth of the granule cell response. In addition, preliminary studies indicate that the growth in synaptic efficacy can be generated when animals are given only one-half hour of daily exposure to a complex environment immediately after each daily recording session. Thus, the effect requires relatively little environmental stimulation and can be seen even when measured twenty three hours after each daily treatment.

In conclusion, a change in synaptic efficacy resembling electrically induced LTE occurs during a time when animals could be expected to be acquiring a "cognitive map" of a novel environment.

191.8 ELECTROENCEPHALOGRAPHIC CORRELATES OF LONG-TERM POTENTIATION. G. Buzsáki. Dept. of Physiology, University Med. Sch. 7643 Pécs, Hungary.

LTP has been widely considered as a model for memory storage. The present investigation examined the effect of high frequency stimulation (HFS) delivered to the commissural/associational systems of the hippocampus on spontaneous EEG activity. Rats were equipped with chronic recording microelectrodes placed in the strata pyramidale and radiatum of CA1 and stimulating electrodes positioned in the contralateral CA1/CA3 regions. They were trained on a VI-40 sec schedule for water reinforcement. During drinking high amplitude (2.0-3.5 mV) sharp-waves (SPW) appeared in the CA1 region reflecting synchronous discharges of a great number of CA3 pyramidal cells and consequent depolarization of the apical dendrites of the CA1 pyramidal neurons (Buzsáki, Leung and Vanderwolf, Soc. Neurosci.8:738, 1982). The number of spikes of predetermined amplitude was counted during the initial 10-sec epochs of drinking. The HFS (400 cps, 20 msec) invariably induced epileptic discharges followed by LTP of the field postsynaptic potential and population spike in CA1. The number of SPWs decreased considerably after the HFS up to 4 hrs and then increased significantly up to 4 days. The time course of changes of evoked and spontaneous activity (SPW) were somewhat different. Following supramaximal single pulses inducing epileptic afterdischarges none of the above changes were observed. LTP failed to have an effect on the frequency of lapping or other behavioral parameters. It was not possible to separate whether the potentiation effect on SPWs was conveyed through the commissural or associational systems (Buzsáki and Eidelberg, Br.Res. 237:283, 1982). These findings are the first indication of long-term changes of spontaneous activity within the hippocampal system. The biphasic depression-potentiation effect is explained by the hypothesis that inhibitory interneurons show shorter LTP than principal cells.

191.9 HUMAN HIPPOCAMPAL FIELD-POTENTIALS EVOKED TO WORDS DURING RECENT MEMORY RECOGNITION TASKS. M. E. Smith*, J. M. Stapleton and E. Halgren. Lab. for Cognitive Neurophysiology, Brain Research Institute, University of California at Los Angeles, and V. A. Southwest Regional Epilepsy Center, Los Angeles, CA.

The sensitivity of medial temporal lobe structures to stimulus familiarity was assessed by recording event-related potentials (ERPs) from chronically implanted depth electrodes during recent memory word recognition. Recordings were obtained bilaterally from amygdala, hippocampus, and parahippocampal gyrus of patients being evaluated for surgical treatment of epilepsy. Stimuli were common nouns, 50% of which were repeats of words presented in previous blocks. Patients performed a forced-choice yes/no recognition task with keypress response. Concurrent ERPs were collected by simultaneous recordings from 23 channels (.1 Hz. - .1 kHz., 7 msec dwell). In associated studies (Stapleton et al., this meeting), ERPs were obtained from the same electrodes during auditory "oddball" tasks.

Visually presented words were observed to elicit large amplitude potentials (50-100 μV), with peak latencies of 250-700 msec and polarity reversals in the area of the hippocampus. Changes observed in response to word familiarity were of two types. One component which appeared similar in morphology and distribution to that observed in oddball tasks decreased in size and latency to repeated words. A second component of earlier latency and different distribution was also noted to decrease amplitude and latency in response to repeats. Keypress response was made 600-850 msec post-stimulus onset. These results suggest that hippocampal activity involved with information processing distinguishes between new words and those stored in Recent Memory. Since the onset of this difference occurs 300-600 msec before response execution, models of memory search operations which specify parallel processes are supported.

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191.10 HIPPOCAMPAL UNIT ACTIVITY ALTERED BY LESIONS OF THE FIMBRIA-FORNIX IN RATS. M. L. Shapiro, R. L. Findling*, and D. S. Olton: Dept. of Psychology, Johns Hopkins University, Baltimore, MD; F. H. Gage, U. Stenevi*, and A. Bjorklund*: Dept. of Histology, Lund University, Lund, Sweden.

Neuronal firing patterns in the CA-1 layer of the hippocampus of intact rats were compared to those of rats given lesions of the fimbria-fornix while the rats traversed a radial maze. Single unit recordings were made through a drivable bundle of microelectrodes implanted into the hippocampus at the level of the hippocampal flexure. Both complex-spike and theta unit firing patterns were altered in rats with lesions of the fimbria-fornix. Complex-spike units were identified by their waveform, composed of a burst of two or more decrementing spikes, and low firing frequency. Complex-spike units recorded from intact rats had discrete place fields, places on the maze where firing frequency was significantly higher than base rate. The place fields in intact rats were not altered by manipulations of intramaze cues (e.g. covering the maze floor). In contrast, complex-spike units recorded from rats with fimbria-fornix lesions had place fields which were either degraded or abolished. Often the complex-spike units in rats with lesions fired without location specificity. Occasionally place fields were observed in rats with lesions, however these were abolished by manipulations of intramaze cues. These results indicate that the neural locus responsible for establishing place fields may be dissociated from those responsible for maintaining the stability of these fields.

Theta units recorded from intact rats were identified by their waveform (composed of near equivalent positive and negative deflections) and bursting firing pattern, which was phase locked to the theta slow-wave rhythm. These units fired with increased frequency during appetitive behaviors (e.g. walking) and decreased frequency during consummatory behaviors (e.g. eating or grooming). Theta units recorded in rats with lesions had wave forms identical to those in intact rats. Furthermore, these theta units fired with increased frequency during appetitive behaviors and with decreased frequency during consummatory behaviors. However, the typical bursting pattern of theta units was never observed. These results indicate that the neural locus controlling theta unit firing frequency may be dissociated from that responsible for organizing the pattern of such activity. Supported by a Research Grant from NIMH, MH24213.

191.11 FUNCTIONAL RECOVERY FROM HIPPOCAMPAL KAINIC ACID LESIONS AND INTERACTIONS WITH SUPERIOR CERVICAL GANGLION. J. P. Kesslak and F. H. Gage. Chemistry of Behavior Program, Dept. of Psychology, Texas Christian University, Ft. Worth, TX 76129, and Dept. of Histology, University of Lund, Lund, Sweden.

It has been demonstrated hippocampal KA lesions of the CA3 area can induce performance deficits on learning tasks which recover over time (Handelmann & Olton, 1981). The time-course of recovery from damage is approximately the same period necessary for sympathetic sprouting after medial septal lesions (Chafetz, Evans & Gage, 1982). The present study was designed to determine if sympathetic fibers may be contributing to recovery of function following hippocampal KA lesions.

Male Sprague-Dawley rats were pretrained on a forced choice alternation task in a T-maze. Following learning to criterion animals received either KA (.3nm/.4ul) or buffered saline injected bilaterally into dorsal and ventral hippocampus. Animals were again tested on the forced choice task till reaching criterion. Thirty days after KA or saline injections half the animals in each group received sympathectomies (SCG) and were retested.

Analysis of variance and post hoc tests indicated; 1)performance deficits on the forced choice task following KA lesions ($p<.001$), 2)following ganglion removal only the combined KA-SCG group showed a significit performance deficit ($p<.001$). The results of the present study indicate sympathetic fibers may play a role in recovery of function from hippocampal damage. Histofluorametric examination of NE fibers tentatively indicate an increase in fluorescence; but quantification of differences between groups is necessary before concluding sympathetic fibers significantly contribute to recovery of function.

191.12 HIPPOCAMPAL PROTEIN PHOSPHORYLATION AND SPATIAL MEMORY. B. Bank* and D. L. Chute[1]. Div. of Life Sciences, University of Toronto, Scarborough, Ont. Canada M1C 1A4.

The purpose of this experiment was to detect changes in synaptic proteins as a result of acquisition of an 8-arm spatial maze task. Twenty-four 400g Charles River male hooded rats were food deprived to a level of 85% of their free feeding weight. Control animals were treated identically to experimental animals, with the exception of actual maze training. Animals were placed daily in an 8-arm radial maze in which each arm was baited with food, and permitted to visit 8 arms in a period of 10 min or less. A correct choice of an arm meant entering an arm which had not been previously chosen. After 2 weeks, only animals performing to a criterion of at least 7 of 8 correct choices were included for biochemical analysis. Twenty-four hours after the last trial, the animals were decapitated, the brains quickly removed and the hippocampus and entorhinal cortex dissected out. The synaptic junctional complex was isolated by Triton extraction. The isolated fraction was incubated with [γ-^{32}P] ATP and analyzed by 2-dimensional polyacrylamide gel electrophoresis. Overall incorporation rates showed a 32% inhibition of protein phosphorylation in the tissue of trained animals compared to controls. This inhibition was reflected in autoradiograms of the 2-dimensional gels as a decrease in phosphorylation of at least three proteins with molecular weights of approximately 50-55, 75-80 and 130K. These changes are consistent with those observed in our lab as a result of other neuroplastic phenomena such as kindling.

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191.13 CORRELATION BETWEEN WORKING MEMORY AND LEVEL OF HIPPOCAMPAL CHOLINE ACETYLTRANSFERASE IN RATS. D.J. Hughey* and E. Friedman* (SPON: S.H. Ferris). Dept. of Psychology, New York University, and Dept. of Psychiatry and Pharmacology, N.Y.U. Medical Ctr., New York, N.Y. 10003 & 10016.

Short-term memory deficits have often been associated with damage of the hippocampus or its extrinsic connections. Moreover, there is evidence that a central cholinergic dysfunction (particularly in the hippocampus and cortex) may play a role in some geriatric memory disturbances. The purpose of this experiment was to examine the relationship between a type of short-term memory and the cholinergic septohippocampal pathway in rats.

An 8 arm radial maze was used. The maze consists of an octagonal center platform from which 8 runways project, like the spokes of a wheel. An abundance of extra-maze stimuli provided spatial orientation cues. A food pellet was placed at the end of each arm. Then a food-deprived rat was placed in the center of the maze and allowed to forage freely.The optimal strategy is to visit each arm only once, thereby collecting all 8 pellets in the first 8 choices. Perfect performance depends on the capacity for short-term retrieval called working memory.

All animals were pre-operatively trained on the maze. After reaching a high criterion of performance, rats were randomly assigned to one of the following groups: unoperated controls, operated controls, or septal lesions. Bilateral septal lesions were produced with cathodal DC current (0.7 mA x 60 seconds). Two post-operative tests were conducted. Each of these consisted of one trial per rat daily for 10 days. The first test period commenced 7-10 days after surgery; the second began 50 days after the surgery.

After the completion of post-operative testing, all rats were sacrificed. The hippocampus of each animal was assayed for choline acetyltransferase (CAT), the synthetic enzyme in the formation of acetylcholine.

Behavioral results showed a typical impairment of radial maze performance in the septal lesioned rats. Operated controls were indistinguishable from unoperated controls. Septal vs. control differences were most pronounced in Test 1. The enzyme assays revealed a significant correlation across groups between maze performance and hippocampal CAT activity ($p<.001$). Within the septal lesioned group there was also a significant correlation between degree of behavioral impairment and level of CAT depletion ($p<.05$).

In conclusion, there appears to be a significant linear relationship between working memory, as measured on the radial maze, and hippocampal cholinergic function.

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191.14 THE INFLUENCE OF BEHAVIOR ON (3H)-CHOLINE UPTAKE INTO ACETYLCHOLINERGIC NEURONS OF THE NUCLEUS BASALIS OF MEYNERT AND THE MEDIAL SEPTAL AREA. G. Wenk, D. Hepler*and D. Olton. Department of Psychology, Johns Hopkins University, Baltimore, MD 21218.

Cholinergic neurons in the basal forebrain, including the nucleus basalis of Meynert and the medial septal area, project topographically to the neocortex and hippocampus, providing a major source of cholinergic input. The present experiments correlated the activity of these cholinergic neurons with behavior by monitoring changes in sodium-dependent high affinity choline uptake in the cortex and hippocampus after the performance of behavioral tasks designed to involved these structures. The tasks included: memory tests on the radial arm maze, active avoidance, and reversal of spatial discrimination on a T-maze. An increase in cortical sodium-dependent high affinity choline uptake following performance in a particular task is taken to indicate increased cholinergic neuronal activity in the nucleus basalis of Meynert, while an increase in hippocampal sodium-dependent high affinity choline uptake is taken to indicate increased cholinergic neuronal activity in the medial septal area. In each experiment, one group of rats composed an untrained control group, to determine baseline levels of sodium-dependent high affinity choline uptake. A second group performed one of the behavioral tasks designed to activate specific brain regions just prior to sacrificing. The rats were sacrificed by decapitation immediately after testing, and the frontal cortex and hippocampus were isolated. Sodium-dependent high affinity choline uptake was assayed according to the method of Atweh et al., (Life Sciences, 17: 1535, 1975). The results suggest that behavior differentially influences cortical and hippocampal cholinergic activity, and are interpreted in terms of the influences of basal forebrain cholinergic structures upon behavior. Supported by grant DAMD 17-C-8225.

191.15 HIPPOCAMPAL LESIONS: BEHAVIORAL RIGIDITY AFFECTS THE APPRECIATION OF EXPERIMENTAL CONTINGENCIES. N. A. Schmajuk* and R. L. Isaacson. Department of Psychology and Center for Neurobehavioral Sciences, SUNY-Binghamton, Binghamton, NY 13901.

Classical conditioning can be described in terms of the contingency between the CS and the UCS. Animals with hippocampal lesions did not have any deficit in classical conditioning, when trained with different combinations of P(UCS|CS), the probability of receiving UCS given the occurrence of CS and P(UCS|$\bar{CS}$), the probability of receiving the UCS given the non-occurrence of CS.

In the first experiment we studied the P(UCS|CS) = P(UCS|$\bar{CS}$) = 0.5 combination. When trained in this condition, hippocampal, cortical, and sham lesioned animals did not differ in performance. However, when hippocampal lesioned animals were trained in an instrumental learning situation with P(UCS|R) = P(UCS|$\bar{R}$) = 0.5, they responded at a higher frequency, displaying a higher P(R) (Devenport, 1980).

This difference between the classical and instrumental results can be explained in terms of how the animal's behavior defines the degree of contingency between the response R and the consequence S. This contingency can be defined according to the absolute probabilities of receiving S, having produced both R and non-R. These absolute probabilities are the intersection of R and S and can be expressed as P(S$\cap$R) = P(R).P(S|R) and P(S$\cap\bar{R}$) = P($\bar{R}$).P(S|$\bar{R}$). Thus, in the instrumental case the degree of contingency is defined by the animal's behavior (Gibbon et al., 1974).

The conclusion is that the differences found in instrumental learning could be the result--not of a deficient learning about contingencies in general--but of a different distribution of the responses.

In a second experiment the distributions of the total time allotted for each behavior and of length of bouts was obtained for hippocampal, cortical, and sham lesioned animals in the open field. These distributions were different for hippocampal lesioned animals when measured in either way.

These behavioral differences may account for some of the differences observed in hippocampectomized animals in instrumental learning, since they would induce lesioned animals to perceive the experimental contingencies in different ways from normal animals.

191.16 WHERE IS THE COGNITIVE MAP? C.A. Barnes and B.L. McNaughton, Dept. of Psych., Univ. of Colorado, Boulder, CO 80309.

Several lines of evidence indicate that the hippocampus is crucially involved in spatial information processing. This has led to the theory proposed by O'Keefe and Nadel (1978) that the hippocampus is the locus of a cognitive map. Consistent with this notion is the observation that the principal inputs to the hippocampus are capable of pronounced long-term synaptic enhancement (LTE) following both their electrical activation at high frequency and exposure to a novel environment (Sharp et al., this meeting). Furthermore there is a correlation between the persistence of LTE and an animal's performance on a spatial memory task (Barnes,1979). If these observations are indeed related, then one would predict that experimental saturation of the LTE mechanism by repeated electrical stimulation should impair cognitive mapping ability.

Ten young (10 mo) and 10 old (25 mo) rats were trained to asymptotic performance on a circular platform maze. This maze requires the animal to find a darkened goal tunnel using extra-maze cues only (odor cues and motor patterns cannot be used to solve the problem). The animals were then prepared for bilateral chronic stimulation of perforant pathway and recording of evoked responses in the fascia dentata. Half of the young and half of the old animals were given 6 high-frequency stimulation sessions distributed over two days. This resulted in a near saturation of LTE. The other half of the animals received the same number of stimulation pulses at a low frequency. No LTE was induced.

Twenty-four hours following the last stimulation session, the animals were retested on the platform. The location of the goal tunnel was, however, rotated 135° away from the original location. This permitted an assessment both of the retention of the original location (initial deviation from original goal location) and the acquisition of a new spatial location (number of errors made following three consecutive daily training sessions with the new position).

There was no difference between treatment groups in the retention of the old spatial habit, whereas there was a large and statistically significant difference in the acquisition of the new one. While the older animals' performance was poorer overall than the younger animals' on this task, both groups were impaired by the high frequency stimulation only on the acquisition phase.

These data suggest that a normally functioning hippocampus is necessary for the formation of a new "cognitive map", but is not necessary for the effective use of a previously established one. Hence it appears that the major input to the hippocampus, while necessary to set up a "map", is not the locus of the map itself.

INTERHEMISPHERIC RELATIONS

192.1 FUNCTIONAL LATERALITY IN THE MOUSE BRAIN: EVIDENCE DERIVED FROM STUDIES OF PAW PREFERENCE AND ROTATORY BIAS IN SHAKER-1 MUTANT MICE. John M. Cooke, Department of Anatomy, University of Massachusetts Medical School, Worcester, MA 01605

Mice homozygous for the gene shaker-1 (gene symbol, sh-1) are spontaneously hyperactive and run in circles. The circling activity is lateralized. Individual mice circle in preferred directions, either clockwise or counterclockwise, at rates which average more than 40 rotations per minute. Preliminary neuroanatomical study of the shaker-1 brain has revealed no obvious morphological abnormality. Consequently, the circling behavior may express an inherent functional asymmetry. The present study was undertaken to determine two separate indices of laterality, namely, paw preference and rotatory bias, in the same individual shaker-1 mice.

Forty-two affected (sh-1/sh-1) and 49 control mice (+/sh-1 or +/+) of the shaker-1 inbred strain were tested for paw preference using chambers similar to those described by Collins (J. Hered. 59:9, 1968). Paw preference was quantified by scoring the number of right paw entries (RPE's) out of a total of 50 reaches made by a fasted individual to obtain food. Rotatory biases were determined for 34 of the 42 shaker-1 mice by calculating the average percentage of clockwise rotations made by each mouse during a minimum of ten three-minute periods of observation.

The distribution of paw preferences among the mutant and control populations was similar. There were approximately equal numbers of dextral and sinistral mice within each group; 21 of the 42 shaker-1 mice and 22 of the 49 controls had RPE's greater than 25. Among individuals which expressed stronger degrees of lateralized behavior, seven mutants and six controls had RPE's greater than 40.

Of the 34 shaker-1 mice which were also tested for circling behavior, 13 circled preferentially clockwise, 21 counter-clockwise. Both paw preference and rotatory bias were stable upon repeated testing of the same individual. Paw preference and rotatory bias were not, however, strongly correlated. Individuals which showed similar paw preferences displayed widely varying values for rotatory bias and vice versa.

Paw preference and rotatory bias constitute two stable, lateralized behaviors which can be easily determined in shaker-1 mice. Their expression in an inbred genetically uniform population may offer a distinct advantage for use of this mutant and possibly other circling mutants of the mouse in studies directed toward understanding the neural mechanisms which underlie lateralized function in mammals.

192.2 STRUCTURAL ASYMMETRIES AND HIPPOCAMPAL MOSSY FIBER VARIATIONS RELATED TO THE STRENGTH OF HANDEDNESS IN MICE. H.-P. Lipp, R.L. Collins*, and W.J.H. Nauta. Mass. Inst. of Technology, Dept. of Psychology and Brain Science, Cambridge, MA 02139 and Jackson Laboratories, Bar Harbor, ME 04609.

The tenacity of a mouse in using its preferred paw has a strong genetic component as demonstrated by the establishment of two mouse lines selectively bred for differential dexterity in a simple manipulation task. One line (HI) has become strongly lateralized, using mostly a preferred paw (left or right), the other (LO) is practically ambidextrous. The use of such selectively-bred lines is a convenient tool in attempts to identify a set of brain variables related to a particular behavior, for every trait showing reliable strain differences must be related somehow to the task. Other genetic breeding paradigms can then be applied to isolate those variables which are critical for the behavior (Schwegler & Lipp, Behav.Brain Res. 7, 1983).

The brains of 6 female HI- and 6 LO-mice were processed for Timm-staining and cut horizontally. At a mid-dorsoventral level, such sections provide an overall view of the mouse brain, allowing easy recognition of left/right asymmetries. In a preliminary morphometric analysis using five sections per mouse brain, we measured cortical thickness at symmetrical locations of the orbitofrontal, somatosensory, and entorhinal cortex; in addition, we determined the volume of the hippocampal formation at the sampling level. These measures were transformed into percentage differences between hemispheres, a difference of more than 10% being judged as major.

Two distinct line differences emerged: 1) 5 HI-mice had a major asymmetry either in the orbitofrontal cortex (OFC) or in hippocampal (HIP) volumes but never in both locations. One animal had only minor asymmetries but uniformly thicker cortex and hippocampus in one hemisphere. LO-mice showed mainly minor asymmetries, except for the entorhinal cortex. A score based on the combined OFC and HIP-percentages showed a highly significant line difference ($p=0.009$). 2) In the ambidextrous LO-line, 5 animals had a bilateral peculiarity in their mossy fiber distribution, never described before: the suprapyramidal mossy-fiber layer appeared to be invaded by pyramidal neurons. This trait was qualitative, either present or lacking, and was found in one HI-mouse, too. Yet, there was still a significant line difference ($p=0.04$, Fisher's Exact). We conclude that a hereditary strong or weak paw preference is apparently associated with a differential development of several localized hemispheric asymmetries, and with subtle bilateral differences in intrahippocampal circuitry.

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192.3 THE EFFECTS OF UNILATERAL DECORTICATION ON BEHAVIORAL ASYMMETRIES AS A FUNCTION OF GENDER, NEONATAL TAIL POSTURE (NTP), AND EXTRA STIMULATION IN INFANCY. M.J. Hofmann*, A.S. Berrebi*, J.S. Gall*, J. Stockler*, D.A. Yutzey, & V.H. Denenberg. Depts. of Biobehavioral Sciences & Psychology, Univ. of Conn., Storrs, Conn. 06268.

Previous research using unilateral cortical lesions demonstrated that different behavioral responses could be obtained from rats with left (L) or right (R) cortical lesions, suggesting an underlying asymmetrical cerebral organization. Denenberg and his colleagues showed that extra environmental stimulation in infancy (handling) could cause changes in the behavioral organization of rats, often resulting in the enhancement of the behavioral asymmetry. Recently, neonatal postural asymmetries in rats were related to adult spatial preferences (SPs) on certain tasks. In addition, females and males differ with regard to behavioral and postural asymmetries. This study examined the relationships among gender, NTP, handling, and unilateral cortical lesions, and their effects on SP and activity it the rat.

Purdue-Wistar rats of both sexes and NTPs (L or R) were handled (H) or not handled (NH) for 20 days following birth. As adults, they were tested for L-R SP and activity in a water-motivated diamond (D-) maze, a water-motivated rotation apparatus, and an open field. Following this, all animals received unilateral decortication (L or R) or a sham lesion. After 30 days recovery, rats were tested again with the presurgical regimen. Following the experiment, rats were sacrificed for histological analyses. Data were treated with a repeated-measures ANOVA.

Comparing pre- vs. postsurgical results, the sham group showed little response to the surgery per se. Female decorticates demonstrated no significant effect in the D-maze, but shifted their rotational preference uniformly to the side contralateral to the lesion. NH male decorticates shifted their SP to the side contralateral to their lesion in the D-maze and the rotation apparatus. In the D-maze, H male decorticates shifted their SP to the side ipsilateral to the lesion. This shift was greater for males with L than for those with R, indicating a cerebral asymmetry. In the rotation apparatus, H male decorticates shifted their SP contralateral to the lesion, but the shift was greater if the lesion was placed contralateral to the NTP, again indicating a cerebral asymmetry. Finally in the open field, there was a uniform decline in the activity of all groups from pre-to postsurgical tests, except for females with a L-NTP. This group maintained high activity levels.

These results indicate: (1) Male and female behavioral asymmetries are organized differently; they are more evident for spatial behavior with males and locomotor behavior for females. (2) Handling changes the behavioral organization of males and induces a neural or behavioral asymmetry.

192.4 INTERHEMISPHERIC REGULATION OF MESOLIMBIC AND NIGROSTRIATAL D2 RECEPTORS. L.H. Schneider[@], E.E. Coons[*#], and R.B. Murphy[$] [@]Department of Biological Psychiatry, N.Y.S. Psychiatric Institute New York NY 10033 and Departments of [#]Psychology and [$]Chemistry, New York University, New York, NY 10033

The specific binding of [^{3}H]-spiroperidol (0.6 nM) to the left and right hemisphere terminal zones of the mesolimbic (olfactory tubercle plus ventral nucleus accumbens: OT+NA) and the nigrostriatal (striatum) dopamine (DA) systems was measured individually in 66 male Sprague-Dawley rats. We have previously reported a highly significant endogenous left > right asymmetry in striatal D2 receptors, and the strong tendency toward an opposite right > left imbalance in OT+NA (Neurosci Lett. 33: 281-284 (1982). We have also presented evidence that these endogenous lateralizations are modulated by electrode implantation into and also by electrical self-stimulation (ICSS) of the ventral tegmental area (VTA), site of mesolimbic DA cell bodies (Soc. Neurosci. Abstr. 8: 656 (1982).

We now report that the modulation of mesolimbic D2 receptors by unilateral VTA ICSS is found more markedly in the OT+NA contralateral to ($p < .005$) rather than ipsilateral to ($p < .09$) the electrode, although the hemispheric OT+NA asymmetry found after electrode implantation only depends in part on the electrode side in relation to the preoperative D2 receptor imbalance. The magnitude of the contralateral < ipsilateral mesolimbic binding asymmetry found after electrical self-stimulation is significantly related to the depth of the electrode ($r=-0.50$, $p < .006$). The interaction of the stimulation with tissue laterality with respect to the VTA electrode was highly significant in the OT+NA ($p < .001$), but not in the striatum.

These data support the existence and influence of reciprocal inhibitory connections between the left and right hemisphere DA /TA cell body groups. That the left and right hemisphere A9 DA cell body groups are similarly interrelated is suggested by our evidence that the endogenous striatal D2 asymmetry is abolished by left or right VTA electrode implantation, regardless of subsequent electrical stimulation treatment. We consider our results imply interhemispheric regulation of mesolimbic and nigrostriatal D2 receptors which could be consistent with the evidence presented from Glowinski's laboratory for interhemispheric control of DA release in the nigrostriatal system of the cat (Cheramy et al, Nature 239: 537-542 (1982).

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192.5 LATERALIZATION OF ACTIVITY IN APOMORPHINE-INJECTED RATS EXPLORING A NOVEL ENVIRONMENT: PREDOMINANCE OF TURNING IN THE CLOCKWISE DIRECTION. H. Szechtman. Department of Neurosciences, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.

There is increasing evidence that an asymmetrical brain organization, both functional and structural, is present not only in humans, but also in a variety of animal species, including rats. This presentation reports that apomorphine reveals a striking lateralization in the behaviour of rats exploring a novel environment. In their investigation of an unfamiliar area (a large sheet of glass 183 x 121 cm, suspended 150 cm above the floor) rats (N= 12) injected with apomorphine (1.25 mg/kg s.c.) turned their head, or the head and the body, for a total of 1717 x 45° during a 60 minute test. (The measurements were taken using the Eshkol-Wachman Movement Notation, with 1 amount of movement= 45°.) Of this amount, 61% was in the clockwise direction (to the right) and only 39% was in the counterclockwise direction (to the left), a highly significant statistical difference ($p < .0001$). Uninjected control animals (N= 12) showed no bias during their exploration of the field. Rats which were familiar with the open field also did not show an overall bias in the direction of turning after an injection of apomorphine, indicating that experience counteracts the revealing effects of the drug. Interestingly, 9 of the 12 apomorphine-injected rats which were unfamiliar with the open field showed a significant bias for turning in the clockwise direction, whereas of the 12 apomorphine-injected animals familiar with the test environment, 4 had a bias for turning in the clockwise and 5 in the counterclockwise direction. (The remaining rats showed no significant bias in the direction of their turning.) These data suggest that during exploration of a strange territory, when the animal is presumably forming a "cognitive map" of the area, the left hemisphere is more active than the right one and that this specialized activity of the left side is exaggerated by apomorphine. (Supported by OMMF and MRC. H.S. is an MRC Scholar.)

192.6 RIGHT HEMISPHERE DOPAMINERGIC CIRCUITRY IS INVOLVED IN ELECTRODERMAL ACTIVITY CONTROL: EVIDENCE FROM HEMIPARKINSONISM. M. Mintz, R. Tomer*, [1]Z. Privorkin*, [2]H. Sroka* and M.S. Myslobodsky. Psychobiology Research Unit, Tel Aviv University, Ramat Aviv 69978, and Depts. of Neurology, [1]Meir and [2]Beilinson Hospitals, Israel.

Attentional deficit in patients with unilateral Parkinson's syndrome (hemiparkinsonism) may be revealed in abnormalities of the P300 wave of visual evoked potentials (Mintz et al., 1982). The present findings corroborate the above study using the analysis of electrodermal activity (EDA). These results also suggest that the right- and left-hemisphere dopaminergic (DA) circuitries have an unequal share in EDA control.

A group of 21 patients was screened for analysis: twelve had strictly unilateral symptomatology (rigidity, tremor and slowing), while in nine there were also initial signs of involvement of the other side. Seventeen patients were on chronic Levodopa medication (125-500 mg/day) and four patients were either drug-free or on anticholinergic medication. Reactive EDA was recorded from the index and ring fingers of both hands, by measuring the resistance changes with 10 μamp constant current. EDA was measured in response to non-signal stroboscopic flashes (baseline session) and during attention task, in which patients were asked to discriminate between, and memorize the number of flashes delivered in two intensities.

Patients were defined as either (a)non-responders (NR), who failed to produce any EDA orienting response or were unresponsive in the first three trials of each session; or (b)responders (R), who produced consistent EDA orienting response, or habituated after at least three trials. Right-sided parkinsonians were largely responders (64.3% during baseline session and 100% during task session), with significantly higher EDA reactivity during the task session ($p < .02$). Conversely, patients with left-sided symptomatology were typically NR (85.7% during baseline, and 83.3% during task session). A test for the significance of a difference between proportions revealed that the occurrence of non-responding differed significantly between the two groups ($p < .05$, two-tailed), in both the baseline and task sessions. Given that unilateral parkinsonian symptomatology correlates with DA-ergic deficiency in the contralateral hemisphere, our findings suggest that bilateral EDA non-responding is associated with right hemisphere deficit. Three patients with right-sided symptomatology were tested both before and following chronic Levodopa treatment. All three showed slightly enhanced EDA reactivity when on medication. It would therefore appear that DA circuitry in the right hemisphere is responsible for bilateral EDA control. Non-responding, observed in medicated right-hemisphere parkinsonians, indicates irreparable damage to this circuitry.

192.7 EVIDENCE FOR LEFT HEMISPHERE DOMINANCE OF VISUAL DISCRIMINATION LEARNING IN THE CHICK. Karen E. Gaston* (SPON: T. J. Mueller). Pitzer College, Claremont, CA 91711.

Hemispheric specialization of the human brain is well-documented, and there is also accumulating evidence of functional brain asymmetries in nonhuman species, especially birds. In the avian brain, the optic nerve fibers from each eye all cross to the contralateral hemisphere; direct visual input can therefore be lateralized to one hemisphere by occluding the ipsilateral eye. It has been shown that monocularly acquired learning in birds is sometimes stored in the form of a unilateral engram, which may or may not be accessible to the "untrained" hemisphere by way of interhemispheric fiber connections. Several recent studies have found that chicks trained monocularly on a visual discrimination task learn somewhat faster through the right eye than through the left eye. This observation suggests that the left hemisphere of the chick brain is the functionally superior, or specialized, hemisphere with respect to visual discrimination learning.

While studies of monocular learning can assess the relative competence of the two hemispheres for a particular task, they do not by themselves reveal the normal pattern of hemisphere function in birds with both eyes open. When visual information is freely available to both eye/hemisphere systems, it is possible either that both hemispheres acquire and store the information or, on the other hand, that the more competent hemisphere dominates acquisition and retention. The present experiment examined these alternatives.

In an automated operant chamber, 20 chicks were trained with both eyes open on a simultaneous 2-choice pattern discrimination. After reaching a criterion of 90% correct responses, each chick was retrained on the same discrimination with one eye occluded. Chicks retrained through the right eye (left hemisphere) demonstrated excellent retention of the discrimination. In marked contrast, chicks retrained through the left eye (right hemisphere) showed no retention at all - they behaved as though they had never seen the discrimination before and they required at least as many trials to re-learn the task as they had to learn it the first time.

The pattern of results suggests that both hemispheres have the capacity for this type of visual discrimination learning, but that the left hemisphere is dominant when learning occurs with both eyes open. In this situation, it appears that only the left hemisphere learns the pattern discrimination and that memory is stored unilaterally and is not available to the right hemisphere. This failure of interhemispheric transfer is remarkable in light of the good transfer we have previously observed in chicks trained monocularly on the same discrimination task. The discrepant findings suggest that interhemispheric interactions are qualitatively different depending on whether or not sensory information is experimentally lateralized.

192.8 LATERALITY IN MONKEYS FOR DISRIMINATING FACIAL EXPRESSION AND IDENTITY. B. A. Vermeire, A. L. Erdmann* and C. R. Hamilton. Div. of Biology, Caltech, Pasadena, CA 91125.

As part of our search for hemispheric specialization in monkeys we test the ability of each hemisphere to discriminate facial characteristics because this ability is well developed in monkeys, it is lateralized in man, and pilot studies indicate it also may be lateralized in monkeys (Hamilton and Vermeire, Behav. Brain Res., in press). Expression as well as identity was tested in this experiment because recent work with human subjects has revealed greater lateralization for facial expressions than for recognition of individuals. Sixteen split-brain rhesus monkeys, eight male and eight female, learned with each hemisphere to discriminate photographs of the faces of conspecifics. Four discriminations required differentiating one monkey from another with facial expression held constant and four additional problems required differentiating one expression from another with the individual held constant. For each discrimination five examples of the positive stimulus and five examples of the negative stimulus were intermixed in a sequence of 80 trials. After the two hemispheres learned the eight discriminations and several months had passed, they were tested separately for retention of the discriminations. Following this, tests were made of generalization of the discriminations to new photographs of the expressions or individuals to determine if the desired categories had been learned and to evaluate the specificity of the categories in each hemisphere.

The results to date show an overall tendency for the right hemisphere to learn more readily than the left, but with 14 monkeys completed this is statistically significant only for the discrimination of expressions by female monkeys. Similarly, there is an advantage for remembering the discriminations with the right hemisphere; with nine monkeys tested this advantage is significant only for the discrimination of expressions. Finally, generalization is also better, although not significant, with the right hemisphere of the seven monkeys which have been tested. In contrast to results from human subjects, no greater hemispheric difference is apparent for discriminating expressions than for discriminating individuals.

In summary, although we have obtained new evidence of hemispheric specialization in monkeys for learning and remembering discriminations based on facial characteristics, the lateralization does not seem as robust or consistent as that found with human beings. There is some indication that the degree of lateralization may depend on the task demands and on the sex of the subjects. Supported by USPH grant MH-34770.

192.9 TRANSCALLOSAL MNEMONIC PROCESSING IS INFERIOR TO INTRAHEMISPHERIC PROCESSING IN MACACA NEMESTRINA. Jeffrey D. Lewine* and Robert W. Doty, Center for Brain Research, University of Rochester School of Medicine, Rochester, New York 14642.

Wearing a lightweight mask equipped with shutters which restrict vision to one eye at a time, monkeys with optic chiasm and anterior commissure (AC) cut via a transsphenoidal approach sit facing two panels. Photographic images are then serially projected on the upper panel at 10-sec intervals. If it is the first time that a given image is seen on that day, the monkey is rewarded for pressing the upper panel; if it is the second presentation of that image, the monkey must press the bottom panel for a reward. The mean delay between first and second viewings is 60 sec (range 10-180 sec). Thus, 0-17 images may intervene between first and second presentation. A total of 280 images is presented daily, 5-7 sessions elapsing before any particular image is seen again. For the monkey reported here 5100 presentations have been made, 2800 of which represent first presentations of a given day, and 2300 representing second viewings. Regardless of which eye was being tested, on 20% of the occasions when the monkey viewed an image for the first time, it erroneously responded as though the image were a second presentation. Chance performance for recognition of a repeat presentation is therefore defined by this "false positive" error rate, i.e., 20% not 50% correct. Thus, if the monkey's ability to recognize repetitions is> 20%, it is demonstrating some memory for the first presentation. The monkey's actual performance as a function of 'seconds since initial viewing' (s) is, for the intrahemispheric condition, given by the equation: % accuracy = 90.5 - 0.11(s); and for the interhemispheric condition by: % accuracy = 74.9 - 0.20(s). The intercepts of these two functions differ at p> 99.9% (F 1,64 = 25.034); the slopes also differ at p> 95.0% (F 1,64 = 4.452). These equations apply regardless of which eye was tested (intrahemispheric condition), or the direction of transfer tested (interhemispheric condition). Although we do not have adequate pre-surgical observations on this animal, these data clearly demonstrate that despite the presence of the entire callosal system, interhemispheric mnemonic processing is significantly worse than intrahemispheric processing at all tested delays (s). In addition, the findings concerning the slopes of the two functions suggest that the interhemispheric process "decays" faster, or is perturbed more readily. It remains to be determined whether the basis for the observed deficiency of the interhemispheric mnemonic comparison is attributable to the absence of the AC, the weakness of a contralateral engram, inefficiency in accessing a unilateral engram, or more than one of these possibilities. The situation can be delineated by preserving the AC and/or by selective, reversible blockade of commissural pathways. (Supported by NSF Grant 5-28606.)

192.10 SPECIALIZED HEMISPHERIC RETRIEVAL FROM LONG-TERM SEMANTIC MEMORY: CONVERGENT EVIDENCE FROM NORMAL AND COMMISSUROTOMY SUBJECTS. D. W. Zaidel* (SPON: D. L. Glanzman). Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The forebrain commissures serve to transmit perceptual and cognitive information from one cerebral hemisphere to the other. In normal subjects with intact commissures it is difficult to determine the extent to which experimentally observed hemispheric differences reflect shared, duplicated, or specialized processes. However, convergent data from patients in whom the forebrain commissures had been sectioned completely can help clarify findings obtained with normal subjects.

In the present study the performance of patients with sectioned commissures was compared to that of normal subjects on a task designed to measure retrieval of semantic information from long-term storage. The task consisted of matching tachistoscopically presented pictorial instances with their natural superordinate categories. Instances were typical or atypical representations of their categories (E. Rosch, J. of Exp. Psychol., 1975, 104:192-233). The stimuli were lateralized briefly to either the left (LVF) or right (RVF) visual half fields and subjects indicated a match or no-match with a key press. Both accuracy and latency scores were recorded and only correct matches were considered in data analysis.

Analysis of the results for latency scores showed (1) a significant interaction between visual half field and the level of typicality in both groups;(2) typical instances were matched faster in the LVF than in the RVF and atypical instances were matched faster in the RVF than in the LVF. There were no visual field differences in accuracy scores.

In view of the similar pattern of functional lateralization observed in commissurotomy and normal subjects these results are interpreted to reflect specialized retrieval from long-term semantic memory in each hemisphere even in the presence of the forebrain commissures. This extends previous observations of hemispheric specialization by suggesting the functional lateralization of long-term storage of experience as well.

192.11 INTERHEMISPHERIC ASYMMETRIES OF CORTICAL AUDITORY EVOKED POTENTIALS USING BRAIN ELECTRICAL ACTIVITY MAPPING. S. Khoshbin*, L. A. Levin*, L. M. Milrod* and M. Hallett. (SPON: S. Wray). Neurology Section, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115.

Cortical auditory evoked responses to frequent (90%) 1000 Hz and infrequent (10%) 2000 Hz 50 msec tone bursts were recorded in 14 normal right-handed subjects ranging in age from 18 to 33. Stimuli were presented at 60 dB above hearing threshold to right and left ears separately, with the contralateral ear masked with white noise. The auditory evoked response was recorded over 450 msec from 16 scalp electrodes using the 10-20 international system. Computerized four-point linear interpolation was done to provide a spatial map of the recorded evoked response over a 48×64 matrix. Significance probability mapping using point-by-point paired two-tailed t-tests was done to demonstrate statistically significant differences in three experimental paradigms. Results were displayed on a television monitor using gray-level coding.

Comparison of right versus left stimulation with 1000 Hz frequent stimuli showed presence of differences ($p < .02$) in the left mid-temporal area from 113 to 123 msec. Similar comparison of the infrequent 2000 Hz tones showed differences ($p < .02$) in the left mid-temporal area from 166 to 180 msec and the right fronto-central from 223 to 250 msec. There were no asymmetries noted in the range expected for the P300 wave.

Comparison of the right versus left hemisphere with same ear stimulation showed consistent asymmetries throughout the recording period in all areas except the anterior to mid-temporal. Responses to infrequent stimuli did not show significant interhemispheric asymmetry, as was predicted by visual inspection of the topographic map prior to statistical analysis.

Our observations show that the response to frequent stimuli demonstrates a polar pattern (left anterior and right posterior positive, and right anterior and left posterior negative), while the response to infrequent stimuli demonstrates an interhemispheric symmetry, with an anteriorly negative to posteriorly positive gradient.

192.12 LINGUISTIC PROCESSING SPEEDS AS A FUNCTION OF HEMISPHERIC DOMINANCE AND DEVELOPMENT, R. H. Bauer. Dept. Psychol., Middle Tennessee State University, Murfreesboro, TN 37132.

A number of processes involved in the rapid searching and naming of visual stimuli and reading were identified, i.e., searching for a match between visual stimuli and visual memory codes, searching for a match between visual stimuli and phonological codes stored in memory, transferring visual stimuli to phonological codes, and the rate of covert responding. On the basis of standardized behavioral tests that measure left and right hemispheric functions, children of 8-, 9-, 10-, 11-, and 12 years of age (N=90) were separated into left-, right-, and neutral-hemispheric dominance groups. They were then tested in a number of tasks which measure the rate of each of the processes listed above. The rates of searching for a match between (a) visual stimuli without graphemic and lexical characteristics and visual memory codes, (b) visual stimuli without graphemic and lexical characteristics and phonological codes, and (c) simple motor responses with the dominand hand did not differ as a function of hemispheric dominance but increased with age. The rates of (a) searching for a match between visual stimuli with graphemic and lexical characteristics and phonological codes stored in memory, (b) transferring visual stimuli into phonological codes, and (c) overt vocal responding were faster in children with left hemispheric dominance and increased with age. In addition, reaction time measures which were faster in children with left-hemispheric dominance were positively correlated with scores on standardized reading tests. Results of the present study indicate that a wide variety of reaction times increase as a function of development. However, only reaction times to graphemic and lexical stimuli and the rate of overt vocal responding differed among hemispheric dominance groups. Reaction times may be a sensitive method for detecting subtle differences in hemispheric functioning in children and, possibly, in adults.

192.13 CEREBRAL SYMMETRY OF VISUAL STIMULUS EQUIVALENCES. R.M. Lazar*, D. Scarisbrick*, G. Kuslansky*, and C. Soares* (SPON: D. Berman). Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367, and Dept. of Psychology and Social Relations, Harvard University.

A substantial literature has supported the view that there are functional differences between the two cerebral hemispheres of humans. The data have suggested that the left hemisphere is specialized for symbolic (e.g., language) function, and the right hemisphere is superior for spatial tasks. A question that remains unanswered, however, is the extent to which both hemispheres may share similar cognitive functions.

Normal, right-handed males were presented arbitrary matching-to-sample tasks, using three sets of Greek-letter stimuli designated Sets A, B, and C. Each set consisted of two stimuli. Viewing stimuli through a tachistoscope, subjects were first taught A-B and A-C matching in which a sample stimulus was presented in the center visual field for 150 msec, followed immediately by a comparison stimulus of similar duration in the left or right visual field. Half the subjects used their right hands to activate switches to indicate "match" or "nonmatch"; the other subjects used their left hands to indicate their responses.

After training, every subject was able to perform B-C and C-B matching, even without feedback on test trials. Response accuracy and the corresponding reaction times following comparison stimulus presentation to the left and right visual fields did not differ, suggesting cerebral symmetry with regard to the formation of simple equivalence classes with visual stimuli. The establishment of arbitrary relations among the Greek-letter forms permitted subjects to form equivalences for which there were no previously-learned labels. The data provide support for bilateral, symbolic thought that can be carried out in the absence of language.

193.1 COLOR CONSTANCY IN THE ASTIGMATIC EYE WITH LONGITUDINAL CHROMATIC ABERRATION: THE NATURAL BASIS FOR THE MCCOLLOUGH EFFECT. Howard C. Howland and Nancy Sayles*. Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The McCollough Effect is a long-term contingent after-effect wherein a subject who has viewed a field of black stripes alternating with a colored background, sees the complimentary color when subsequently viewing a field of black and white stripes of the same orientation. It is generally thought to be due to the adaptation of a population of neurons which respond to both color and orientation.

We believe that the McCollough effect is due to a normal color-constancy mechanism operative in persons with normal amounts of astigmatism. Such persons cannot focus parallel lines in orthogonal meridians simultaneously. Accordingly, when they view sets of black and white orthogonal stripes, one or both sets of such stripes must be defocused. Due to the normal chromatic aberration of the eye, either the red or the blue component of the white light forming the image will be more severely defocused than the other. This will result in a color separation of the two images, yielding colored stripes. The effect is, of course, direction-specific.

This hypothesis explains a) why it is a monocular effect (anisometropia), b) why it is a directional effect (astigmatism), c) why we don't ordinarily notice the McCollough effect (color constancy), d) why some persons do not see the effect (no astigmatism), e) why the usual McCollough stimulus presentation "overdrives" the color constancy mechanism (It employs long viewing times of intensly saturated colors), and f) the natural basis of the effect.

We are conducting experiments with subjects having varying degrees of astigmatism to test aspects of this hypothesis and will present data to confirm or refute it.

193.2 REDISUAL VISION IN HEMISPHERECTOMIZED PATIENTS. A. Ptito*, M. Lassonde et M. Ptito. Groupe de neuropsychologie, Université du Québec à Trois-Rivières, Trois-Rivières, Québec, G9A 5H7, Canada.

It has traditionnally been assumed that damage to the occipital cortex will result in permanent blindness in the contralateral part of the visual field. These past years, however, an important aspect of research on blindness of cortical origin has been to demonstrate that the patient, even if blind in his subjective experience can respond to visual stimulations presented in his blind field. The present study was thus undertaken to investigate the existence and extent of such residual vision in hemispherectomized patients. Three levels of visual analysis were studied: 1. stimulus detection (SD); 2. movement analysis (MA); 3. pattern discrimination (PD). The experiments were carried out in a light-tight chamber and the Ss were dark adapted for a 30 min. period. Stimulus presentation was computer controlled and ocular movements were constantly monitored. The first experiment (SD) involved the presentation on an oscilloscope of a stationary visual stimulus (2^{o}). The oscilloscope position was varied randomly along the horizontal meridian. The S's task consisted in pointing at the location where he (she) believed the stimulus was presented. The second experiment (MA) required S to discrimate motion: a) in step 1, S had to indicate whether the stimulus (grating: 2c/deg.) presented in his (her) blind or intact field was stationary or moving; b) in step 2, two gratings were presented in the blind and/or the intact field and S's task consisted in telling whether the stimulus velocity was the same or different; c) in step 3, S had to indicate whether the gratings were moving in the same or opposite direction. The last experiment (PD) involved two and three-dimensional pattern discrimination (letters, shapes, numbers and familiar objects) presented simultaneously in the blind and intact fields. Results showed that stimulus detection in the blind field is preserved in all Ss. Motion detection and discrimination were also possible in all patients. However, the discrimination of stimulus direction was failed. Concerning pattern discrimination, only one subject performed the tasks successfully. It therefore seems that some residual vision is present in the blind field of hemispherectomized patients. These residual capacities are sufficient to allow motion detection and velocity comparisons. They are limited however when finer discrimination tasks (direction or pattern) are required. The results are discussed in terms of differential involvement of cortical and subcortical mechanisms.

193.3 PITCH OF AM-SIGNALS: EVIDENCE FOR A CORRELATION ANALYSIS IN THE HUMAN AUDITORY SYSTEM. G. Langner. Zoologie, TH-Darmstadt, Schnittspahnstr.3, 6100 Darmstadt, FRG.

The "residual" pitch P of AM-signals(sinusoidal amplitude modulation) was measured by a matching procedure with a "pure" tone. If the carrier frequency, f_c, is a harmonic of the modulation frequency, f_m, (i.e. $f_c/n_H=f_m$, n_H integral) P corresponds to f_m. As experiments with nonharmonic AM-signals revealed P is neither defined by the modulation envelope nor by a subharmonic of the carrier since it deviates systematically from f_m as well as from f_c/n_H (Schouten,J.F. et al.,J.Acoust.Soc.Am.34:1418,1962).

It is evident that the same discrepancy is found in the time domain since the period $\tau_p=1/P$ deviates from $\tau_m=1/f_m$ as well as from $n_H\cdot\tau_c=n_H/f_c$. Pattern models of an auditory pitch perception could comply with this effect. They are based on the assumption that the auditory system analyses the spectral pattern of the AM-signal either on the basilar membrane or central in the auditory system (Goldstein,J.L.,Audiology 17:421, 1978; Terhardt,E. et al.,J.Acoust.Soc.Am.71:671,1982; Wightman,F.L.J.Acoust.Soc.Am.54:407,1973).

In contrast, the present pitch measurements with 5 human subjects suggest that the auditory system does not resolve the spectral pattern, but performs a cross correlation between the envelope and the carrier. As a result the pitch period τ_p may be expressed by:

$$\tau_p = n \cdot \tau_c + b$$

(τ_c, carrier period; n, integer). Furthermore, the results suggest that the values b are multiples of an auditory time constant τ_1 = 0.4 ms.

The same evidence comes from a new interpretation of old pitch measurements (Schouten et al., see above; Smoorenburg,G.F.,J.Acoust.Soc.Am.48:924,1970). The auditory time constant is also supported by measurements with continuous variations of f_c. The resulting pitch curves revealed steps where τ_p was a multiple of 0.4 ms.

Adequate neuronal correlation mechanisms were described for the auditory system of the Guinea fowl. The envelope cycles of AM-signals trigger neuronal oscillations with periods multiple to 0.4 ms. This activity coincide at the midbrain level with neuronal activity phase coupled to the carrier (Langner,G.,Soc.Neurosci. Abstr.43.8,1982;Exp.Brain Res.1983, in press).

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193.4 DISORDERS OF TIME PERCEPTION IN BRAIN INJURED PATIENTS, ESPECIALLY APHASICS. J. Ilmberger* and E. Pöppel. Institute of Medical Psychology, University of Munich, FR Germany.

Human time perception can be described by different categories, each one probably being based on different mechanisms. Using such a classification (Pöppel, Handbook of Sensory Physiology, Vol. VIII, 1978, p. 713) various aspects of time perception were studied in aphasic patients, control patients with right hemisphere lesions and control subjects. Observations on fusion and order threshold and on temporal reproduction are reported here. Contrary to previous reports, auditory fusion threshold was found to be the same in the two patient groups and in the control subjects. The measurement of order threshold gave a different picture (order threshold marks the interstimulus interval which is needed for a correct identification of the temporal order of successive stimuli). Patients with injuries of the left hemisphere show a significant increase in auditory order threshold (90 ms on average); their visual order threshold is comparable to the control group. Patients with right hemisphere lesions show a significant increase of visual order threshold: their auditory threshold is in the normal range. The deficit of auditory order threshold of the left-hemisphere-patients may be relevant for their aphasia. Voice onset time for instance has a value up to 50 ms; if order threshold is prolonged in patients due to a central lesion beyond this value, difficulties in speech processing may be expected. It has been suspected for a long time that temporal integration can be tested with experiments on temporal reproduction. Durations of about 2 to 3 sec usually are reproduced correctly; shorter intervals are overestimated, longer intervals underestimated. This observation was confirmed in control subjects and right-hemisphere-patients. Most of the left-hemisphere-patients showed a deficit in reproduction experiments, usually overestimating also long intervals. If a basic time-keeping mechanism is tapped with this experiment, one has to conclude that aphasic patients in addition to an increased order threshold show a deficit in temporal integration which may be reflected on a linguistic level. (Supported by grant Po 121/3,6,8 of the DFG).

193.5 BIMANUAL PERFORMANCE OF PARKINSON PATIENTS: SIMULTANEOUS VS. CONCURRENT TASKS. L. Z. Podbros.[1] Dept. of Psychology, SUNY at Stony Brook, Stony Brook, NY.

Parkinson patients generally show greater difficulty relative to normals in bimanual than in unimanual activity. The present investigation, which is part of a larger study of Parkinson dysfunction, compared performance on two types of bimanual responding: bimanual execution of the same tasks (simultaneous) and bimanual execution of different tasks (concurrent). An individual research strategy was employed with eight male idiopathic Parkinson out-patients. Patients were administered 37 tasks. In order to compare performance across tasks, standardization data were obtained from comparable normal controls, and raw scores converted to z scores. On the Purdue Pegboard, which involves both conditions, all patients showed greater decrement for simultaneous than for concurrent performance. In order to compare the bimanual skills across tasks, z scores were combined into clusters. Seven patients clearly showed greater decrement for simultaneous than for concurrent performance.

Since bimanual concurrent activity is harder than simultaneous for normals, it is surprising that Parkinson patients show relatively less difficulty with it. An explanation may be found in consideration of patients' performance: in contrast to normals, patients performed the concurrent tasks in consecutive order; however, they could not resort to this strategy for the simultaneous tasks, and hence performed more slowly. Findings are discussed in terms of Parkinson patients' postural instability and increased reliance on visually-directed movement.

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193.6 COGNITIVE PERFORMANCE IN PATIENTS WITH TEMPORAL LOBE EPILEPSY. J. A. Walker, Cognitive Neuropsychology Laboratory, Good Samaritan Hospital and Medical Center, Portland, OR 97210.

Patients undergoing unilateral temporal lobectomy for siezure relief were tested both before and three months after surgery in a battery of tasks designed to measure elementary cognitive operations. The tasks measured the patient's performance in such areas as access to physical, phonological, and semantic features of the test items. The tasks chosen included letter matching, physical or linguistic compatibility/incompatibility, mirror reading, semantic priming, mental rotation, and verbal and nonverbal memory scanning. Several of the visual tasks were presented in both macular and lateralized forms to compare memory skills in the damaged and undamaged hemisphere.

The results indicate that, although there are wide individual differences across patients in these tasks, reflecting different patterns of deficient and normal performance, individuals show an internally consistent pattern. For most patients, there was little or no change in the pre to postoperative period, indicating that surgery, per se, had little influence in determining the patient's pattern of results. In contrast, side of lesion, extent of lesion, and location within the temporal lobe appear to be critical in determining an individual's pattern of elementary cognitive operations.

193.7 INFORMATION PROCESSING AND ENCODING STRATEGIES IN ALCOHOLIC KORSAKOFFS. G.B. Marcil* (SPON: J. Montplaisir). Dept. de psychologie, Université de Montréal, Montréal, Québec.

An information processing deficit is believed to underlie the amnesia associated with Korsakoff's Syndrome. The purpose of the first experiment of this study was to determine if Korsakoff patients are capable of processing verbal information on a semantic level which requires an indepth analysis of the properties of the material. Conversely, they may rely on a more superficial analysis such as attending to the phonetic characteristics of words. It was hypothesized that the less elaborate the encoding strategy, the more prone to decay is the trace. Korsakoffs (n 10), chronic alcoholics (n 10) and normal subjects (n 10) were instructed to memorize lists of words. Exposure rates (2 sec or 8 sec/stimulus), types of recall (cued by semantic categories or free recall) and post-acquisition intervals (15 sec or 60 sec) were combined factorally. The results demonstrated that semantic cueing, regardless of temporal factors, was the main determinant in improving recall among Korsakoff subjects. In contrast, free recall resulted in inferior performances. It seems that Korsakoffs, in a free recall condition where they must spontaneously encode the material, adopt a less sophisticated strategy. The consequence is a deficient retrieval of improperly stored information.

The second experiment evaluated the percentage of residual mnemonic traces of the same subjects 30 sec, 15 min, 1 hr and 24 hrs following the original learning of a list of words. Amount of retention was inferred by the number of trials required to relearn the list to criterion. The types of errors on a recognition test were also examined. Results indicated that Korsakoffs did not retain significant amounts of information since they did not demonstrate a decrement in relearning trials as a function of longer learning-relearning time intervals. Qualitatively, errors were similar in the three groups. However, in the Korsakoff group, the quantity of errors did not significantly drop over time. The finding that the Korsakoffs made semantic errors is evidence of limited spontaneous semantic encoding which was not stable enough to allow selective retrieval or recognition of pertinent information.

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193.8 NEURAL POPULATION MODELING AND PSYCHOLOGY: A REVIEW. D. S. Levine, Department of Mathematics, University of Houston, Central Campus, Houston, TX 77004.

The development of neural modeling as it relates to psychology is traced from the early 1940's to the present. The evolution of the field has been from descriptions of randomly connected neurons transmitting all-or-none signals to analyses of structured multi-level networks whose dynamics involve several different spatial and temporal scales.

The cybernetic revolution of the 1940's led to the incorporation into digital neural models of such concepts as linear threshold logic, redundant computation, and information. Each of these concepts has more recently been synthesized with learning to generate a set of adaptive neural models. Concurrently, a variety of data from neurophysiology and from experimental psychology suggested ways to incorporate continuous and nonlinear effects into models.

Since the late 1960's, there has been much activity in the design of rules for modifiable synapses in models of learning or conditioning. There has also been much activity in the design of lateral inhibitory networks that model sensory pattern storage. The development of models of these effects is outlined, together with that of multi-level networks that combine modifiable synapses and lateral inhibitory anatomies. These multi-level networks model such psychological effects as reinforcement, attention, coding of feature detectors, and interactions between short-term and long-term memory.

194.1 SPATIO-TEMPORAL MAPPING OF DEVELOPMENTAL DIFFERENCES IN EVENT-RELATED POTENTIALS IN YOUNG CHILDREN DURING INFORMATION PROCESSING. J.T. Marsh, R.J. Strandburg, W.S. Brown*, R.F. Asarnow* and D. Guthrie*, Dept. of Psychiatry, UCLA School of Medicine, LA, CA 90024.

Event-related potentials (ERPs) were recorded from young (YNG) and older (OLD) children (mean ages 8.5 and 11.5 yrs) during the performance of a complex information processing task to establish developmental norms against which a variety of clinical cohorts being run in our laboratory could be assessed.

The task involved the discrimination of a randomly placed target letter in 3 or 12 letter arrays which are presented for 50ms, 0.5sec after a 60dB warning tone. ERPs were recorded from a widely distributed array of scalp electrodes and averaged over a 1.5sec interval.

Response to warning tone: YNG subjects produced a longer latency, lower amplitude auditory ERP (P1-N1 only) compared to the well defined P1-N1-P2 complex obtained in the OLD group. A midline CNV was apparent at the onset of the ERP. In OLD subjects CNV continued to develop after the warning tone and resolved rapidly after the visual stimulus. Relying less on the auditory warning and visual target stimuli, YNG subjects produced a larger, almost tonic negativity. Though both groups exhibited a lateralized slow negativity at frontal leads, in YNG subjects, a much larger and prolonged hemispheric asymmetry (R>L) was observed.

Response to visual stimulus: In YNG subjects, exogenous components were larger (esp. the P1-N1 wave) and slightly delayed at posterior leads while endogenous components were considerably smaller and later at all leads. This suggests immaturity in perceptual mechanisms and less efficient strategies or neural substrates for higher level information processing and discrimination. A pronounced, lateralized enhancement of P1 and N1 was seen at the right posterior lead in the OLD subjects which was not apparent in thee YNG group suggesting that YNG subjects are not processing information in the same way as OLD subjects. Finally, late positive Slow Wave activity resolves more slowly in YNG subjects, perhaps indicating lack of certainty or closure and continued processing.

194.2 EVENT-RELATED POTENTIAL (ERP) INDICES OF DEVELOPMENTAL LAG IN CHILDHOOD SCHIZOPHRENIA. R.J. Strandburg, J.T. Marsh, W.S. Brown*, R.F. Asarnow* and D. Guthrie*. Dept. of Psychiatry, UCLA School of Medicine, LA, CA 90024.

The authors have previously reported a number of ERP concomitants of an information processing impairment observed in schizophrenic (SCH) children. When compared with age-matched (OLD) normals, differences were observed in the magnitude and spatial distribution of the CNV, N1, P3 and Slow Wave components. These suggest deficits in the ability of SCH children to (1) mobilize and direct attention and (2) locate and identify target stimuli.

In this study ERPs from SCH children (age 11.5yrs) were compared with those of children 3 years younger (YNG) to determine if these deficits might represent developmental delays. ERPs were recorded during the performance of a task involving the discrimination of a randomly placed target in multiple letter arrays presented for 50ms, 0.5sec after a warning tone. A widely distributed array of scalp electrodes was used and ERPS were averaged over a 1.5sec interval.

The SCH group resembled YNG subjects (while at the same time differing from OLD children) in 4 respects: both exhibit sluggish and poorly stimulus coupled CNV activity; both show a large, prolonged negative shift over right frontal cortex; both fail to produce the lateralized visual N1 enhancement seen in OLD subjects over right temporal cortex; the SCH children demonstrate an even greater degree of amplitude and latency attenuation late positive components (coupled with even more pro-longed late Slow Wave activity) than was seen in the YNG relative to the OLD group.

However, SCH subjects do not show the large amplitude, slightly delayed early components (visual P1-N1-P2) seen in YNG subjects nor were they capable of modulating N1 and Slow Wave activity in response to variations in task demand as was the case with both normal groups. Thus, important ERP differences exist between SCH and YNG subjects which suggest that while inefficiencies in (1) the mobilization of general attention and (2) complex information processing may reflect developmental delays in the SCH children, they also exhibit specific attentional deficits not seen in either of the normal groups.

194.3 HUMAN CORTICAL PATTERNS OF "P300" POTENTIALS TO NOVEL VISUAL ITEMS. Merle Prim+, George Ojemann, and Ettore Lettich*. Dept. of Neurological Surgery, Univ. of Wash., Seattle, WA and Dept. of Psychology, Western Wash. Univ., Bellingham, WA.(+)

The scalp electroencephalographic evoked potential (EP) change associated with novel, attended information is a positive wave peaking at about 300 msec after onset of the attended item, the "P300". The effects of behavioral manipulation on this EP have been extensively investigated, but little is known about the brain mechanisms that generate it. We report here EP's recorded (to a neck reference) directly from human cortex through subdural electrodes placed for the diagnostic evaluation of medically intractable epilepsy, in 8 cases, during a "visual odd-ball" task, comparing averaged EP's for the 31% counted novel diamonds interspersed among repeated squares (diamonds turned 45^0), each shown for 100 msec at 4 second intervals. Electrode location varied as determined by clinical factors, but was verified by x-rays.

An augmented P300 to novel items was recorded from scalp Cz leads in 6 of 8 cases. In the case where subdural electrodes overlay a previous anterior temporal resection, EP patterns were of low amplitude, indicating little subdural volume conduction. Large positive-negative-positive complexes for both novel and repeated items were often discretely localized to single subdural electrodes, especially in either temporal lobe. These were only partly reflected in simultaneous recording from overlying scalp in the one case where this was done. Localization of these EP's was unrelated to the site of that patient's epileptic focus. Alterations in the EP's to novel, compared to repeated items include: 1. An augmented "P300" in lateral temporal cortex in 5 of the 6 cases with recording there. The maximum P300 augmentation was localized to anterior-midtemporal cortex in 4. 2. An augmented N300 in medial temporal cortex, in phase with the augmented P300 for lateral cortex in the same patient, in 2 of the 5 cases with recordings there. 3. An augmented N300 in medial parieto-occipital cortex, in phase with an augmented P300 from lateral parietal cortex in the patient with recordings in that plane. 4. Diminution of EP's to novel items from medial frontocentral sites. These findings indicate phase-reversal of P300 between medial and lateral sides of the posterior half of cerebral hemisphere, as suggested by earlier studies of P300 localization through medial temporal implanted electrodes (Halgren et al, Science 210:803; Woods et al, Conf. on Cog. Neurosciences, 1982). But contrary to those studies, our data indicate that the generator is much more widespread than just the hippocampus (or even limbic cortex). Similar preliminary data have been obtained using novel auditory items.

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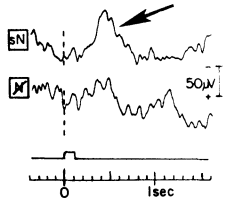
194.4 ELECTROCORTICOGRAPHIC (ECoG) CORRELATES OF NAMING, READING AND VERBAL MEMORY. G. Ojemann and E. Lettich*. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

ECoG correlates of language that have behavioral specificity (present during language tasks and not spatial tasks using the same visual input) and anatomic specificity (present at sites independently related to language functions by electrical stimulation mapping, and not at unrelated sites) were measured in awake patients undergoing craniotomy under local anesthesia for the treatment of medically intractable epilepsy. Such averaged ECoG changes during silent naming (with verbal output delayed to a cue) were previously reported by us (Fried, Ojemann and Fetz, Science 212:353) to include simultaneously recorded slow potentials at frontal, and desynchronization at posterior language sites, suggestive of thalamo-cortical activity in human cortical language mechanisms. We have now studied additional cases, silent reading, and in one unique case, the relation of ECoG changes with silent naming and reading to cortical sites related by stimulation mapping to naming, reading, short-term verbal memory, orofacial motor control, and measures of language understanding (mapping techniques described in Ojemann and Mateer, Science 205:1401).

In these new cases, slow potentials lasting up to 1 sec, specific to silent naming at naming sites have been identified in both posterior frontal and superior temporal lobes, the former a correlate of effector readiness in a final motor pathway for speech, the latter perhaps a correlate of a system common to sequential orofacial movements and speech sound decoding. Desynchronization at other posterior sites was quantified by spectral density measures, with decreased variance in the 10-11 Hz band 700-1200 msec after onset as an anatomically and behaviorally specific change during silent naming. These patterns are also present in ECoG changes during silent reading, but with a different localization, paralleling the differential localization of naming and reading with stimulation mapping. A unique averaged ECoG potential identified at a site related by stimulation only to short-term verbal memory, and present only during tasks requiring recent memory, is shown in the figure (upper trace: ECoG with silent naming, second: spatial task using the same 100 msec visual input (marked in third trace), as recorded from the memory site. Arrow identifies the unique potential). This appears to be an ECoG hallmark of recent memory, perhaps indicating when it is active during language processes.



Supported by NIH Grants NS 04053 and NS 17111.

194.5 BRAINSTEM AUDITORY EVOKED POTENTIALS AND DICHOTIC LISTENING: DO THEIR ASYMMETRIES CORRELATE? R.A. Levine*, D. Isenberg*, and P. McGaffigan* (SPON:E. Keithley). Eaton-Peabody Lab., Mass. Eye and Ear Inf., Boston, MA, 02114

Significant asymmetries of the brainstem auditory evoked potentials (BAEPs) occur in peaks I(-), III(+), and IV(+) when the BAEPs (recorded from a midline pair of electrodes) from right ear stimulation are compared to the BAEPs from left ear stimulation (Levine, R.A., McGaffigan P. Electroencephalogr Clin Neurophysiol, 1983 (in press)). For all three components with significant asymmetries, the peaks from right ear stimulation were larger than for left ear stimulation. The asymmetry is stronger for right-handed than left-handed individuals. The dichotic listening test is a behavioral auditory task that also shows a right-left asymmetry. The asymmetry for dichotic listening also favors the right ear and is stronger in right-handed individuals. Is the asymmetry observed in the BAEPs related to that found in the dichotic listening test? To answer this question, the two tests were done on the same 32 normal subjects (half male, half female; half right-handed, half left-handed), and their results compared. The Wexler-Halwes dichotic listening test was administered in the standard manner. The BAEPs were obtained by presenting monaural clicks (10/sec, 70dB SL) first to one ear then the other ear. The amplitudes of peaks I(-), III(+), and IV(+) for left ear stimulation were then subtracted from the corresponding amplitudes for right ear stimulation. As expected, significant right-left asymmetries were found for both the dichotic listening test and the BAEPs. The correlation coefficients between these amplitude differences and several measures of asymmetry on the Wexler-Halwes dichotic listening test were then determined. For none of the three peaks was there a strong correlation ($p<.05$) with any of the measures of asymmetry in dichotic listening. When right-handers and left-handers were considered as separate groups, a weak correlation ($p=.05$) was found for peak I(-) in right-handers only; otherwise, no significant correlations were found. These results suggest little relationship between the asymmetries of this dichotic listening test and the BAEPs.

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194.6 LATERALIZATION CUE IN THE DETECTION OF SIGNAL IN NOISE. P.W.F. Poon, J.C. Hwang, and K.T. Tam*, Dept. of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

The use of lateralization cue in the detection of brief acoustic signals embedded in background noise was accessed in 4 human subjects using psychophysical procedures that involved headphone presentation of binaural sounds.

The acoustic stimulation consisted of a burst of noise (20 to 20 kH_z white, 100 msec long) that served as the masker, and a simultaneous train of clicks (derived from 0.1 msec rectangular pulses, repeated at 7 H_z) that served as the signal. While the noise masker was centralized throughout the experiment, the click signals could be either centralized or lateralized to various extent by manipulating the interaural time difference. In each trial, the subjects were required to indicate, under a two-alternative force-choice paradigm, the presence of the click signals that had been delivered (at 50% probability of occurrence across trials) along with the noise masker. Their ability to detect the masked signal was determined as a function of interaural time differences of the clicks (0 to 1.0 msec) at fixed click-to-noise intensity ratios.

The results invariably showed that the subjects' ability to detect the presence of the clicks in noise was poorest when both the signal and masker were centralized and improved progressively when the signals became increasingly lateralized from the centralized masker towards either side. This suggested that lateralization cue was utilized in the detection of the signal in noise delivered in the present manner.

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194.7 ABDOMINAL EMG ACTIVITY DURING SPEECH, J. Hoit-Dalgaard* R. Lansing, B. Plassman* and T. Hixon*. Depts. of Psychol. and Speech & Hearing Sciences, Univ. of Arizona, Tucson, AZ 85721.

EMG activity of the abdominal wall during speech was studied in normal adult subjects. The investigative protocol was designed to determine the effects of electrode placement, body position and lung volume on speech-related abdominal EMG levels.

Ten young adults, five females and five males, served as subjects. Five pairs of electrodes were applied to the right side of the abdomen; one pair each on the upper and lower lateral aspects to monitor the combined activity of the external and internal oblique, and transverse abdominis muscles, and three pairs placed rostrally to caudally along the rectus abdominis. Magnetometers, fixed to the rib cage and abdomen, transduced anterior-posterior diameter changes into linear representations of torso shape and lung volume which were recorded by inkwriters along with the EMG tracings. The protocol consisted of vital capacity maneuvers, isovolume maneuvers, muscle separation maneuvers, and a variety of speech and reading tasks performed in the supine and upright positions.

Data analysis revealed that EMG levels recorded in the supine position were lower overall than the upright EMG amplitudes. In the supine position most subjects showed an increase in lateral EMG levels during speech by the time lung volume fell below FRC. Rectus activity could be seen only when a subject reached very low lung volumes. An increase in lateral EMG levels at utterance onset was exhibited by fewer than half of the subjects when supine, and then only inconsistently.

Abdominal EMG levels recorded in the upright body position were higher than those recorded in supine. Unlike the supine data, speech initiation was associated consistently with an immediate rise in lateral EMG amplitudes. Lateral EMG continued to augment and rectus activity rose above resting levels when the subject spoke below FRC. Finally, most subjects in the upright position evidenced brief "shut downs" of active regions to below resting levels when inspiring between spoken breath groups. These shut downs appear to be tied to the speed of inspiration.

Absolute EMG levels and changes in EMG levels were substantially greater in the two lateral EMG signals than in the three rectus EMG signals under both resting and speaking conditions. In contrast, laughter was accompanied by large EMG bursts from all electrode sites. Coughing and throat clearing showed similar patterns.

This study elucidates the regional differences in abdominal EMG activity during speech and shows that EMG levels are position and lung volume dependent. Our data documenting the presence of abdominal activity in speech directly contradict those of Draper, Ladefoged and Whitteridge (1959).

194.8 SOURCES OF LATE COMPONENTS OF THE BRAIN MAGNETIC RESPONSE, F. Richer, R.A. Johnson* and J. Beatty. Department of Psychology, University of Calif., L.A., Ca. 90024.

The ability to localize tangential current sources in the cortex with neuromagnetic recordings was used to investigate the origin of late components of the brain event-related magnetic fields (ERFs) in perceptual discrimination tasks. We have recorded a set of late components in the ERF which are modality-specific, originating in separate locations in auditory and visual oddball-counting tasks. Attended rare stimuli presented randomly in a sequence of standard stimuli differing in frequency or visual pattern configuration elicited components with latencies as high as 450 msec poststimulus with a rather restricted scalp distribution reversing polarity over a few centimeters. We have shown that emitted and evoked late fields originate in the same location for a particular modality. The late magnetic response is sensitive to the probability of the oddball stimuli and to the complexity of the discrimination performed. The field is best accounted for by sources located in each hemisphere and there are slight inter-individual variations in the location and strength of these sources. In the auditory modality, the late components appear to originate in superior temporal cortex and are oriented in a ventro-dorsal axis about 3 cm below the scalp. Sources in the left hemisphere are more posterior than sources in the right hemisphere, which is consistent with the known anatomy of auditory cortices. Late visual ERFs cannot be recorded from the locations in which late auditory ERFs are observed and appear to originate in a more posterior location in both hemispheres. The latency and elicitation parameters of one of the late components appear to correspond to that of the P300 complex of the event-related potential observed at the scalp.

194.9 DEXAMETHASONE SUPPRESSION TEST AND MOOD FOLLOWING STROKE

J.R. Lipsey*; R.G. Robinson, and G.D. Pearlson (SPON: J.R. DePaulo) Dept. of Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

46 patients admitted to rehabilitation hospital following thromboembolic stroke or intracerebral hemorrhage were evaluated with standardized depression rating scales (Hamilton, Zung, modified PSE) and were given 1 mg overnight dexamethasone suppression tests (DST). An Overall Depression Score (ODS) (range 0-15) was calculated from the Hamilton, Zung, and PSE scores; and DSM III diagnoses of major depression (MD) were determined from PSE responses. Patients also were categorized as to presence or absence of moderate to severe disturbance of mood, sleep, or appetite (MSA) (at least 1 of the 3). The following tables show DST results for each clinical category.

	DST+	DST-		DST+	DST-		DST+	DST-
ODS≥9	14	5	MD+	11	3	MSA+	21	10
ODS<9	11	15	MD-	15	17	MSA-	5	10
	(p<.05)			(p<.05)			(p<.05)	

Patients with positive DSTs had significantly higher Hamilton (p<.0125), Zung (p<.025), PSE (p<.0125), and ODS (p<.01) scores than patients with negative DSTs. Patients with positive DSTs were not significantly different in terms of age, race, sex, socioeconomic status, marital status, psychiatric history, time since stroke, or cognitive impairment than patients with negative DSTs. However, positive DSTs were significantly associated with greater functional impairment.

194.10 CAN POST-STROKE DEPRESSION PRODUCE A "PSEUDO-DEMENTIA"?

R.G. Robinson, K.L. Kubos*, J.R. Lipsey*, L.B. Starr* and T.R. Price. Dept. of Psychiatry, Johns Hopkins Univ. Sch. Med. and Dept. of Neurology and Psychiatry, Univ. MD. Sch. Med., Baltimore, MD 21205.

Patients (N=30) with a single CT scan verified stroke lesion of either the right or left hemisphere were evaluated for intellectual impairment and depression. Stroke patients with significant intellectual impairment (Mini-Mental Exam score (MME) below 23) (N=17) were not significantly different from patients without impairment (N=13) in their age, sex ratio, years of education, or lesion location along the A-P axis. The volume of the stroke lesion however was significantly larger in patients with MME scores below 23 as compared with those patients with scores above 23 (6.1 ± 5.2 SD vs. 2.4 ± 4.3 SD). Non-depressed patients with intellectual impairment showed a highly significant correlation between the volume of brain lesion and the degree of intellectual impairment ($r = -.94$ $p < .001$). Patients with intellectual impairment and symptoms of major depression (N=7), however, showed no significant correlation between lesion volume and Mini-Mental score ($r = -.12$ NS). In addition, all patients with major depressive symptoms showed intellectual impairment (7 of 7) while patients without major depression showed intellectual impairment in less than half of the cases (10 of 23) ($\chi^2 = 6.8$ $p < .05$).

Finally, the lesion volume was significantly smaller in patients with depression and intellectual impairment as compared to those with intellectual impairment and no depression (3.1 ± 1.3 SD vs. 8.6 ± 5.2 SD $p < .05$). One patient treated with nortriptyline for depression showed a significant increase in MME score after the depression was improved.

These data suggest that intellectual impairment in non-depressed stroke patients is related to lesion volume. Depressed stroke patients, however, appear to have a different etiology for their intellectual impairment. The depression itself may contribute to the intellectual decline in some patients and may represent another form of treatable dementia.

194.11 DIFFERENCES IN RECOGNITION MEMORY AMONG PATIENTS WITH ALZHEIMER'S DISEASE, HUNTINGTON'S DISEASE OR ALCOHOLIC KORSAKOFF SYNDROME.

M. Moss, M.S. Albert, M. Payne* and N. Butters. Dept. of Anatomy. Boston Univ. School of Medicine, Depts. of Psychiatry and Neurology, Massachusetts General Hospital and Psychology Service, Boston V.A. Medical Center, Boston, MA.

Memory loss constitutes one of the more striking cognitive impairments in various dementing disorders. While emphasis has primarily been placed upon the documentation of such deficits, only recently has attention been paid to the elucidation of their possible disease-specific nature. In order to adequately assess and compare memory function in such groups of patients, particularly in those with severe dementia, tasks which are simple to perform, yet sensitive to memory dysfunction, must be used. We have developed such a task by modifying one which was designed to assess spatial recognition memory in non-human primates (Rehbein, 1983) and administered it to patients with Alzheimer's Disease (AD), Huntington's Disease (HD), or alcoholic Korsakoff syndrome (KS) as well as to normal control subjects.

The task requires the subject to identify, in successive trials, a new stimulus among an increasing set of previously presented familiar stimuli displayed on a board with a 5 x 6 matrix. Five different classes of stimulus material; spatial, verbal, color, pattern and faces, were presented for five sets of trials each. In the verbal condition, 16 stimuli were presented successively regardless of the number of errors committed. In all other conditions, the presentation of the new stimulus was terminated after the first error. In the verbal condition, patients were also asked to recall the stimuli after a 15 sec. and 120 sec. delay.

For recognition, results showed that the AD and KS groups were impaired relative to the normal group on each of the five stimulus conditions. However, the group of HD patients, though impaired on four of the conditions, were unimpaired as compared to normals when verbal stimuli were used. There were also significant differences between groups on delayed recall of the verbal stimuli. At 15 sec. the AD, HD and KS groups, though not significantly differ- from each other, were impaired relative to the normal group. However, at 120 sec, the KS and HD groups performed significantly better than did the AD group. These results suggest that the rate of forgetting in patients with AD is greater than that in patients with either HD or KS.

The normal performance on verbal recognition by the HD group together with the observed difference in forgetting rate among the groups 1) helps account for recent evidence that memory performance of patients with HD is enhanced with the availability of verbal mediation (Butters et al, 1983) and 2) lends support to the notion that AD, HD and KS patients have qualitatively different memory impairments. Supported by Grants AG02269, NS16367 and NS19416.

195.1 PUTATIVE NEUROTRANSMITTERS IN NEOSTRIATAL NEURONS: A LIGHT AND ELECTRON MICROSCOPIC STUDY. Ronald H. Bradley, S.T. Kitai and J. Y. Wu*, Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824-1316, *Baylor College of Medicine, Houston, TX 77030.

We have previously shown at the light level that rat neostriatal projection neurons contain substance P(SP) and methionine enkephalin(ME). Using a double labeling method with retrogradely transported wheat germ agglutinin(WGA) and GAD immunocytochemistry we found that rat neostriatal neurons also contain GABA(Neurosci. Abs. 48:19,'83). In order to ultrastructually characterize these immunocytochemically identified SP, ME and GABA containing neostriatal neurons we utilized a modified "avidin:biotin" technique. Antisera to SP, ME, and GAD were used and anti-GABA-transaminase (GABA-T) was used to compare GAD and GABA-T staining patterns.

To increase somatic concentrations of neurotransmitters, colchicine was injected into the lateral ventricles. Following 48hrs survival time, rats were perfused according to our previous procedures(Neurosci. Abs. 48:19,'83). The brains were removed and sectioned at 60μm with a Vibratome. Sections were rinsed in MSB buffer, incubated 24 hrs with SP, ME, GAD or GABA-T antiserum and washed with tris buffered saline(TBS). Sections were incubated 2hr with biotinylated IgG, rinsed with TBS, incubated with reagent-peroxidase, rinsed with TBS and reacted with DAB. The sections were rinsed with TBS, osmicated, rinsed in TBS, enbloc stained and embedded in Epon-Araldite and viewed unstained in a JEOL 100CX.

SP, ME and GAD staining was localized in medium-sized neostriatal neurons(soma size:10-15μm) possessing a smooth, round and unindented nucleus and a thin rim of immunoreative cytoplasm. Immunoreactivity was observed in primary dendrities, dendritic spines and presynaptic terminals. ME and GAD staining was localized primarily in symmetrical boutons while SP staining was localized in asymmetrical terminals. GABA-T staining was localized in medium(10-16μm) and large(25-30μm) sized neostriatal neurons. The medium sized neurons had either an unindented nucleus or deeply invaginated nucleus and a thin rim of immunoreactive cytoplasm. GABA-T staining was observed in soma, dendrites and dendritic spines.

These results indicate that SP, ME and GAD staining is localized in somata, dendrites and dentritic spines ultrastructually characteristic of medium-spiny neostriatal neurons. GABA-T staining is observed in neostriatal neurons which ultrastructually resemble the large aspiny, medium spiny and aspiny cell types. The lack of GABA-T staining in presynaptic terminals suggests that GAD localization is a better marker for GABA neurons than GABA-T localization. (Supported by NIH Grant RR 05772 to R.H.B. and NS 14866 to S.T.K.).

195.2 MORPHOLOGY AND SYNAPTIC CONNECTIONS OF A GIANT ASPINY INTERNEURON IN THE RAT NEOSTRIATUM. C. J. Wilson, H. T. Chang and S. T. Kitai, Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824.

Intracellular recordings were obtained from a sample of giant aspiny neurons identified by their appearance after staining by intracellular injection of horseradish peroxidase and preparation for light and electron microscopic examination. The neurons corresponded to the giant interneuron with radiating dendrites described by Kolliker in 1896, but denied by most subsequent authors. The somata were multipolar or fusiform and could be up to 50 μm in their maximum dimension. They had only a few very stout and straight dendrites that branched and radiated to extend up to 600 μm from the soma. Some dendrites terminated with tufts of fine branched processes that could exhibit varicosities or spine-like appendages. The axons of the cells bifurcated into approximately equal caliber branches that each repeatedly branched in the same way. These formed a dense plexus of very fine varicose fibers extending throughout the dendritic field of the cell and somewhat beyond. There was no main axon that could be identified throughout this plexus and no branch that left the area of the dendritic field. Electron microscopic examination of the axonal field showed it to form numerous synaptic contacts both at the varicosities and along the intervaricose segments. The latter could be as small as 0.1 μm in diameter. The synaptic vesicles were of the smallest diameter class observed in neostriatum and were highly pleomorphic. Most of the postsynaptic elements were shafts of dendrites.

The cells exhibited prominent short latency EPSPs in response to stimulation of thalamus, substantia nigra or cerebral peduncle. The EPSPs were judged to be monosynaptic on the basis of their latency invariance with changes in stimulus intensity. They were similar to those evoked in neostriatal spiny projection neurons by the same stimulation. Only one or two action potentials were evoked by maximal stimulation, and the cells did not exhibit a high rate of spontaneous activity in urethane-anesthetized animals.
(Supported by NIH Grants NS 17294 to C.J.W. and NS 14866 to S.T.K.).

195.3 MORPHOLOGY OF SOMATOSTATIN NEURONS IN THE HUMAN PUTAMEN; A CORRELATIVE IMMUNOCYTOCHEMICAL-RAPID GOLGI STUDY. R.S. Williams, P.E. Marshall* and D.M.D. Landis. Neurology Service, Mass. General Hospital, Boston, Ma. 02114.

In the putamens of young adults without neurological disease, high quality immunocytochemical (ICC) preparations were obtained with antisera to somatostatin using the Avidin-Biotin technique. Neurons with somatostatin-like immunoreactivity (SLI) had a distinctive morphology. Cell somas were small to medium-sized, and oval to fusiform in shape (12-20 um minor axis,15-30 um major axis). There were 2-4 (50% had 3) stout primary dendrites which often exited from opposite poles. Subordinate dendrites of second to fourth branch order were stained also; they were sparsely branched and many segments were exceptionally long (>150 um). Axon-like processes were tentatively identified on most cells, based upon: 1). A tapering initial segment emerging from the soma or a primary dendrite , 2). A beaded appearance, 3). A meandering course radially outward from the cell soma and 4). Sparse numbers of beaded collaterals emerging at near right angles. We compared the morphology of SLI neurons to the neuron subtypes identified in rapid Golgi impregnations of the same and other human brains. The morphology of SLI neurons is different from that of typical medium spiny, large aspiny or a distinctive ("clewed", "spidery") subclass of small to medium-sized aspiny neurons, as described by others in a variety of species. The dendritic and axonal morphology of SLI neurons corresponds best to a small to medium-sized cell with dendrites that are both relatively aspiny, or invested sparsely in patchy fashion with spines of highly variable morphology. Our findings in the human striatum contrast with those in the rodent where ICC-electronmicroscopic studies indicate that SLI neurons are aspiny (DiFiglia and Aronin, J. Neurosci. 2:1267,'82), and suggest that the presence or absence of spines is a less reliable criterion for defining the class-characteristic morphology of SLI neurons in the human striatum. (Research supported in part by NIH grants MH 34079 and NS 16367.)

195.4 A GOLGI STUDY OF THE NORMAL HUMAN NEOSTRIATUM. G.A. Graveland, M. DiFiglia, R.S. Williams, (SPON: N.Aronin) Dept. of Neurol., Mass. Gen. Hosp. Boston, MA 02114.

As a background for the study of neuropathologic changes associated with basal ganglia disease, the human neostriatum was examined in Golgi impregnations (Kopsch or rapid methods) of normal tissue obtained within 18 hours postmortem from 28 adult cases (ages 22-99). At least 6 types of neurons were observed. The most frequent was a medium size neuron (15-18μm) with numerous dendritic spines which appeared mostly on secondary and distal branches. The longest dendrites from each of 25 well impregnated cells (N=3 cases) were examined for spine types and their density distribution. The overall density of spines was 7.7/10 m dendrite length. Spines appeared about 25 μm from the soma and maintained a maximum density (X=9.6/10 μm) 60-200 μm from the cell body. Thin, stubby and mushroom shaped spines were present at a frequency of 74, 16 and 10% respectively. The axons of spiny neurons were long with up to 5 collaterals arising and branching within the dendritic field of the cell. A smaller population of medium size neurons with relatively low spine density was observed in the same preparations. Aspiny neurons of medium size (14 to 18 μm) were seen frequently. The dendrites were smooth, varicose and in most cases highly branched and curly. The axons within 70 μm of the soma, gave rise to a profuse arborization of fine beaded processes which frequently extended beyond the dendritic field. Large neurons (30 μm in size) with either smooth, highly branched, varicose dendrites and short axons or sparsely branched, long, thick, spiny, dendrites were observed less frequently. A heterogeneous group of small neurons (10-12 μm) was relatively numerous in our series. Cells were usually unipolar or bipolar with somatic spines. Dendrites were thin, sparsely branched, and exhibited varicosities and/or spines. Many long dendritic appendages appeared in some neurons. Axons were thin and poorly arborized. Four categories of afferent fibers were also recognized: 1. large diameter fibers (>1.0 μm) with numerous smooth branching processes; 2. thick axons with large bulbs (2 μm in size); 3. axons (0.5-0.7 μm) with short appendages, and 4. thin axons (0.5 μm) with fine beaded processes. The present findings confirm and extend recent Golgi observations in the human caudate (Eder et al, Acta Morphol. Acad. Sci. Hung. 28, 1980; Braak and Braak, Cell Tissue Res. 277, 1982). Although many similarities to the monkey (Fox et al, J. Hirnforsch. 13 1971; DiFiglia et al, Brain Res 114, 1976) exist, the human neostriatum exhibits overall a greater variation within cell types and quantitative differences in the morphology of spiny neurons. Supported by grants NS 16367 (M.D.), H.D. Foundation (M.D.), and the Stone Fund (G.A.G).

195.5 NEURONAL ORGANIZATION IN THE MONKEY NEOSTRIATUM: A QUANTITATIVE LIGHT MICROSCOPIC STUDY USING SEMI-THICK SERIAL SECTIONS. M. DiFiglia and G.A. Graveland* Dept. of Neurology, Massachusetts Gen. Hosp., Boston, Ma. 02114.

Golgi studies of the neostriatum in a number of species have shown that the population of small to medium size neurons visible with Nissl stains is composed of at least 4 cell types, of which spiny neurons are thought to be the most numerous. Aspiny neurons however are observed relatively frequently in the monkey and human brain (DiFiglia et al., Brain Res. 114 1976; Graveland et al., Neurosci. Abst. 1983). Ultrastructual studies of individually identified neurons using the combined Golgi-electron microscopic method (DiFiglia et al., J. Neurocytol. 8, 1980, DiMova et al, Neurosci. 5, 1980; Somogyi and Smith, Brain Res. 178, 1979) and intracellular filling with HRP (Wilson and Groves, J. Comp. Neurol. 194, 1980; Bishop et al., Neurosci. 7, 1982) have shown that medium size spiny neurons and aspiny neurons can be distinguished by features characteristic of their nuclei and cytoplasm. The nuclei of spiny neurons are unindented whereas those of spiny cells exhibit shallow and/or deep enfoldings. In the present study the occurrence and distribution of neurons with nuclear indentations was examined in random samples of monkey (N=3) caudate-putamen. Epon-embedded blocks (1.5 mm^2) of well-fixed tissue were cut with an ultramicrotome (LKB Nova). Sections, 0.5 µm thick, were mounted serially (10 to 20 sections) on glass slides, stained with toluidin blue and coverslipped. An area approximately 250 μm^2 from a middle section in each series, was selected and drawn at 440X with camera lucida. All neurons with nuclei present (approximately 100 cells per sample) were recorded and blood vessels and fiber bundles were included for orientation. With the drawing as a guide, sections preceding and following the drawn section were examined at 100X. Five consecutive sections were usually sufficient to assess nuclear morphology. Results showed that neurons of small to medium size with indented nuclei accounted for 26.4% ($\bar{X}$=26.4, SD ± 7.6, n=10) of the total neuronal population sampled (1065 neurons). Nuclear indentations were seen in all large neurons (<30 µm) which accounted for an additional 1%. The number of small to medium cells with nuclear indentations was significantly greater ($p<.02$) in the caudate ($\bar{X}$=31.2, SD ± 6.3) than in the putamen ($\bar{X}$=21.6, SD ± 5.9). These findings suggest that a considerable proportion of the small to medium size neurons in the monkey neostriatum may belong to the aspiny category and that they may be more numerous in the caudate than the putamen. Results may be helpful in evaluating the organization of neostriatal neurons in neuropathological and experimental in vivo and in vitro preparations. Supported by NIH Grant NS 16367 (MD) and a grant from the Hereditary Disease Foundation (MD).

195.6 DEVELOPMENTAL RELATIONSHIPS BETWEEN OPIATE RECEPTORS AND DOPAMINE IN THE FORMATION OF CAUDATE-PUTAMEN PATCHES. D. van der Kooy Neurobiol. Res. Group, Department of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

"Patchy" distributions of various neurotransmitters, receptors and enzymes are seen in sections through the rat caudate-putamen. Some of these patchy markers change their distribution during development. In particular, the dopamine fibers from the midbrain are distributed in the caudate-putamen in a patchy manner perinatally, before becoming diffusely distributed over the early postnatal weeks. On the other hand opiate receptors are diffusely distributed prenatally but become organized in patches perinatally. We studied the anatomical relationship between opiate receptors and dopamine fibers during the first week after birth when both markers are localized to patches.

Adjacent, fresh frozen sections through the caudate-putamen of a day 5 rat pup were processed to reveal opiate receptors using ^{3}H-etorphine autoradiographic procedures and glyoxylic acid induced endogenous dopamine fluorescence using the SPG procedure. At this age, patches of dopamine fluorescence are seen most clearly in the lateral portion of the caudate-putamen, whereas opiate receptor patches are seen throughout the region. Especially in the dorsal two-thirds of the lateral caudate-putamen the opiate receptor and dopamine patches appear to be continuous. In addition, when the same sections were later Nissl stained, the opiate receptor and dopamine patches appeared to avoid the "islands" seen at this age which contain especially high densities of neuronal cell bodies.

In order to test if dopamine released from the fibers in caudate-putamen patches was important in inducing the formation of opiate receptor clusters, we injected pregnant rats daily with 2.5 mg/kg of haloperidol from 12 days after conception until they gave birth. Prenatal haloperidol treatment has previously been shown to result in a dramatic decrease in pup caudate-putamen dopamine receptors in the post-natal period. However, haloperidol did not seem to block the development of opiate receptor patches in the caudate-putamen as assessed in the pups during the first few postnatal days. Thus, the dopamine patches in the caudate-putamen do not seem to direct the formation of opiate receptor patches, although preliminary knife cut data suggests some influence from caudal brain areas may be necessary for the maintenance of opiate receptor patches.

195.7 MEDIO-LATERAL TOPOGRAPHY OF CORTICO-STRIATAL TERMINAL FIELDS IN RHESUS MONKEY. L.D. Selemon and P.S. Goldman-Rakic. Sect. Neuroanat., Yale Univ. Sch. of Med., New Haven, CT 06510.

Kemp and Powell (1970) proposed that in primates cortico-striatal projections are topographically arranged along the antero-posterior (A-P) axis of the striatum such that frontal cortex projects to anterior striatum whereas more posterior areas of cortex project to posterior striatum. However, recent studies challenge this model by showing that some areas of cortex project throughout the entire A-P axis of the striatum rather than to one A-P domain (Goldman and Nauta,'77; Yeterian and Van Hoesen,'78). In the present study, the topography of cortico-striatal terminal fields was re-examined. Thirteen rhesus monkeys were injected either with ^{3}H-amino acids or HRP in twelve different cortical sites. Animals were perfused 2-7 days later; the brains were processed either for autoradiography or HRP histochemistry. Distributions of silver grains in the striatum, representing cortico-striatal terminal fields, were examined.

The major finding of this study is that all cortico-striatal projections are segregated into parasaggital territories that are aligned along the medio-lateral axis of the striatum. Areas projecting to medial regions of both caudate and putamen include orbito-frontal cortex, anterior cingulate gyrus, and superior and inferior temporal gyri. The dorsal bank of the Sylvian fissure projects medially as well but to the putamen only. In contrast, lateral sectors of caudate and putamen receive afferents from the posterior parietal cortex and posterior bank of the superior arcuate sulcus. Cortical projections from pre- and post-central gyri are located laterally but are restricted largely to the putamen. Finally, striatal projections from prefrontal cortex on the dorsal and ventral banks of the principal sulcus, as well as from the dorsolateral convexity of the frontal lobe, are located between lateral and medial zones, in central regions of the caudate and putamen. Likewise, the posterior bank of the inferior arcuate sulcus projects to central regions of the putamen.

These findings indicate that the topographic organization of cortico-striatal terminal fields is medio-lateral rather than antero-posterior and add to the growing body of evidence that there is a "division of labor" between the caudate nucleus and the putamen. While terminal fields from association cortices are distributed to both caudate and putamen, projections from sensori-motor cortices and from premotor cortex are limited almost entirely to the putamen. These findings may prove useful to understanding the biochemical and functional heterogeneity of the neostriatum, as well as the consequences of disease in selected regions of the neostriatum. (Supported by MH00298, MH38546 and a grant from the Hereditary Disease Foundation)

195.8 AXON COLLATERALS IN THE EFFERENT PROJECTIONS OF THE NEOSTRIATUM IN THE RAT. Louise D. Loopuijt* and Derek van der Kooy (SPON: V.K. Singh) Department of Anatomy, Faculty of Medicine, University of Toronto, Toronto, Ontario M5S 1A8 Canada

It has been well established, that the efferent projections of the neostriatum have their terminals in the globus pallidus and the substantia nigra (predominantly its pars reticulata). There is circumstantial evidence that the projections to the globus pallidus represent collateral axons, that originate from striatonigral projections.

The present study was undertaken to obtain more direct evidence on the collateralization of these striatal efferents. Therefore, retrogradely transported fluorescent tracers were used: in rats the substantia nigra was stereotactically injected with either nuclear yellow (NY) or propium-iodide (PI) and the globus pallidus with 4-acetamido, 4'-isothio-cyanostilbene -2, 2' disulfonic acid (SITS). Since the striato-nigral fibres run through the globus pallidus, SITS was chosen for injection into this nucleus, because it is not taken up by fibres of passage. The amount of tracer injected into the substantia nigra with either NY or PI was 0.4 µl and into the globus pallidus 0.5 µl. Observations of histological sections of this material in the fluorescent microscope revealed that the whole globus pallidus and the major part of the substantia nigra had been reached by the tracer. Moreover, cell bodies retrogradely labeled with the fluorescent tracer injected in either terminal site were seen throughout the striatum. In part of the nucleus accumbens, cell bodies were only labeled with the tracer that was injected into the substantia nigra. The major portion of the striatum contained cell bodies that were either retrogradely double labeled (with SITS and either PI or NY) or singly labeled with one tracer. These different labeled cell bodies appeared to be intermingled.

Thus, the striatonigral efferents contain axons with collaterals that terminate in the globus pallidus, except for the fibres that originate in the nucleus accumbens. It cannot be excluded, however, that there are fibres that project on either the substantia nigra or globus pallidus without collaterals to the other target nucleus.

195.9 INTERHEMISPHERIC INPUTS TO THE NEOSTRIATUM: THE EXISTENCE OF DIVERGENT CORTICOCAUDATE PROJECTIONS IN THE CAT. C. Shiota*, R.S. Fisher, M.S. Levine, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, UCLA, Los Angeles, CA 90024.

Interhemispheric inputs to the caudate nuclei (Cd) were determined by axonal uptake and retrograde transport of multiple labels. In six adult cats, wheat germ lectin-bound horseradish peroxidase (WG-HRP) was pressure-injected into the left Cd while a fluorescent marker (bis-benzamide (Bb) or nuclear yellow (NY)) was injected into the right Cd. Four additional cats that served as controls received bilateral label injections into the ventricles and/or white matter and neocortex overlying the Cd. 24-72 hrs after injection, cats were sacrificed (aldehyde fixation). Coronal brain sections were processed with peroxidase histochemical methods compatible with the retention of Bb and NY.

As in our earlier assessments of the Cd inputs using WG-HRP alone, all of the retrograde markers used in this study labelled neuronal somata (WG-HRP) or nuclei (Bb and NY) in the same five brain sites: neocortex (Cx), intralaminar thalamus (Th), substantia nigra (SN), mesencephalic raphe nuclei (Ra), and globus pallidus (GP). Such labelling patterns were not evident in control animals. In counterstained material (ethidium bromide), the cytological characteristics of neurons labelled with Bb and NY were similar, if not identical, with those of neurons labelled with WG-HRP. Leakage of fluorescent labels into glial cells was often evident in the SN and Th (but not in the Cx).

Three brain regions (Cx, SN, and Ra) projected bilaterally to provide Cd inputs. Only the Cx contained multiply labelled neurons (e.g., WG-HRP in the neuronal cytoplasm and Bb or NY in the nucleus). Neurons with divergent corticocaudate axons were found bilaterally in the precruciate, cingulate, postcruciate, and prorean gyri. The complement of neocortical cells with divergent axonal collaterals was quite small (<1% of labelled Cx neurons) regardless of the markers used for their demonstration. The double-labelled cells were small-to-medium sized pyramidal neurons interspersed randomly in the neocortical laminae providing Cd inputs (layers 3-5 in adult cats).

These results are important because they provide a morphological basis for the functional interhemispheric coupling of the caudate nuclei shown in numerous biochemical studies. Common input to neurons (as in the Cx, SN, and Ra) projecting exclusively to the ipsi- or contralateral Cd may contribute to such coupling. However, direct inputs from Cx neurons with divergent axonal projections to both Cd could provide a simpler and more synaptically secure coupling mechanism.

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195.10 POSTNATAL DEVELOPMENT OF GABA NEURONS IN CAT: VENTRAL FOREBRAIN, PERIPALLIDAL REGIONS, AND VENTRAL TEGMENTAL AREA. R.S. Fisher, M. S. Levine, A.M. Adinolfi, C.D. Hull and N.A. Buchwald. Mental Retardation Research Center and Brain Research Institute, UCLA, Los Angeles, CA 90024.

In a series of kittens and adult cats, indirect peroxidase-antiperoxidase methods were used to demonstrate neuronal elements containing glutamate decarboxylase (GAD), the synthetic enzyme of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). This report is a light microscopic analysis of the distribution and postnatal development of GABA neurons in the ventral forebrain (olfactory tubercle-OT, islands of Calleja-IC, and nucleus accumbens-NA), peripallidal regions (vertical and horizontal limbs of the nucleus of the diagonal band-VL and HL, substantia innominata-SI, and lateral hypothalamic area-LHA), and the ventral tegmental area-VTA). Similar GAD-positive neuronal elements were previously reported in the basal ganglia. Stained punctae were GAD-positive terminals (primarily Gray Type II, axosomatic and axodendritic contacts). Stained cell bodies were GAD-positive neurons.

GABA neurons and terminals were evident in each of the examined brain sites in both kittens and adult cats. In contrast to the neostriatum, diffusely distributed GABA terminals were more prevalent in the neuropil of the OT and AC and formed dense linear arrays around cells and dendrites in the IC. Medium-sized GABA neuronal somata and dendrites were evident prominently in all of these regions. The large cells of the IC were also stained. As in the pallidal segments, many densely stained medium-to-large fusiform neurons were evident in all of the peripallidal regions. However, terminal organization varied across these brain sites. In the VL and HL numerous densely stained terminals were found, but linear and circular arrays of terminals were not as prominent as in pallidum. SI and LHA contained considerably fewer GABA terminals than the pallidum. The more diffuse terminal organization of the rostral peripallidal regions was maintained. The VTA was similar in appearance to the caudal peripallidal regions. It contained fewer, more randomly organized GABA terminals than the substantia nigra. As in the basal ganglia, the major developmental trends in all of these brain sites were: 1) terminal addition, 2) terminal organization, and 3) terminal elaboration (increasing terminal diameter and staining density). The incidence of stained cell bodies also increased with age. Terminal distribution was roughly mature by 30 days of age, but terminals were not elaborated fully by 90 days of age.

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195.11 PROJECTION NEURONS OF THE NUCLEUS ACCUMBENS: AN INTRACELLULAR HRP LABELING STUDY IN THE RAT. H. T. Chang and S. T. Kitai, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Neurons of the nucleus accumbens (NAC) project via the medial forebrain bundle (MFB) to ventral pallidum (VP), lateral hypothalamic areas, ventral tegmental area (VTA), substantia nigra (SN), and retrorubral field. Although these projection cells are reported to be medium in size, their complete soma-dendritic morphology have not been demonstrated. In this study we sought to identify the morphology of NAC projection neurons by the technique of intracellular recording and intracellular labeling with horseradish peroxidase (HRP) in male Sprague-Dawley rats anaesthetized with Urethane and Ketamine. Bipolar stimulating electrodes were placed in VTA, SN and/or MFB in order to antidromically activate NAC projection neurons. Electrodes were also placed in amygdala and/or thalamus to orthodromically activate NAC neurons. Bevelled glass micropipettes containing 4% HRP in Tris buffer (0.05M, pH=7.6) and 0.5M potassium methylsulfate were used for recording. Both antidromically activated NAC cells and those could only be activated orthodromically were labeled by iontophoretic application of HRP (3-7nA, 5HZ, 3-10 min 50% duty cycle). The criteria for antidromic activated action potentials include all-or-none at threshold stimulation, constant latency and collision with intracellularly evoked spikes. Although some cells could not be antidromically activated, their HRP-labeled axons were traced into VP, and thus were included in the present sample. The somata of NAC projection cells were round or polygonal in shape and medium in size (10-20μm). The dendrites radiated into a stellate-shaped dendritic field with a radius of 200-250μm. Four to 6 primary dendritic stems (width, 2-5μm) usually branched within a 50μm radius from the soma into 2° dendrites, some of which terminated without further branching. Frequently, however, 3° and 4° branchings occurred within short distances from the first branch point such that the terminal dendritic branches were 50-150μm in length. The surfaces of somata and proximal dendrites (within a 20μm radius from the soma) were generally smooth. More distal dendrites, however, were densely covered with both pedunculated and sessile spines. The axon usually arose from the soma and often took a tortuous course rostrally for up to 200μm before recurving back caudally toward VP. Many fine axon collaterals arose from the initial 150μm length of the main axon and arborized extensively within the parent dendritic domain. The main axons, some dividing into two branches within NAC, could be traced into VP in which terminal collaterals were observed. The overall appearance of these NAC projection cells resembled the medium spiny projection neurons of the neostriatum. (Supported by NRSA F32NS06951 to H.T.C. and NIH Grant NS 14866 to S.T.K.).

195.12 CYCLIC NUCLEOTIDE DISTRIBUTION WITHIN THE RAT STRIATOPALLIDAL PROJECTION SYSTEM. S.K. Ufkes* & M.A. Ariano (SPON: W.G. Bradley). Anatomy & Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Previous immunohistochemical studies have documented the medium spiny neuronal localization of cyclic nucleotides within the rat striatum (J. Cell Biol. 91: 287, 1981). Substance P (SP) and metenkephalin (ME) have also been localized within medium spiny cells and implicated as transmitters in the striatopallidal efferent pathway (Neurosci. 6: 1291, 1981). In order to investigate a possible cyclic nucleotide-neurotransmitter relationship within the striatopallidal neurons, these cells were identified through retrograde transport, and characterized using immunohistochemical techniques.

The fluorescent dye Evans blue (EB) was injected stereotaxically into rat brains which were processed for immunohistochemistry (Neurosci., in press). Tissue sections were examined with ultraviolet optics using a 540 nm/590 nm filter set to visualize EB-labeled striatopallidal neurons. The EB-filled cells were oval, 15 μm diameter and displayed unlabeled nuclei. SP- and ME-containing elements were detected with specific rabbit polyclonal antibodies (ImmunoTech, Inc.). Cyclic nucleotide immunoreactive cells were identified with antisera synthesized in our lab. 485 nm/520 nm filter combination was used to detect the fluorescein-labeled immunofluorescent striatal elements. The immunohistochemical staining patterns of the cyclic nucleotides, ME and SP were similar and in agreement with previously published descriptions (J. Cell Biol. 91: 287, 1981; Acta Histochem. Suppl. B24: 97, 1981; J. Neurocytol. 12: 325, 1983).

Of the cyclic GMP reactive elements, 98.2% were identified as striatopallidal efferent neurons, and 97.4% of the striatopallidal neurons contained cyclic GMP. 99% of the cyclic AMP-reactive cells co-localized with striatopallidal neurons, and 95% of the identified neurons contained cyclic AMP. Of the SP and ME immunoreactive elements, 96% were coincident with striatopallidal neurons, while 87.5% and 83.7% of the striatopallidal neurons contained SP or ME, respectively.

The large proportion of striatopallidal neurons which contain SP, ME, and the cyclic nucleotides supports a link between the primary neurotransmitters and the secondary messengers. Our findings demonstrate a 28.9% coincidence of cyclic AMP-staining within striatonigral neurons, in contrast to the 99% coincidence of cyclic AMP present within the striatopallidal system. This variance may reflect a preferential utilization of the cyclic nucleotides in distinct modes of information processing in these different pathways.

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195.13 WEAVER MUTATION HAS DIFFERENTIAL EFFECTS ON THE DOPAMINE INNERVATION OF THE LIMBIC AND NON-LIMBIC STRIATUM. S. Roffler-Tarlov and A.M. Graybiel, Depts. Neurol. & Anat., Tufts Univ. Sch. Med., Boston, MA 02111 & Mass. Inst. Tech., E-25, Cambridge, MA 02139.

The dopamine (DA)-containing projection to the striatum contains mesolimbic and nigrostriatal components considered to be functionally and anatomically distinct. The mesolimbic DA system projects mainly to the ventral striatum, which includes regions related to limbic forebrain: the nucleus accumbens (NAc), olfactory tubercle (OT), and ventral caudoputamen (CPv). The nigrostriatal DA system projects to the large remainder of the caudoputamen (CP), which is called the dorsal striatum and is interconnected with sensorimotor regions. Here we show that these major DA-containing systems are differentially affected in an inherited disorder and, in particular, that mesolimbic projections to the NAc and associated medial OT are differentially spared whereas the nigrostriatal projection is severely affected. The weaver mouse (wv) carries an autosomal recessive mutation well known for its effects in the cerebellum and recently shown to produce a severe DA deficiency in the forebrain (Schmidt et al., J. Neurosci,'82). We have measured the DA content of retina, midbrain and the three divisions of the striatum (CP, NAc and OT). Catecholamines were extracted from samples dissected from serial brain slices (or whole retina) and were separated and measured using HPLC followed by electrochemical detection. The 15 weaver (wv/wv) mice and 15 littermate controls (+/wv) examined were on a C57BL6/CBA background and were 1-9 mos. old. Homozygous (+/+) controls have not yet been studied. Values were expressed as pmoles DA/mg protein (mean ± SEM). DA in weaver retina was normal (7.0±.6 in controls, 7.9±.5 in weavers). DA depletion was highly nonuniform in the striatum. DA was reduced 68% in the CP, was entirely conserved in the NAc, and was reduced 32% in the OT. The values were: for CP, 245±19 in controls, 78±5 in weavers; for NAc, 239±16 in controls, 234±14 in weavers; and for OT, 228±10 in controls, 156±10 in weavers. Midbrain DA was reduced by 33% (6.9±.5 in controls, 4.6±.4 in weavers). Neither the pattern nor the extent of these DA losses changed from 1-9 mos.

Catecholamine glyoxylic acid fluorescence in the striatum was studied in 11 weavers and 13 controls. All striatal subdivisions fluoresced brightly in controls. The pattern of residual fluorescence in weavers recalled maps of the limbic striatum: fluorescence was weak in the dorsal CP, gradually increasing ventrally; was very strong in the NAc; and was intermediate in the CPv. There was also a mediolateral gradient in the OT, fluorescence appearing normal medially but weak laterally.

We conclude that DA fiber systems innervating limbic and non-limbic striatum can be influenced separately in an inherited disorder. Funded by NIH 2S07-RR05598; EY02866; NSF BNS8112125; and by the Wills Foundation.

195.14 LIGHT AND ELECTRON MICROSCOPIC IDENTIFICATION OF IMMUNOCYTOCHEMICALLY LABELED SEROTONINERGIC AXONS IN THE SUBSTANTIA NIGRA OF MONKEYS. G. Holstein*, T. Pasik and P. Pasik (SPON: J. Weinberger). Dept. Neurol., Mount Sinai Sch. Med., New York, N.Y. 10029

Monkeys (M. fascicularis) were perfused briefly with 4% pure formaldehyde and 0.25% purified glutaraldehyde in 0.12M phosphate buffer. Some animals received an intracerebral 3 µl injection of 2.5% colchicine solution 16 hr before perfusion. Others were Pargyline (75 mg/kg) and L-tryptophan (100 mg/kg) i.p., three and one hr, respectively, prior to sacrifice. Vibratome 40 µm sections were incubated in a 1:500 or 1:1000 dilution of rabbit antiserum raised against a serotonin-BSA conjugate, and further processed with the PAP technique. No reaction was visible in control sections incubated with the same antiserum preabsorbed with serotonin-BSA conjugate or coprecipitated with this conjugate plus rabbit anti-BSA.

With light microscopy, immunoreactive axons were seen in both regions of substantia nigra, although predominantly in pars reticularis. These processes were fine (0.5-1.0 µm), and gave rise to a profusion of thinner branches (0.1-0.4 µm). Varicosities, 0.7-1.5 µm in size, were visible along the lengths of all axonal processes, spaced irregularly from 1 to 5 µm apart. Pale reaction was apparent in cells of the pars compacta at the lower, but not the higher serum dilution, contrary to the heavily stained neurons of the raphe nuclei present at both dilutions.

Using serial section electron microscopy, labeled profiles of variable size were observed containing dark particulate reaction product, about 50 nm in diameter. Small dark mitochondria and round vesicles, 50-100 nm in diameter, were scattered throughout the immunoreactive elements. Synaptic contacts between labeled terminals and large dendritic elements were indicated by restricted regions of membrane thickening, enlargement of the cleft and presence of vesicles adherent to the presynaptic membrane. Often the surfaces of these dendrites were completely covered with synaptic membrane specializations, only one of which involved a labeled axon. Most of the synaptic articulations appeared asymmetrical, and occasionally subjunctional dense bodies were visible under the postsynaptic membrane. Sometimes immunostained profiles formed synapses with dark dendrites.

These observations offer positive identification of serotoninergic processes and terminals in monkey substantia nigra. Although the projections from dorsal and median raphe nuclei to this structure have been demonstrated previously, some disagreement has persisted about the relative extent of afferent input to the two subdivisions. The present findings support the contention that pars reticularis, not pars compacta, receives the predominant serotoninergic innervation. Aided by NINCDS grants #NS-11631 and F32 NS-06954, and the Am. Parkinson's Disease Assoc.

195.15 MORPHOLOGY OF NIGROSTRIATAL NEURONS INTRACELLULARLY STAINED WITH HRP. I. Grofova*, H. Kita and S. T. Kitai (SPON: G. Lew), Dept. of Anatomy, Mich. State Univ., E. Lansing, MI 48824 and Dept. of Anatomy, Univ. of Tennessee, Memphis, TN 38163.

Antidromic responses of rat substantia nigra(SN) neurons to striatal stimulation were intracellularly recorded and the cells were subsequently labeled by iontophoretic injection of HRP. We report here light microscopic data on dendritic arborizations and axons of both physiologically characterized nigrostriatal cells and non-characterized SN projection neurons. The former cells were considered dopaminergic because of their low conduction velocities (antidromic latency from striatum longer than 7 ms). Such criteria were not available for the latter cells, but they were mostly located in the pars compacta (SNC) and the ir axons were traced well beyond the boundaries of the SN.

The majority of nigrostriatal cells were localized within the SNC. They had smooth medium-sized polygonal somata and one or two thick ventrally oriented dendritic stems branching within the pars reticulata(SNR). Several thin and varicose dendrites remained within SNC and made up a large discoid dendritic field. The axons often looped through the SNC before leaving the nucleus, but intrinsic axon collaterals have not been so far seen. A number of non-characterized SNC neurons showed similar morphological features. One slowly-conducting nigrostriatal neuron was localized within the SNR. This cell had a thick dorsally oriented dendritic stem ascending into the SNC. Three primary dendrites branched and coursed for long distances within the SNR. The axon coursed rostromedially through the SNC and exhibited prominent varicosities, but did not collateralize. These morphological features were shared with a non-characterized SNR neuron found in the posteromedial region of SNR, an area known to contain dopaminergic nigrostriatal cells. A few non-characterized cells lying along the dorsal border of SNC had a different soma-dendritic and axonal morphology. Their dendrites radiated into both ventral tegmentum and SNC and their axons emitted recurrent collaterals.

These observations indicate that the SNC contains a rich plexus of varicose dendrites consisting of extensively overlapping dendritic arbors belonging to slow conducting, possibly dopaminergic nigrostriatal neurons located in both SNC and SNR. The nigrostriatal dopaminergic neurons situated in the SNR are morphologically distinct from the nigrothalamic and nigrotectal SNR cells as well as from the nigrostriatal SNC neurons. Finally, the SNC projection neurons probably consist of at least two different cell types. One with dendrites remaining within the confines of SN and without axon collaterals, and the other with dendrites invading the tegmentum and with recurrent axon collaterals. (Supported by NIH Grant NS 19483 and BRSG Grant 0637).

195.16 ACETYLCHOLINESTERASE-CONTAINING NEURONS IN HUMAN NEOSTRIATUM: A STUDY OF NORMAL AND ALZHEIMER-DISEASED BRAINS. A. Parent, C. Csonka* and P. Etienne*. Lab. of Neurobiology, Fac. Med., Laval Univ., Québec and Douglas Hospital Res. Ctr, Montréal, Canada.

The cellular localization of acetylcholinesterase (AChE) in human basal forebrain was studied using the Karnovsky and Roots histochemical procedure. Of the 5 brains examined so far, 2 were from patients who had suffered from senile dementia of the Alzheimer type (one case of early and another of late onset), whereas the others served as controls (age ranging from 66 to 84). After an autopsy delay of less than 24 h., a brain slab which included nucleus basalis (substantia innominata) and part of the basal ganglia, was dissected out and keept in 10% buffered formalin with DMSO for 60 h. The tissue block was then sectioned on a freezing microtome and the sections (30 um thick, 1 out of 2) were reacted for AChE. The adjacent sections were stained with cresyl violet.

In each brain the background AChE staining in neostriatum was sufficiently low to allow the visualization of large and intensely-stained AChE neurons scattered trhoughout the caudate nucleus and putamen. These AChE neurons vary in shape from fusiform with one thick process emerging from one pole of the cell body, to multipolar with 3 to 6 straight and thinner processes. Measurements made in one control brain reveal that the perikarya had a maximum diameter of 40.10 ± 5.93 um (mean ± standard deviation) and a minimum diameter 27.75 ± 4.03 um. Comparisons with adjacent cresyl violet-stained sections suggest that the AChE neurons do not represent more than 5% of the total striatal cell population. Thus, these highly reactive neurons appear equivalent to similar AChE cells disclosed previously in the neostriatum of rat, cat and monkey after AChE inhibitor (DFP) pretreatment. In these species the striatal AChE neurons were shown to be intrinsic cholinergic elements. On the other hand, in the 2 humain brains examined in which the classical neuropathological signs of Alzheimer's disease (abundant neuritic plaques, neurofibrillary tangles and granulovacuolar degeneration) were present, the number, morphological characteristics and staining intensity of the large striatal AChE neurons were found to be unaltered, despite a marked loss (most striking in early onset case) of AChE neurons in the adjoining nucleus basalis.

These findings suggest that large intrinsic cholinergic neurons exist in human neostriatum and that these elements, in contrast to those of nucleus basalis, are not affected in Alzheimer's disease.

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195.17 THE OUTPUT ORGANIZATION OF THE SUBSTANTIA NIGRA IN PRIMATE. Smith*, Y., A. Mackey*, L. De Bellefeuille* and A. Parent (SPON: M. Filion). Lab. of Neurobiology, Fac. Med., Laval University, Québec, Canada, G1K 7P4.

The exact cellular origin and degree of collateralization of the efferent projections of the substantia nigra (SN) in the squirrel monkey (Saimiri sciureus) were studied using the fluorescence retrograde double labeling method introduced by Kuypers and Van der Kooy. Three combinations of fluorescent tracers were utilized: (1) Evans blue and DAPI-primuline, (2) Fast blue and Nuclear yellow, and (3) True blue and Nuclear yellow.

In a first series of experiments (6 monkeys), one tracer was injected in the caudate nucleus whereas the complementary tracer was delivered in the putamen. After these injections numerous clusters of nigrocaudate and nigroputamen cells occur at all rostrocaudal levels in the substantia nigra pars compacta (SNc). These cell clusters were closely interlocked and distributed according to a complex mosaic-like pattern. Although clearly separated from one another in the transverse plane, each of the nigrocaudate and nigroputamen cell clusters appear to run in continuity from section to section when examined along the rostrocaudal axis. This suggests that these clusters may in fact be part of a complex three-dimensional array of tubular neuronal aggregates. Surprisingly, very few SNc neurons were found to project to both caudate nucleus and putamen.

In a second series of experiments (10 monkeys), fluorescent tracers were injected in the 3 major target structures of the substantia nigra pars reticulata (SNr): thalamus, superior colliculus and midbrain tegmentum. After these injections numerous double-labeled neurons were observed in SNr, in contrast to what has been found in SNc after caudate-putamen injection. Our data reveal that the largest number (about 60%) of SNr branching neurons are those projecting to both the thalamus and the midbrain tegmentum; that a moderate number (20-30%) of SNr neurons send collaterals to the thalamus and the superior colliculus; whereas only a small number (about 10%) of SNr neurons project to both the superior colliculus and the midbrain tegmentum. Such a high degree of axonal branching is a neuronal characteristic that the SNr elements obviously share with those of the internal pallidum in the same species.

These findings reveal that, in regard to their efferent projections, the two major subdivisions of SN in primate are organized quite differently. Whereas the SNc cells occur in the form of numerous nigrocaudate and nigroputamen neuronal subunits that display a complex mosaic-like arrangement, the SNr neurons appear clearly more multipotential and not compelled to such a rigid topographical organization. (Supported by grant MT-5781 of the Medical Research Council of Canada).

195.18 6-OHDA LESIONS OF THE MEDIAL FOREBRAIN BUNDLE MODIFY PATTERNS OF GLUCOSE UTILIZATION IN THE BASAL GANGLIA DURING STIMULATION OF THE SUBSTANTIA NIGRA. H. H. Holcomb, H. D. Everist, P. M. Gross, M. Kadekaro, and A. Pert. National Institute of Mental Health, Bethesda, Md. 20205

Unilateral electrical stimulation of the substantia nigra increases local cerebral glucose utilization in the ipsilateral globus pallidus, entopeduncular and subthalamic nuclei. We have assessed to what extent nigral dopamine neurons contribute to this metabolic activation. Employing the quantitative autoradiographic 2-^{14}C deoxyglucose method we have measured local rates of glucose utilization in the globus pallidus, entopeduncular and subthalamic nuclei in six groups of animals:])sham stimulation, 2) unilateral nigral stimulation, 3) nigral stimulation in animals pretreated with haloperidol (0.1 mg/kg i.v., 20 minutes prior to stimulation), 4) unilateral lesion of the medial forebrain bundle with 6-OHDA, 5) 6-OHDA lesion + nigral stimulation, 6) 6-OHDA lesion + nigral stimulation + haloperidol. Stimulation of substantia nigra in animals pretreated with haloperidol increased glucose utilization in the ipsilateral globus pallidus, entopeduncular and subthalamic nuclei as compared to non-treated, stimulated animals. In non-stimulated animals 6-OHDA lesions alone produced a slight increase in metabolic activity of the ipsilateral globus pallidus (20%). Nigral stimulation of 6-OHDA treated animals did not increase glucose utilization in the entopeduncular or subthalamic nuclei. Not further increased by nigral stimulation, pallidal glucose metabolism remained the same as in 6-OHDA lesioned animals without stimulation. Pretreatment with haloperidol in 6-OHDA lesioned animals receiving nigral stimulation, eliminated ipsilateral increases in glucose utilization in globus pallidus. Nigral dopamine neurons appear to be essential for metabolic activation of the entopeduncular and subthalamic nuclei in nigral stimulated animals. In contrast, the globus pallidus did not require such input. This finding suggests that elevated pallidal glucose metabolic activity is due either to the effects of dopamine denervation or stimulation of non-dopaminergic fibers. In 6-OHDA lesioned animals, haloperidol may suppress metabolism in the globus pallidus by binding to cells rendered hypersensitive to this dopamine antagonist.

195.19 ^{14}C DEOXYGLUCOSE STUDIES OF THE NORMAL RAT STRIATUM SHOW HETEROGENEITY AND REGIONAL DIFFERENCES IN GLUCOSE UTILIZATION. Lucy L. Brown and Leslie I. Wolfson*. Dept. Neurol., Albert Einstein College of Medicine, Bronx, NY 10461.

Histochemical and autoradiographic receptor binding studies of the striatum have revealed an organizing principle of "islands" and "mosaic-like compartments" of opiate receptors, cholinesterase, and afferent systems (e.g. Herkenham, M. & Pert, C., Nature, 291:415, 1981). We noted that striatal glucose utilization (GU) measured autoradiographically in rats appeared to be heterogeneous. Thus we used an image analyser densitometer (Cambridge Instruments) to determine if the heterogeneity in normals showed any consistent pattern. Eight rats were injected with 14C 2-deoxy-D-glucose (DG) with no previous treatment. Seven other rats were injected with DG 20min following apomorphine (APO), 2.5mg/kg i.p. APO was used as a functional manipulation of the striatum to determine if changes in the normal pattern could be detected. The DG procedures of Sokoloff, et.al. (J. Neurochem., 28:897, 1977) were used. "Islands"of high-normal GU (123±5 umols/100g /min) and larger regions of low-normal GU (80±4 umols/100g/min) were observed throughout the striatum of individual animals. This relatively large difference between high and low regions represents a significant heterogeneity of GU in the striatum. The "islands" formed an arc shape in the dorsal striatum. Other consistent patterns were seen across animals, forinstance, a low-normal region in the dorsomedial striatum and in a thin strip under the corpus callosum. This thin strip correlated with an area which contains a high level of opiate receptors. The high-normal "islands" were similar to islands of corticostriate afferents (Sharp, F.R., & Evans, K., J. Comp Neurol., 208: 255,1982) and nigrostriatal afferents(Olson, L., et.al., Brain Res.,44:283,1972). APO increased GU in the low-normal regions of the ventromedial striatum, but not above high-normal values. The drug decreased GU in the dorsomedial striatum. The results suggest a heterogeneity of striatal organization which can be functionally analysed and further emphasize the importance of "islands" and cell clusters (Mensah, P.L., Brain Res. 137:53, 1977) as an organizing principle in the striatum. The localized specific changes with APO treatment suggest that discrete regional analysis provides a further analytic tool for DG studies, especially if the topographic organization of afferents is considered. Systemic APO has a complex effect on striatal GU and may reflect specific afferent system activity as well as a direct effect on dopamine receptors in the striatum.

196.1 DISTRIBUTION OF AXON COLLATERALS OF IDENTIFIED MEDIAL VESTIBULOSPINAL AXONS IN THE UPPER CERVICAL SPINAL CORD OF THE CAT. F. Fleming* and P. K. Rose. (SPON: F. J. R. Richmond) Dept. of Physiology, Queen's University, Kingston, Ontario, Canada K7L 3N6.

Dorsal neck muscle motoneurons receive a powerful monosynaptic inhibitory connection from the vestibular complex. Wilson and Maeda (J. Neurophysiol. 37: 346, 1974) have shown that this connection is mediated by the medial vestibular spinal tract which originates bilaterally from the vestibular nuclei and travels in the ventromedial funiculus. The goal of the present study was to describe the fine structural characteristics of individual medial vestibular axons using intra-axonal injections of horseradish peroxidase.

All of the axons described in the present report travelled in the ventromedial funiculus contralateral to their origin within the vestibular nuclear complex and were monosynaptically excited by stimulation of the vestibular nerve. The distribution of individual collaterals was determined from reconstruction of collateral branches seen on serial histological sections. Each axon gave rise to more than one collateral, usually spaced 1 to 2 mm apart. All collaterals branched extensively within lamina IX and ventral parts of lamina VIII. There were two distinct regions of termination: 1) the ventromedial nucleus containing dorsal neck muscle motoneuron cell bodies and 2) the spinal accessory nucleus which contains trapezius motoneuron cell bodies. All well-stained collaterals had numerous boutons, usually 250 boutons per collateral. When viewed in the horizontal plane, the distribution of boutons was not uniform, Instead most boutons were concentrated into several small zones. This distribution was accentuated by a special arrangement in which a single terminating branch gave rise to a cluster of five or six boutons. These clusters consisted of a single large central bouton surrounded by a number of smaller boutons.

The results of the present experiments indicate that the medial vestibular spinal tract has a major termination zone within the contralateral ventral horn in a region occupied by the cell bodies of dorsal and lateral neck muscles. Although previous anatomical studies have not reported such a projection, our results are consistent with electrophysiological experiments which describe disynaptic IPSP's in dorsal neck muscle motoneurons following stimulation of the contralateral vestibular nerve.

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196.2 PROJECTIONS OF NECK MUSCLE SPINDLE AFFERENTS INTRA-AXONALLY STAINED WITH HORSERADISH PEROXIDASE IN THE UPPER CERVICAL SPINAL CORD OF THE CAT. S.A. Keirstead* and P.K. Rose. Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada K7L 3N6.

Electrophysiological studies have shown that stimulation of neck muscle spindle afferents evokes small composite monosynaptic EPSP's (350-3100 uV) in dorsal neck muscle motoneurons (Brink et al., J. Neurophysiol. 46: 496, 1981). Furthermore, no reciprocal or crossed disynaptic IPSP's have been detected in these motoneurons (Rapoport, J. Physiol. 289: 311, 1979). In the present experiments we have examined the intraspinal projections of neck muscle spindle afferents anatomically since electrophysiological studies have provided little positive information about the segmental role of dorsal neck muscle spindles.

Axons of "primary-like" endings of dorsal neck muscle spindles were impaled near the dorsal root entry zone of the upper cervical spinal cord, and injected with horseradish peroxidase. The course of the axons and the projections of their collaterals were reconstructed from serial histological sections cut in the sagittal plane. Stained axons gave rise to collaterals at intervals from 0.6 to 5.2 mm (average, 3.4 mm).

All collaterals descended along the medial border of the dorsal horn and arborized extensively upon reaching the intermediate horn, in a region which included the central cervical nucleus (CCN). This projection gave rise to a dense termination zone which accounted for 70 to 80% of the boutons from any single collateral. From this intermediate region, the main branch of collaterals from biventer cervicis muscle spindle afferents coursed ventrally and gave rise to boutons along the medial border of the ventral horn and in the ventromedial nucleus, deep in the ventral horn. In addition to these projections, collaterals from splenius muscle spindle afferents had a lateral branch in the ventral horn, which terminated in the region of the spinal accessory nucleus. The rostrocaudal extent of the diffuse ventral arborizations varied, but usually did not exceed 1000 um.

The prominent projection of neck muscle spindle afferents to the CCN which contains precerebellar neurons could provide a route by which information from neck proprioceptors can be transmitted to the cerebellum. The relatively minor projections from neck muscle spindle afferents to regions containing dendrites and somata of neck muscle motoneurons, combined with the wide intercollateral spacing may, at least in part, explain the small monosyaptic EPSP's which have been observed in dorsal neck muscle motoneurons.

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196.3 TOPOGRAPHIC COMPARISON OF MESENCEPHALIC PROJECTIONS TO THE INFERIOR OLIVE, VESTIBULAR NUCLEI AND UPPER CERVICAL CORD IN THE CAT. S.J. Spence*, J.A. Saint-Cyr, and M.T. Stechison*. Playfair Neuroscience Unit and Departments of Anatomy and Surgery, University of Toronto and Toronto Western Hospital, Toronto, Ontario M5T 2S8.

Mesencephalo-olivary projections have been shown to arise importantly from parvocellular red nucleus (RNp), interstitial nucleus of Cajal (INC), nucleus of Darkschewitsch (ND), subparafascicular and parafascicular nuclei (sPf and Pf), nucleus of the pre-rubral field (NPRF) and nucleus of the fields of Forel (NFF) (Saint-Cyr and Courville, JCN 198, 1981). Some of these regions are also known to send fibres to the vestibular nuclei (VN) and upper cervical cord (C.Sp.). To assess the topograhic distribution of these cells with respect to their efferent projections, retrograde transport experiments were performed using lectin-HRP (WGA-HRP) (0.5- 2.0%; 0.05-0.9 µl) and the fluorescent dyes fast blue and nuclear yellow (1.0-3.0%, 2.6-4.8 µl) in a series of 25 cats. The largest injections were confined to the C.Sp. Only those cases wherein injections were restricted to the defined targets (IO, VN, C.Sp.) were used to obtain the following data.

No single mesencephalic region was found to project soley to the IO. Oculomotor and magnocellular red nuclear neurons were uniquely labeled after VN and C.Sp. injections respectively. Both IO and VN received inputs from certain pretectal nuclei, while common sources of afferents to IO and C.Sp. arose from ND and nucleus of the posterior commissure. The nucleus of Edinger-Westphal projected to both C.Sp and VN. Principal mesodiencephalic cell groups providing inputs to all three targets included RNp, INC, NPRF, sPf, Pf and NFF.

The olivary afferent cell groups formed a compact distribution which was densest in the rostral mesencephalon. In contrast, cells projecting to the VN and C.Sp. were more diffusely distributed, and were found over a greater area both laterally and caudally in the mesencephalon, although they were also more densely concentrated in the rostral mesecephalon.

This study suggests that the inferior olive may be in receipt of corollary information arising from mesencephalic areas also known to project to vestibular and upper cervical centres. Double labeling studies are in progress to identify such axonal collaterals. In addition, further details with regard to the bilaterality of these projections are under analysis.

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196.4 POSTSYNAPTIC POTENTIALS OF LUMBAR MOTONEURONS EVOKED BY CUTANEOUS C-FIBER VOLLEYS IN THE CAT. K.Endo*, Y.Hori* and W.D.Willis (SPON: J.E. Bottenstein). Marine Biomed. Inst. & Depts. of Physiol. & Biophys. and Anatomy, Univ. TX Med. Branch, Galveston, TX 77550.

Postsynaptic potentials evoked by cutaneous C-fiber volleys (C-PSPs) in lumbar motoneurons were examined in unanesthetized decerebrated cats. The spinal cord was transected at the lower thoracic level. Motoneurons were identified by antidromic spike potentials following stimulation of peripheral muscle nerves. Thesural or tibial nerve was stimulated at strengths sufficient to activate A or A and C afferent fibers with three pulses at 33 Hz. The duration of the stimuli was either 100 µs or 1 ms for activation of A and C fibers, respectively. The afferent A and C volleys were monitored. C-PSPs were obtained by subtracting PSPs evoked by maximal A volleys from PSPs evoked by A and C volleys.

A total of 34 flexor (PBST,DP) and 27 extensor (GS,PL,FDHL) motoneurons were tested. C-EPSPs were evoked in 18 (54%) flexor and 12 (44%) extensor motoneurons, while C-IPSPs were evoked in 8 (24%) flexor and 9 (33%) extensor motoneurons, respectively. The mean latencies of C-EPSPs and C-IPSPs were 292 msec (N = 20, range: 180-630 msec) and 273 msec (N = 11, range: 190-390 msec), respectively, indicating that the fastest C-fiber afferents involved had a mean conduction velocity of 0.8 m/sec. In the early part (400-800 msec after stimulation) of C-PSPs, an increase in membrane conductance was observed. This fact shows that presynaptic terminals producing C-PSPs in motoneurons are located on the soma or proximal dendrites.

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196.5 FACILITATION AND DEPRESSION OF Ia-MOTONEURON EPSPS DURING SHORT BURSTS IS DETERMINED BY EPSP AMPLITUDE. W.F. Collins, III, M.G. Honig and L.M. Mendell. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

We have examined the frequency dependence of EPSPs at 27 single medial gastrocnemius (MG) Ia-motoneuron connections in intact and acutely transected (T13) cats under Nembutal anesthesia. We electrically stimulated the Ia fiber impaled in a dorsal rootlet and recorded the resulting EPSPs in the motoneuron (MN)(Honig et al., J. Neurophysiol. 49:). We previously reported (Collins et al., Neurosci. Abstr. 8:) that facilitation/depression behavior during long trains (250 stimuli) of intermediate frequency (50-100Hz) is correlated with EPSP amplitude and with MN rheobase (Rh). We have extended these studies by delivering brief, repetitive high frequency bursts (32 shocks at 167Hz; 128 bursts; 2-30sec between bursts). We examined changes in EPSP amplitude occurring during the burst by averaging responses in successive bursts. At most connections (90%) the first EPSP in the burst was larger than the average EPSP in a control low frequency (18Hz) train, due to potentiation from the repeated bursting (Beswich & Conroy, J. Physiol., 180:) and/or the presence of a small degree of depression in the 18Hz train. This ambiguity prompted us to measure facilitation/depression relative to both the 18Hz average and the first EPSP in the burst. The results were qualitatively similar and we report only the 18Hz comparison. In control experiments we found the amplitude of later EPSPs in the burst (beyond the first 4 or 5) to be independent of the extent of any initial potentiation. The ratio of the average amplitude of the 30th and 31st EPSPs to the mean amplitude at 18Hz varied from 0.63 (depression) to 1.94 (facilitation) with a highly significant negative correlation between this ratio and EPSP amplitude (18Hz). Plotting these ratios as a function of MN Rh (range 1.7-31nA) revealed a significant positive correlation. Since amplitude and Rh were themselves correlated, it is difficult to decide which is primary in determining facilitation/depression. We have further analyzed these data using the method of partial correlations. This revealed a highly significant contribution of EPSP amplitude to the variability in facilitation/depression behavior at different connections. However, at present we cannot rule out a small additional contribution to facilitation/depression variability from MN properties as indicated by Rh. We conclude that whatever process regulates EPSP amplitude seems likely to influence facilitation/depression behavior. Our results indicate that these properties are non-uniformly distributed on MNs of different Rh. Thus variability in intrinsic properties of the synapse may be principally responsible for variations in EPSP amplitude on different groups of MNs. Supported by NIH grants NS06407 (WFC), NS06427 (MGH) and NS16996 and NS14899 (LMM).

196.6 DETERMINATION OF SYNAPTIC EFFICACY AT INDIVIDUAL Ia-MOTONEURON CONNECTIONS. L.M. Mendell and W.F. Collins, III. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.

Single Ia afferent fibers often discharge in high frequency bursts (Loeb & Duysens, J. Neurophysiol. 38) subjecting the EPSPs they evoke in α-motoneurons to temporal summation. Assuming EPSPs of uniform amplitude, modelling predicts smooth depolarization to a steady DC level with superimposed fluctuations due to each EPSP (Calvin, Brain Res. 39). Increasing the afferent discharge frequency should elevate the steady level of depolarization (Harrison & Taylor, J. Physiol. 312). We have stimulated single medial gastrocnemius (MG) Ia fibers using an intrafiber electrode in the dorsal root and recorded the EPSPs produced in a MG motoneuron in anesthetized cats (Honig et al., J. Neurophysiol. 49). We observed that the DC level did not always increase monotonically to some steady level during the burst (167Hz, 32 shocks). Rather an initial increase was followed by a decline due largely to a decrease in EPSP amplitude during the burst (Collins et al., this volume). At other connections EPSP amplitude increased during the burst producing a greater level of steady depolarization than anticipated from the amplitude and shape of the initial EPSP. Furthermore, at some connections steady state DC level decreased for bursts of increasing frequency due to greater depression of EPSP amplitude during the burst. To quantitate these effects we have calculated the mean amplitude of the synaptic response by dividing the integral of the response waveform (e.g. a burst or a single EPSP) by its duration. Mean amplitude of EPSPs obtained during low frequency stimulation (18Hz) averaged about 1/3 of their peak amplitude. In contrast mean amplitude of the first 15 EPSPs in the burst was only 15% smaller on the average than the peak amplitude of the low frequency run at that connection (n=13). The extent to which the mean amplitude of the burst approached or even exceeded the peak amplitude of the low frequency run depended both on EPSP shape (i.e. half width) and the magnitude of depression/facilitation during the burst. We observed that even when peak amplitudes of EPSPs averaged during low frequency stimulation at 2 connections were very similar, the mean amplitudes of the bursts could differ substantially. Mean amplitude may provide a better estimate of synaptic efficacy than peak amplitude because no single connection is sufficiently strong to discharge to motoneuron (see also Barrett & Crill, J. Physiol. 293). It follows that estimates of relative synaptic efficacy among connections may differ from those computed from peak amplitudes of EPSPs obtained during low frequency stimulation. Such assessments may depend on the specific stimulation conditions (e.g. frequency of stimulation), EPSP shape and facilitation/depression behavior. Supported by NS14899, NS16996 (LMM) and NS06407 (WFC).

196.7 IPSP ACTIVITY INDUCED IN LUMBAR MOTONEURONS BY MEDULLARY RETICULAR FORMATION STIMULATION DURING ACTIVE SLEEP. P.A. Boxer*, F.R. Morales, S.J. Fung and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

Previous experiments performed in the chronically prepared cat have demonstrated that stimulation of the pontomesencephalic reticular formation induces a state-dependent hyperpolarizing potential in both brainstem and spinal cord motoneurons exclusively during active sleep (AS) (J. Neurophysiol., 44:372, 1980; Brain Res., 248:267, 1982). The more caudal area of the reticular formation in the lower medulla has traditionally been implicated in the regulation of sleep and wakefulness, and in descending motor inhibition. The present experiment was designed to examine the contribution of this medullary area to the control of motor functions during sleep and wakefulness. Accordingly, the effects of electrical stimulation of the nucleus reticularis gigantocellularis (NGC) upon the membrane potential of lumbar motoneurons was examined in six cats.

Each animal was prepared with a permanent head and spinal cord implant in accord with established procedures for intracellular recording in the chronic cat (Physiol. Behav., 27:355, 1981). Sleep and waking states were identified on the basis of standard polygraphic criteria. Intracellular recordings were obtained from antidromically identified lumbar motoneurons (n=39) with glass microelectrodes filled with 2M K-citrate or 3M KCl. Various loci within the NGC (P8 to P10, L1 to L2, H-6 to H-8) were stimulated with a monopolar electrode (2 to 4 pulses, 800 Hz) at a low intensity (20-80 uA). A small hyperpolarizing potential with a peak latency of 40 msec arose in some motoneurons following NGC stimulation during quiet sleep and wakefulness; in the remaining cells no corresponding potential was observed. However, during active sleep, in all cells, NGC stimulation produced a larger hyperpolarizing potential with a peak latency of 40 to 45 msec and an amplitude of 2 to 8 mV. The passage of hyperpolarizing current and/or the iontophoretic injection of chloride ions through the recording electrode diminished or reversed this potential, indicating that it was a chloride-dependent inhibitory postsynaptic potential.

These results suggest that the NGC exerts a weak inhibitory influence on lumbar motoneurons during quiet sleep and wakefulness. However, during AS, motoneuron inhibition of NGC origin is omnipresent and considerably more potent. These data indicate that the NGC exerts a function during AS which has not previously been demonstrated electrophysiologically; this function may be related to the atonia of AS and/or the phasic patterns of inhibition which are present during this state.

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196.8 "Compartment Nuclei" of the Cat Medial Gastrocnemius Muscle. O.I. Weeks and A.W. English (Spon: R. McBride). Dept. of Anatomy, Emory University, Atlanta, GA 30322

A "compartment nucleus (CN)" consists of a group of spinal motor neurons which supply individual primary muscle nerve branches of a muscle nerve. Each primary muscle nerve branch (PMNB) innervates a specific subvolume or compartment of a single muscle.

The spatial and size distributions of motor neurons which supply PNMB of the cat medial gastrocnemius (MG) muscle were examined to test the following hypothesis: that the anatomical characteristics of each MG compartment nucleus mirror images a corresponding lateral gastrocnemius (LG) muscle compartment nucleus. The hypothesis predicts a rostrocaudal topographic organization of MG compartment nuclei reversed to those of LG. The more rostral CN of MG would therefore supply more distal muscle compartments and the more caudal CN would supply more proximal muscle compartments.

Individual PMNB of the MG nerve were isolated, microdissected, cut and the proximal stump soaked in a 30-50% horseradish peroxidase (HRP) solution in 1.6 mg % hyaluaronidase. As a control in each case, the entire contralateral MG nerve was similarly cut and soaked in HRP. A 48-72 hour survival period followed. Spinal segments L6-S1 were serially sectioned and processed for HRP using tetramethyl benzidine (TMB). The distribution of retrogradely labeled cells supplying each nerve branch was compared to the distribution of the entire contralateral MG motor nucleus. These data were also compared to data on the spatial and size distribution of motor neurons in the LG motor nucleus.

Preliminary results indicate significant overlap in the distribution of motor neurons in each compartment nucleus. However, a clear rostral to caudal topographic arrangement for each CN exists for proximal to distal muscle compartments. Rather than a reversed topographic organization of each CN of MG, in comparison to corresponding LG compartment nuclei, the arrangement mimics that of LG. However, for each compartment nucleus of MG, relative to the corresponding CN of LG, there is a more lateral, ventral and caudal positional shift. Like LG compartment nuclei, the most rostrally located CN of MG consists of the largest sized motor neurons and the more caudally placed compartment nucleus a larger percentage of the smallest sized motor neurons.

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196.9 Anatomical Organization of Long Ascending Propriospinal Neurons in the Cat Spinal Cord. J. W. Tigges, A. W. English and P. R. Lennard, Yerkes Regional Primate Research Center and Emory University, Atlanta, GA 30322.

Long ascending propriospinal neurons (LAPN's) are ascending interneurons which connect the lumbar and cervical regions of the spinal cord. The anatomical organization of the cells of origin of LAPN's were examined using the retrograde transport of wheat germ agglutinin - HRP (WGA-HRP) conjugate. Injections of 1-4% WGA-HRP dissolved in saline were made into the cervical spinal cord in pentobarbital anesthetized cats using a double-barrelled glass micropipette, one barrel of which was used to inject small quantities (0.05µl) of WGA-HRP and the other to record a field potential elicited by suprathreshold stimulation of the superficial radial nerve. The pipette was advanced obliquely through the dorsal columns until a large negative field potential either disappeared or reversed direction. Analysis of these injection sites indicated that the label was placed primarily into the intermediate zone (lamina VII). After 48-72 hours survival, animals were perfused with 3.5% glutaraldehyde and the lumbar cord sectioned serially and reacted for demonstration of HRP. Two injection paradigms were compared: injections into the cervical enlargement (C_5 - T_1) and injections rostral to the enlargement (C_{3-4}). Labelled cells were found both ipsilateral and contralateral to the injection site in both the dorsal and ventral horn at all lumbar levels. A large population of moderate-sized, spherical cells were found in the dorsal horn (laminae IV-VI), mainly ipsilateral to the injection site. Injections into the dorsal columns and dorsal column lesions, which were used as controls, indicate that at least some, but not most of these cells are secondary dorsal column neurons injected in the electrode tract. Large multipolar cells were labelled in the ventral part of lamina VII and, in rostral lumbar segments, also in laminae VIII and IX. In the lumbar enlargement (L_{4-7}) a substantial number of labelled cells were also noted in lamina VIII. All of these ventral horn cells were found more frequently contralateral to the injection sites and some could be noted to decussate at their segmental level of origin. Controls indicate that these neurons were probably not labelled by damage along the electrode tract. Analysis of the distribution of labelled cells indicates that lower lumbar LAPN's may project more strongly to upper cervical levels where they may interact with short descending propriospinal neurons to exert an effect on cervical motor output. Upper lumbar LAPN's may project more strongly to the cervical enlargement than the C_{3-4} region and thus exert their effect more directly.
Supported by USPHS NS17531

196.10 INTRINSIC ORGANIZATION OF A MOTONEURON POOL. E. Theriault and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

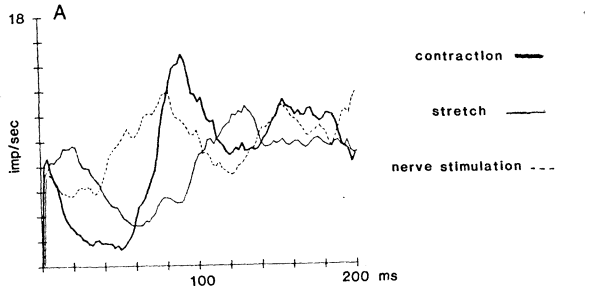
The cutaneous trunci muscle (CTM) in the rat is a vast sheet of skeletal muscle that underlies back and flank skin, and is reflexly activated by pinching or heating the overlying skin. Functionally the muscle is compartmentalized, in that a localized nociceptive stimulus to the skin elicits a reflex contraction focussed at the muscle region below the stimulus. This reflex behavior however, does not depend upon a segmentally organized central circuitry; while the sensory input is indeed segmental (T4-L4) all the CTM motoneurons are located in the cervical cord, and the motor nerves originate from the brachial plexus. Could there be an intrinsic anatomical organization of the CTM motoneuron pool that relates to the functionally compartmentalized nature of its reflex activation? The 3-5 major motor nerves were shown to innervate essentially longitudinal bands of muscle; this is in contrast to the more transversely oriented reflexly-activated areas of the muscle. Retrograde transport of HRP revealed that the CTM motoneurons occupy a crescentic region in the most ventral portion of the ventral horn between segments C6 and T1. The motor nerves supplying the most dorsal muscle band were found to originate from a narrow column of motoneurons located at the medial edge of the crescent, but extending over the entire length of the pool. The more lateral muscle nerves, supplying progressively more lateral bands of the muscle, originated from similar columns in more lateral regions of the crescent. Thus motoneuron columns within the CTM pool seem to correspond to longitudinally-oriented muscle bands. Small nerve twigs within the muscle were then dissected out, their muscle fields determined, and the twigs soaked in HRP. The labelled motoneurons again were arranged in columns but these occupied only portions of the major motoneuron columns. Injections of HRP directly into the muscle gave results consistent with the HRP uptake being into one, or a few, nerve twigs. These findings suggest that the behavioral compartments of the CTM are supplied by sub-groups of motoneurons that are probably short portions of the major columns; the portion length reflects the extent of the muscle compartment in the longitudinal axis, and its medio-lateral location, the location of the muscle compartment in the transverse axis. Perhaps during development this spatial organization of the motoneuron pool facilitates access by sensory information to appropriate sub-populations of motoneurons.

(Supported by N.I.H. Grant NS 14951-02, M.S. Society of Canada Research Student.)

196.11 MUSCLE RECEPTOR INPUT AND INHIBITION IN DSCT. C. E. Osborn and R. E. Poppele, Lab. of Neurophysiol., Univ. of Minnesota, Minneapolis, MN 55455.

During stimulation of peripheral nerves DSCT units may respond with one of 3 patterns of either inhibition or excitation (Knox et al., J. Neurophysiol. 40: 626, 1977). Monosynaptic excitation is the least common (20%), but the only response for which muscle receptor input has been examined (Oscarsson, Physiol. Rev., 45:495,1965). We have therefore investigated the receptor input responsible for polysynaptic inhibition or excitation, in DSCT units by recording activity during muscle stretch and contraction. Activity of isolated DSCT units was recorded from the dorsolateral funiculus, and cross-correlated with the Poisson-distributed pulse train (mean rate 8/sec) used in producing stretch, contraction of the gastrocnemius-soleus muscle, or stimulation of the muscle nerve. For most of the 79 units recorded, contraction was the most potent stimulus, and inhibition the most common response. The cross-correlogram illustrated below was typical of the responses of the largest class of DSCT units (40%). These units were inhibited during contraction, stretch and nerve stimulation, although ususally the response to stretch was weak. Activity in Golgi tendon organs (GTO's) is probably responsible for these responses. The inhibition is due to an activation of afferent fibers as evidenced by the fact that electrical stimulation of the nerve produces the same response as contraction or stretch. The weaker response to stretch would also be expected from an effect due to GTO's. The results suggest that GTO's provide a potent, inhibitory source of input to the DSCT.

Supported by NIH grant NS 07147



197.1 DEVELOPMENTAL PROTEIN MALNUTRITION IN THE RAT: CORTICAL SINGLE UNIT ACTIVITY. P.J. Morgane, W.C. Stern, W. Pugh*, and O. Resnick. Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545 and Burroughs Wellcome, Research Triangle Park, NC 27709.

It was reported previously (Stern et al., Developmental Brain Res., 1983) that adult rats born to dams fed a low protein (8% casein) isocaloric diet starting 5 weeks prior to mating showed the following changes in spontaneous activity of single neurons when compared to 25% casein controls: a) a 25% slower mean discharge rate in frontal cortex and b) 70% fewer fast firing neurons. The present study examined a more severe protein malnutrition condition (6% casein) and evaluated the effects of dietary reversals at birth or adulthood on frontal cortex unit activity. Methods consisted of anesthetizing male Sprague-Dawley rats from the malnourished, control and diet reversal groups (total n = 37 rats, 700 neurons) with urethane followed by extracellular recording of frontal cortex units using glass micropipettes. Computer analyses consisted of average discharge rates, mean interspike interval (ISI), variability of the ISI (SD/mean) and percentage of discharges occurring in bursts or pauses.

The 6% casein group produced effects slightly greater than that previously seen for the 8% casein fed rats, i.e., an approximately 30% decrease in discharge rates, a tripling of the mean ISI, more slow firing cells, and less bursting activity than the 25% casein controls. When the 8% and 25% casein data were analyzed according cortical depth, the most profound differences were noted from 600-1200 microns below brain surface (layers III and IV) where average discharge rates (2-4/sec) were only half that of the 25% control (6-9/sec).

The effects of diet reversal at births (8%→25% diet or vice versa) which were continued until adulthood showed that introduction of the 8% casein diet at birth produced effects on frontal cortex activity comparable in magnitude to when the 8% diets were started prior to gestation. However, restoration of a normal diet at birth to an in utero malnourished rat pup failed to reverse the malnutrition effect. Dietary reversals for 2-3 months in adulthood demonstrated that the 25%→8% condition showed no appreciable change from the 25% control group. Similarly, the 8%→25% adult reversal did not alter the 8% diet induced changes. Thus, the rat brain is vulnerable to the effects of protein malnutrition starting in utero and at birth, but not starting in adulthood. Dietary restoration introduced as early as at birth following gestational malnutrition is an insufficient means for normalizing cortical neuronal activity in adulthood. (Supported by grant HD06364 and funds from Burroughs Wellcome.)

197.2 PRENATAL ASPIRIN: EFFECT ON AMBULATION AND ONE-WAY AVOIDANCE IN MICE. C. L. Randall, R. F. Anton*, G. E. Hoffmeyer*, and C. J. Wallis. Veterans Adminstration Medical Center and Medical University of S.C., Charleston, S.C. 29403

Aspirin (acetylsalicylic acid, ASA) is one of the most common over-the-counter drugs ingested by pregnant women. Animal studies have shown aspirin to be a potent teratogen. However, in humans, the literature is not clear. Prospective clinical studies are difficult to conduct, since many commercial preparations contain ASA. Aspirin use may be denied upon inquiry when, in fact, it was ingested. Given the fact that aspirin is teratogenic in animals and possibly humans, it is of interest to determine whether aspirin use during pregnancy alters behavior in the offspring. We measured spontaneous ambulatory activity and one-way active avoidance behavior in mice exposed to ASA in utero.

Pregnant C3H mice were injected (s.c.) from Gestation Day 14-parturition with either buffer, 150, or 250 mg/kg ASA. A separate buffer group was run with each aspirin dose because they were not run simultaneously. The data were analyzed accordingly. Litter size, birth weight, sex ratio, and pup viability were recorded at parturition. At 20-25 days of age, 1-2 females/litter were tested for ambulatory behavior in an automated open-field apparatus for 4 consecutive 5 minute periods. Males (1-2/litter) were tested at 35-40 days of age in an automated one-way avoidance chamber for 5 blocks of 10 consecutive trials.

The results demonstrated a significant increase in gestation length in the 250 mg/kg dose group as compared to buffer but no difference on this measure in the 150 mg/kg group. The activity and avoidance data were analyzed as a 2-factor ANOVA with repeated measures with equal N using each subject as an individual unit. Individual comparisons were made by Newman Keuls test. Data analysis revealed that ambulatory activity decreased significantly across blocks of time in all groups ($p<0.01$). There was no effect of 150 mg/kg aspirin on ambulatory activity, while 250 mg/kg aspirin reduced ambulatory activity in all time blocks ($F(1,22)=12.02$, $p<0.01$). There was no significant interaction between time and treatment. The one-way avoidance task failed to reveal a significant difference between the 150 mg/kg and buffer groups in the percent avoidance responses made in each block of trials or total avoidance responses. One way avoidance testing in the 250 mg/kg group and their buffer group is in progress as are teratologic studies at these doses. These data imply that aspirin may be a behavior teratogen in mice. (Supported by the Veterans Administration and grant #AA04574 from NIAAA.)

197.3 GANGLIOSIDES ALTER FUNCTIONAL NEONATAL DEVELOPMENT: INCREASED TURNOVER OF CNS MEMBRANE PROTEIN.
S.P. Mahadik and Stephen E. Karpiak, Division of Neuroscience, NYS Psychiatric Institute and Depts. of Biochemistry & Psychiatry, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Various studies report that exogenously administered gangliosides enhance 1) neurite outgrowth in vitro; 2) peripheral nerve regeneration and 3) CNS neuronal regeneration. We have examined the effects of exogenous gangliosides on neonatal rat learning behavior. Rat pups were given injections (daily: sub.cu) of 2.5 mg of total brain ganglioside (average composition: GD1b 16%; GT1 19%; GM1 21%; GD1a 39%). Controls received saline. The neonates were trained and tested for learning on a multi-directional avoidance paradigm on postnatal (PN) days 11,12 & 14. It was found that the rats treated with ganglioside acquired the learned behavior at a significantly faster rate than controls ($p<0.01$). Further, in testing the rats for retention of the behavior (after skipping a day with no-training) it was found that the rats injected with gangliosides showed 15-20% improved performance, whereas controls showed a 10% loss in memory for the task ($p<0.01$). We have also tested the effectiveness of individual ganglioside species [supplied by Fidia Research Laboratories, Abano Terme Italy]. These include GM1, GD1a, GD1b, and GT1b (sub.cu.:0.5mg/rat/day). Preliminary results show that GM1 and GD1b were as effective as the total ganglioside injections in enhancing the behavioral performance of the rat pups. GD1a and GT1b were not effective.

To study the possible mechanisms by which gangliosides exert their effect(s), we studied the extent of glucose utilization in the biosynthesis of cytosolic and membraneous proteins in the CNS. Rat pups (n=16) treated (supra) with total gangliosides were given i.p. injections on PN days 10 & 15 of 10uCi [μ-14]C-Glucose and killed 3hrs later. Saline treated pups were similarly injected (n=16). Cytosolic and membrane fractions were isolated from brains (minus cerebellum) and [14]C counts incorporated into proteins were determined. At PN days 10 & 15 the levels of [14]C in cytosolic proteins were the same for both groups, whereas levels in membrane proteins were slightly increased at PN day 10, and 30% increased at PN day 15. Total brain wet weight, as well protein levels in cytosolic and membrane fractions were similar for all groups. Weight gain for both groups was identical. Our data suggests that ganglioside injections alter membrane protein turnover. This may reflect accelerated neuronal maturation and/or an increase in neuronal synaptogenesis and dendrogenesis.

197.4 PRENATAL EXPOSURE OF GUINEA PIGS TO A 2450-MHz CW MICROWAVE FIELD. Mary Ellen O'Connor and David A. Bartsch*. Bioelectromagnetics Research Laboratory, Department of Psychology, University of Tulsa Tulsa, OK 74104.

Time mated female guinea pigs were exposed on gestational days 18 through 25 to 2450-MHz CW microwave radiation. The power density of the field averaged 28 mW/cm^2 and was endured for 60 minutes on each of the 8 days. Ambient temperature was 20.5°-21.7°C and relative humidity was maintained at 50%. Pre- and post-exposure rectal temperatures were taken. Pre-exposure temperatures averaged 37.6°C and post-exposure temperatures averaged 39.5°C. Caeserean sections were performed on day 60. The number of live offspring were noted. Measures of body mass, external skeletal development, and crown rump length were taken. Following the gross skeletal examination the brain was removed and assessed for mass. One fetus per litter was placed in alcohol for further histological examination of skeletal maturity. No abnormalities were observed in either the exposed or the control litters. Analysis revealed no differences in brain mass, body mass, or body length. Compared to data on the teratogenic effects of heat stress -- the results are somewhat surprising. It would appear that the temperature change (of nearly 2°) is not the critical factor in inducing abnormalities. Previous reports indicate that guinea pigs are more teratogenically susceptible than other rodents and also that some abnormalities should be expected to occur at the temperatures that were used in the present study. The heat that resulted from exposure to microwave radiation apparently is not as stressful as heat induced by other means. Data will be compared to measures taken on guinea pigs exposed under identical conditions but at intensities that will raise the maternal temperature to nearly 42°C.

197.5 DIETARY DEPRIVATION OF LINOLENIC ACID IN DEVELOPING RHESUS MONKEYS: EFFECT ON VISUAL FUNCTION BUT NOT DISCRIMINATION LEARNING. M. Neuringer*, C. Van Petten*, W. E. Connor*, and L. Barstad* (SPON: L. Gronke). Oregon Health Sci. Univ., Portland 97201, and Oregon Regional Primate Res. Ctr., Beaverton 97006.

Linolenic acid (LA) is the dietary precursor of docosahexaenoic acid (DHA), a major fatty acid in synaptic and photoreceptor membranes. In rats, dietary deprivation of LA produces depletion of DHA from brain and retina, reduction of electroretinogram amplitudes, and impaired learning of a visual discrimination. We are examining the effects of dietary LA deprivation during prenatal and postnatal development in rhesus monkeys.

Semipurified diets low in LA (safflower oil as only fat source, LA < 0.3% of total fatty acids) were fed to one group of adult female rhesus monkeys throughout pregnancy and to their infants from birth. A control group of mothers and infants received similar diets supplying ample LA (soy oil as only fat source, LA = 8% of total fatty acids).

In the plasma phospholipids of adult females fed the low-LA diet, the percent composition of LA fell to 5% of the levels in the control group, but DHA was conserved relative to LA and remained at approximately 75% of control values. In the plasma phospholipids of the LA-deprived infants, LA was undetectable and DHA levels were dramatically and progressively depleted, falling from 50% of control values at birth to 15% at 4 weeks, 9% at 8 weeks, and 6% at 12 weeks of age. In other plasma lipid classes, DHA was undetectable by 12 weeks.

In order to assess visual function, we measured the infants' visual acuity at 4 and 8 weeks with the forced-choice preferential looking method. Compared to control infants, the LA-deprived infants' acuity thresholds were reduced by 30% at 4 weeks ($p < .05$) and by 50% at 8 weeks ($p < .0001$); their acuity failed to improve significantly between 4 and 8 weeks, whereas control infants demonstrated the expected two-fold improvement.

Starting at 6 weeks of age, the learning ability of LA-deprived infants was tested in a reversal learning task. We used a spatial, rather than visual, discrimination in order to assess learning apart from visual loss. The number of trials and errors required to learn 6 successive reversals did not differ between deprived and control infants. A previous study reported a learning deficit in LA-deprived rats, but used a visual discrimination. If rats and monkeys are affected similarly by LA deprivation, then the previous study's results were probably due to the visual nature of the task rather than an inability to learn.

These results suggest that LA may be a dietary essential fatty acid, and that DHA may have a specific function in photoreceptor membranes. (Supported by NIH grant AM-29930.)

197.6 EXPOSURE TO ETHANOL IN UTERO ALTERS THE AREAL DISTRIBUTION OF THE INNER MOLECULAR LAYER IN THE DENTATE GYRUS OF ADULT RATS FOLLOWING UNILATERAL ENTORHINAL CORTEX ABLATION: A QUANTITATIVE APPROACH USING IMAGE PROCESSING. S.L. Dewey, M.D. Cassell, and J.R. West, Department of Anatomy, University of Iowa, College of Medicine, Iowa City, IA 52422.

Characteristic abnormalities of the fetal alcohol syndrome (FAS) include a host of facial and organ system malformations and central nervous system dysfunction. Previously, we reported that prenatal ethanol exposure permanently altered both the hippocampal mossy fiber teminal field (West et al., Science, 211: 856, 1981) and the post-lesion Acetylcholinesterase (AChE) staining pattern in the dentate gyrus of adult rats (Dewey et al., Soc. Neurosci. Abstr., 8 (1982) 182. Utilizing the Timm's sulfide-silver staining technique, we chose to ivestigate whether the post-lesion staining pattern observed in the dentate gyrus of the hippocampal formation was altered in animals that received 35% ethanol derived calories (EDC) throughout days 1-21 of gestation and unilateral entorhinal lesions as adults. Following a survival period of at least 30 days, animals in the lesioned groups were transcardially perfused and processed for Timm's histochemistry. The hippocampi of the selected coronal sections were photographed at 4X using Kodak Plus X Pan 35mm Film. The mounted photographic negatives were presented to the camera of the image processing system (EyeCom II/PDP-11/34) by backlighting from a UL700 light table. Following digitization of the mage, the contrast range in the image was expanded such that the differences in the staining pattern were enhanced. By first manually tracing the outline of the molecular layer, the area of the inner molecular layer was determined as a percentage of the total molecular layer. Animals exposed to ethanol in utero showed a significantly ($p < 0.01$) larger inner molecular layer area as compared to normal lesioned controls. These changes are reminiscent of those observed following unilateral entorhinal cortex ablation in younger (rather than mature) rats. Supported in part by grant AA03884 to J.R.W.

197.7 A SELECTIVE ENZYMATIC LESION IN THIAMIN DEFICIENCY. G.E. Gibson, H.J. Ksiezak-Reding*, K.-F.R. Sheu,* V. Mykytyn,* and J.P.Blass. Cornell University Medical College, The Burke Rehabilitation Center, White Plains, NY 10605.

Thiamin deficiency is a convenient animal model of dementias in man and of selective vulnerability to metabolic insults. Thiamin deficiency was produced in male Wistar rats (40-50 g) that were fed a thiamin deficient diet and injected daily with the centrally-acting thiamin antagonist pyrithiamin. After thirteen days, the animals developed gross neurological symptoms (tremors, seizures and Wooley-White reflexes). Daily injections of thiamin for seven days reversed the behavioral deficits. The forebrains of both symptomatic and reversed rats were used to determine the effects of thiamin deficiency on the in vitro production of CO_2 and acetylcholine from [U-^{14}C]glucose, and the activities in the brain of thiamin dependent enzymes [pyruvate dehydrogenase complex (PDHC), 2-oxoglutarate dehydrogenase complex and transketolase] as well as other non-thiamin dependent key energy metabolism enzymes (hexokinase, NAD-glutamate dehydrogenase, phosphofructokinase and PDH-phosphatase). The thiamin dependent enzymes were assayed in the presence of saturating quantities of thiamin pyrophosphate (TPP), so that any reduction should reflect loss of apoenzyme. In symptomatic animals, ACh (106.5 ± 6.0% of control value) and CO_2 (98.5 ± 2.5%) production in low K^+ buffer remained normal. However, the brains from symptomatic animals did not show a K^+-stimulated increase in ACh or CO_2 production. Values for both variables with low and high K^+ incubation were normal in reversed animals. The non-thiamin-dependent enzymes hexokinase (114.4 ± 7.9%), NAD-glutamate dehydrogenase (97.0 ± 3.0%), phosphofructokinase (97.4 ± 6.6%) and PDH-phosphatase (105.9 ± 7.8%) were the same as controls at all stages. PDHC was unchanged in symptomatic (96.4 ± 4.1%) or reversed (107.0 ± 3.0%) animals, whereas the 2-oxoglutarate dehydrogenase complex declined in symptomatic (64.4 ± 4.5%) but not in reversed (104.2 ± 6.0%) animals. Transketolase as assayed in the presence of TPP was 35.4 ±2.2% of controls in symptomatic animals, and was still diminished to 69.5 ± 1.5% of control after thiamin treatment for seven days. A similar decline in transketolase was observed in the absence of TPP. Thus, pyrithiamin-thiamin deficiency caused a selective loss of transketolase in reversed animals.

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197.8 NEURONAL MIGRATION IN PROTEIN MALNOURISHED RATS, William A. DeBassio* and Thomas L. Kemper* (SPON: C. Curcio). Depts. of Neurology and Neuropathology, Boston University School of Medicine, Boston City Hospital, Worcester Foundation for Experimental Biology, Shrewsbury.

In the mature rat neurogenesis continues in the subependymal layer of the anterior lateral ventricle and the majority of these cells are destined for the internal granule layer of the main olfactory bulb. This postnatal neurogenesis and migration allows us to study effects of low protein diets on generation and migration of neurons in rats.

Rats were fed 8% casein (low protein) or 25% casein (control) isocaloric diets for 5 weeks prior to mating and maintained on these diets until 35 days of age when they were injected intraperitoneally with 5 microcuries per gram body weight of tritiated thymidine as a marker of neurogenesis. The rats were sacrificed at 1, 3 and 6 days and the left anterior forebrain and olfactory bulb was blocked, embedded in epon plastic and serially sectioned in sagittal plane at a thickness of 2 microns. Sections containing the migratory stream were dipped in NTB-2 (Kodak) emulsion and processed according to standard autoradiographic techniques. The migratory stream of each animal was parcellated into sections and counts were made of tagged cells at specific levels along the stream. At 1 day significant differences were seen with control cells showing more heavily tagged cells farther along the stream. However, at 3 days significantly more tagged cells were seen farther along near the bulb in the experimental group, suggesting that these cells were traveling along more rapidly. At 6 days there were no differences along the stream indicating the rapidly moving cells had arrived at their destination. Calculation of the migratory rates were 600-700 microns/day in the experimental and 450 microns/day in the control group.

The early migration lag at 1 day in experimental animals may be related to a longer cell cycle, which occurs in malnourished animals. Our study suggests the brain in the malnourished animals generates cells which migrate more rapidly. This may be an adaptive mechanism allowing earlier maturation of neurons.

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197.9 DEVELOPMENT IN RAT PUPS ARTIFICIALLY REARED WITH A FORMULA SIMILAR TO RAT'S MILK. J. Diaz, C.R. Stamper, N.S. Auestad and J. Edmond, Dept. of Psych., Univ. of Wash., Seattle, WA,98195 and Dept. of Biol. Chem., UCLA Sch. of Med., Los Angeles, CA,90024.

The efficacy of rearing rat pups away from their mother and siblings, and feeding them exclusively via chronic intragastric cannulas, relies on the formula. We have demonstrated that a formula considered an adequate replacement for mother's milk (the "Messer" formula), when used in the artificial rearing procedure yielded pups with significantly lighter brains and heavier livers than their normally reared siblings. These effects were detected within 24 hours of artificial rearing. Closer examination of this formula revealed major differences in its components compared to mother's milk. This experiment examined the effectiveness of a formula which more closely resembles mother's milk.

Four day old female Long-Evans rats were assigned by weight to a normally reared (NR) group or an artificially reared (AR) group. Both groups were subdivided according to the age of sacrifice: day 5,6,8 or 12. AR animals were reared using a formula (RMS-2) developed by J. Edmond and N. Auestad at UCLA. This formula represents a substantial improvement over current rat milk replacements in that the protein/carbohydrate ratio, as well as the osmolarity and the profile of amino acids are all very similar to that of mother's milk. At sacrifice, each animal's brain, liver, kidney and spleen were removed and weighed. The brain was dissected and the cerebellum weighed.

The table summarizes the percent difference of AR to NR:

	Day 5	Day 6	Day 8	Day 12	(* p<.05)
Body Weight....	-3%	-11%*	-16%**	-6%	(** p<.01)
Whole Brain....	-2%	-4%	-12%**	-11%**	
Cerebellum.....	=	-3%	-17%**	-16%**	
Liver.....	-3%	-4%	-3%	+33%**	
Kidney.....	-3%	-6%	-7%	+16%**	
Spleen.....	-25%*	-42%**	-36%**	+60%**	

The incidence of gastrointestinal complications in the AR animals, which has been <5% with the Messer formula was 43% in this experiment.

With the Messer formula, brain weights decreased within 24 hours and liver weights increased within 48 hours of artificial rearing. With the RMS-2 formula, there were no differences in these organs at these times. Short term artificial rearing with the RMS-2 formula yielded animals that are not substantially different from their NR siblings. These data indicate that the RMS-2 formula is a significant improvement over the Messer formula in the artificial rearing procedure. However, the data for animals artificially reared for longer than 2 days indicate that relatively long periods of this procedure will require further modifications of the RMS-2 formula.

197.10 EFFECTS OF HIGH AND LOW L-TRYPTOPHAN DIETS IN THE THIRD TRIMESTER OF MOTHER RATS. M. Sakuma, *S. Gershon*, Dept.Psychiat., Wayne State University, 951 E. Lafayette, Detroit, MI 48207

(Spon:W.P.Clarke,Ph.D.)

The effects of prenatal exposure to changes in indole and catecholamines were studied in the brains of newborn rats of dams given three different tryptophan diets for a week prior to delivery. It was found that: (1) The mean brain weight of 1-day old pups of the maternal low tryptophan diet group was significantly more elevated than that of controls. The brain weight of pups of the maternal high tryptophan diet (3.0%) group was indistinguishable from that of the controls (0.19%); (2) The concentration of brain tryptophan, 5-HT, 5-HIAA, DOPA, DOPAC, and HVA in low tryptophan and high tryptophan diet groups in 1-day old pups were significantly higher than concentrations of the controls when brain weight of pups was taken into account. Brain levels of indole and catecholamines of the high tryptophan diet group surpassed the levels of the low tryptophan group. At the same time, the plasma levels of tryptophan in both diet groups were significantly higher than those of the controls, whereas 5-HT levels and dopamine metabolites in all diet groups were indistinguishable statistically. Thus, our findings of the low tryptophan diet are consistant with the suggestion of prior studies that the maternal supply during low tryptophan diet can cause high concentration of pup's brain metabolites. These findings in pup brain along with pup plasma tryptophan levels provide evidence of a possible difference in metabolism of pups.

NEUROTOXICITY II

198.1 EFFECTS OF PRENATAL LOW LEVEL LEAD EXPOSURE ON MAXIMAL ELECTROSHOCK SEIZURES AND CARBONIC ANHYDRASE ACTIVITY IN NEONATAL RATS. J. C. Coleman*, J. W. Kemp* and D. M. Woodbury (SPON: P. Burgess). Dept. of Pharmacology, Univ. of Utah Sch. of Med., Salt Lake City, Utah 84132.

Recent studies performed in this laboratory have demonstrated that prenatal, low-level exposure to lead causes alterations in patterns of electrolytes and transport ATPases in the neonatal rat (Coleman, et al., Fed. Proc. 42:1134, 1983). To correlate these changes with possible adverse developmental effects of lead exposure, further studies were performed to analyze seizure activity in neonatal rats by the maximal electroshock seizure (MES) test. Also, carbonic anhydrase (CA) activity was determined in the cerebral cortex and the cerebellum. Female Sprague-Dawley rats were exposed to lead, as lead acetate, via their drinking water. The concentrations of lead used were 0 (control), 40, 80, 160 or 320 mg/l. The females were exposed to the lead for a minimum of eight weeks before mating with normal males. Subsequent offspring were tested on postnatal days 3, 6, 9, 12 or 15. The pups were first subjected to the MES test. The electroshock was given by corneal electrodes with a current of 100 ma for a duration of 0.2 seconds. The degree of seizure response was observed by another member of the laboratory to prevent bias. The intensities of seizure responses were quantified as follows: running seizures were assigned a rating of 1; forelimb clonus was 2; forelimb flexion was 3; hindlimb flexion was 4; and hindlimb extension was assigned a 5. The results of this study show that lead-exposed neonatal rats were more susceptible to the MES test, i.e., lead-exposed rats of the same age as controls had more intense seizures and, hence, were hyperexcitable. This effect was greatest at earlier ages and was approaching control values at day 15. Others have proposed that the lead-induced hyperexcitability is due to excessive myelination. If this were the case, the activity of the glial enzyme, CA, should be increased. However, in lead-exposed pups the cerebellar CA activity was reduced between days 3 and 9; whereas in the cerebral cortex, CA activity was reduced only after the 9th day. Previous observations in this laboratory have demonstrated an inverse relation between cerebellar CA activity and brain excitability. Since the observed reduction in CA activity in the cerebellum of lead-exposed rat pups between 3 and 9 days of age corresponds to the increase in excitability noted in this period but does not correlate with the reduction of CA activity noted in the cerebral cortex, the data support the previous observations of a role of cerebellar CA in susceptibility to seizures. The lead-induced alterations in both the cerebral cortical and cerebellar CA patterns during neonatal development suggest that the intricate balance between the cerebral cortex and cerebellum is upset and this may account for the increased excitability seen in the lead-exposed neonatal rat. (Supported by NINCDS Grant No. NS-15767 and NIGMS Grant No. GM-00153).

198.2 DEVELOPMENT OF LOCOMOTOR ACTIVITY IN RAT PUPS IS ALTERED BY POSTNATAL EXPOSURE TO CADMIUM. Patricia H. Ruppert and Karen F. Dean*. Neurotoxicology Division, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

Alterations in locomotor activity are a common finding after developmental exposure to toxicants. One type of activity change involves hypoactivity during the neonatal period and hyperactivity during the juvenile or adult period. This biphasic effect on locomotor activity was found in figure-eight mazes following postnatal day (PND) 5 exposure to triethyltin (Reiter et al., Neurobehav. Toxicol. Teratol. 3:285-293, 1981). Exposure of rat pups to 4 mg/kg cadmium on PND 4 was reported recently to produce gross brain damage and juvenile (PND 30) hyperactivity in figure-eight mazes (Wong and Klaassen, Toxicol. Appl. Pharmacol. 63:330-337, 1982). The present study examined the ontogeny of this cadmium-induced alteration in locomotor activity. On PND 5, rat pups (Long Evans; Charles River) received a single, s.c. injection of saline, 1, 2, or 4 mg/kg cadmium chloride as the base. The volume of injection was 2 µl/g. A within-litter design (N=10 litters) was used for dosing; one male and one female from each litter received each dose and, therefore, each litter contained all treatments. Pups were weighed on PND 5, 10, 15 and 20. Preweaning motor activity was assessed during 30-min sessions in figure-eight mazes from PND 13-21. From PND 13-15, activity of control animals increased both across days and throughout each of the 30-min sessions. A peak in activity occurred in controls on PND 16, after which both within-session and between-session habituation developed. Pups exposed to 4 mg/kg cadmium (which also reduced preweaning growth and produced brain pathology) did not show a peak in activity on PND 16 or subsequent within-session habituation. Initially, high-dose animals were hypoactive (PND 14-16) but this progressed to hyperactivity on PND 20-21. Preweaning hypoactivity may represent the initial stage in the development of juvenile or adult hyperactivity induced by toxicant exposure. Therefore, age at testing is a critical variable in interpreting alterations in locomotor activity following developmental exposure to toxicants.

198.3 CARBON MONOXIDE INDUCED HYPOXIA IN UTERO ALTERS THE POSTNATAL DEVELOPMENT OF ENDOGENOUS NOREPINEPHRINE CONTENT IN THE CEREBELLUM OF THE RAT. J.E. Storm and L.D. Fechter. Dept. Envir. Hlth. Sci., The Johns Hopkins Univ., Baltimore, MD 21205.

Previous work has shown that prenatal hypoxia interferes with the normal development of the central nervous system, leading to changes in susceptible regions that persist into the postnatal and adult periods. One such change is decreased cell number in the cerebellum of 21 day old rats exposed to prenatal carbon monoxide (CO) induced hypoxia. We report here that alterations in the progressive postnatal development of norepinephrine (NE) content in the cerebellum also occurs following prenatal hypoxia.

Sperm positive female Long Evans hooded rats were placed in one of three chambers supplied with either compressed air or compressed air containing 150 or 300ppm CO, and maintained ad libitum until parturition approximately 22 days later. On the day of birth dams and their litters were removed from the chambers and then maintained in a colony room under normal air conditions for the remainder of the experiment. One male pup from each litter was sacrificed on postnatal days 14, 21, 28 and 35. Their cerebelli were rapidly removed, frozen over dry ice and kept at -80°C until assayed for NE content. Unexposed adult male cerebelli were taken for comparative purposes. Final sample sizes were 7 or 8 for each group at each age. Tissue was weighed and homogenized in 10 vols. 0.1N perchloric acid and spun at 3000xg 15 min. NE was extracted from the supernatant and measured using HPLC with electrochemical detection according to a method modified from that of Hefti, et al. (Br. Res., 195:123, 1980).

Prenatal CO induced hypoxia significantly increased mean cerebellar NE content (Two way ANOVA; treatment $F(2,80)=4.03$, $p<.05$). These increases were clear at day 14 and persisted at every age through day 35. No differences were evident between the 150 and 300ppm groups so that at each age, mean NE content in both CO exposed groups was 10-20% greater than that of the corresponding air exposed group. The general pattern of progressive increases in NE content during the postnatal period was the same for air and CO exposed groups (Two way ANOVA; day $F(3,80)=6.76$, $p<.01$, treatment by day interaction $F(6,80)=0.09$, N.S.).

Gradual postnatal increases of cerebellar NE content are one measure of noradrenergic nerve terminal growth and development in that region. The higher levels of NE observed in CO exposed groups over that of air exposed groups at each age suggests that the development of normal innervation of the cerebellum by the extrinsic noradrenergic neurons of the locus coeruleus, or their function, has been altered by prenatal hypoxia.

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198.4 5-THIOGLUCOSE PREVENTS GOLD THIOGLUCOSE LESIONS BUT NOT TRIAMINE LESIONS IN THE VENTROMEDIAL HYPOTHALAMUS. D. F. Brown* (SPON: D. D. Rigamonti). Division of Combat Casualty Care, Letterman Army Institute of Research, Presidio of San Francisco, CA 94129.

Recent reports have shown that the triamine, 3,3'-methylimino-bis-(N-methylpropylamine) (MIMPA), produced lesions in the hypothalamus of a variety of rodents, very similar to those produced by gold thioglucose (GTG) in the ventromedial hypothalamus (VMH) of mice (Levine and Sowinski, J Neuropathol Exp Neurol. 41: 54-66, 1982; Exp Neurol. 79: 462-471, 1983). These authors suggested that MIMPA is a general toxin causing brain necrosis in areas possessing a weak blood-brain barrier. Since MIMPA and GTG are not related chemically but produced nearly identical brain damage, they concluded that GTG causes hypothalamic lesions purely by means of penetrating the blood-brain barrier. In direct conflict with this idea is the evidence that GTG lesion formation is, 1) not found in the median eminence, 2) sensitive to plasma insulin, glucocorticoid and estrogen levels, hormones involved with carbohydrate metabolism, 3) specific for neural cells and not vascular tissue in the VMH, 4) prevented by glucose transport inhibitors, 5) blocked by abdominal stress, 6) not found in gerbils, 7) increased by a glucose injection, and 8) abolished by mechanical VMH damage. In view of these conflicting ideas, we investigated whether or not 5-thioglucose (5TG), an antimetabolic glucose analog, would prevent the development of GTG and/or MIMPA lesions in the mouse VMH. Female Swiss-Webster mice approximately 3 months of age were used in this study. They were housed at 23 C, on a 12-hour light:12-hour dark photoperiod, fed Purina mouse and rat chow, and given tap water freely. All mice were given 5TG (500 mg/kg) intraperitoneally (IP). Thirty minutes later, either GTG (500 or 870 mg/kg, IP) or MIMPA (870 mg/kg, subcutaneously) was administered. The mice were decapitated 24 hours after the GTG or MIMPA was given. The brains were fixed in Bouin's fluid, subjected to routine histology, stained with hemotoxylin and eosin, and examined under the light microscope. Both control groups, GTG only or MIMPA only, developed typical bilateral VMH lesions. On the other hand, mice challenged with 5TG and GTG did not possess lesions in the VMH, while animals given 5TG and MIMPA displayed typical VMH damage. 5TG is a potent glucoprivic agent in mice and rats. Presumably, 5TG binds to glucoreceptors in the hindbrain, producing a pronounced hyperphagia. Our results suggest that 5TG prevented GTG lesion formation by binding to glucoreceptors in the VMH. MIMPA lesions were not inhibited by 5TG, implying that glucoreceptors are not involved with triamine VMH destruction. Moreover, the data demonstrated that although MIMPA and GTG cause similar VMH damage, the mechanism by which each lesion occurs is apparently quite different.

198.5 SLOW AXONAL TRANSPORT FOLLOWING SINGLE HIGH-DOSE ACRYLAMIDE ADMINISTRATION. B. G. Gold*, J. W. Griffin and D. L. Price (SPON: A. Pestronk). Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Acrylamide (AC) produces a distal axonopathy when administered in repeated doses. The mechanism by which AC elicits its neurotoxicity is unknown. Because the axonopathy is associated with distal neurofibrillary pathology, it has been suggested that AC may affect the slowly transported components of the cytoskeleton. A deficit in slow transport is also suggested by our recent observation that a single high dose of AC completely inhibits axonal outgrowth following nerve crush. However, previous studies of slow transport have produced conflicting results. The present study was designed to detect any direct effects of AC on slow transport prior to the development of confounding structural changes.

AC (75 mg/kg, ip) was given to rats 24 hours prior to isotope injection. The lumbar ventral horns of AC-treated and age-matched controls were injected with [^{35}S] methionine. Animals were sacrificed six days following labeling and sciatic nerves removed, cut into 3-mm segments, homogenized, aliquots counted, and the proteins separated by gradient SDS-PAGE. Radioactivity in individual proteins was demonstrated by gel fluorography. In order to determine whether a selective defect exists in the transport of individual slow component polypeptides, the radioactivity of the neurofilament 145 kd protein (NF 145), tubulin, and actin was quantified by cutting, dissolving, and counting their individual bands. Transport velocities were expressed as half velocities (HV); HV for each protein were calculated from plots of cumulative radioactivities by extrapolating to the position along the nerve reached by 50% of the radioactivity.

Inspection of the plots of total radioactivity within nerve segments revealed no apparent abnormality in slow transport peaks. HV, calculated from these data, demonstrated a significant decrease (26%) in slow transport between AC and control groups; HV were reduced from 2.3 to 1.7 mm/day. HV of NF 145 protein were significantly decreased from 1.93 to 1.51 mm/day (21%). Tubulin and actin HV were similarly reduced (27% and 25%, respectively) from 2.24 to 1.64 mm/day and 2.22 to 1.67 mm/day, respectively. Morphological studies demonstrated no gross abnormalities (i.e., Wallerian degeneration) during the time of study.

These studies demonstrate a modest nonselective defect in the transport of slow component proteins following single high-dose AC intoxication in nerves displaying no evidence of Wallerian degeneration. Future studies will elucidate the possible role of these transport defects in the production of abnormal regenerative properties of nerves from AC-intoxicated animals.

198.6 EFFECTS OF CHRONIC FENFLURAMINE TREATMENT ON THE SEROTONERGIC SYSTEM OF THE YOUNG RAT. P. F. Warren*, M. A. Peat, C. J. Schmidt* J. W. Gibb. (SPON: J. A. Madsen) Dept. of Biochem. Pharmacol. & Toxicol., University of Utah, Salt Lake City, UT 84112.

Seven day old male Sprague-Dawley rats were housed in a twelve hour light-dark cycle with lactating mothers. At two weeks of age the pups were injected (s.c.) twice daily for 14 days with either 2.5 mg/kg fenfluramine, 7.5 mg/kg fenfluramine, 5 mg/kg d-amphetamine or saline. Rats were sacrificed by decapitation 24 hours, 3 weeks, 5 weeks or 7 weeks after the last injection and cerebral cortex, neostriatum and hippocampus dissected out. Tissues were frozen at -70°C before analysis. Adult male Sprague-Dawley rats were administered a similar dosage schedule. Tryptophan hydroxylase (TPH) activity was measured by a $^{14}CO_2$ trapping procedure (Life Sci., 25:1373, 1979) and serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan (TRP) concentrations by HPLC-fluorescence (Anal. Biochem. 128:275, 1983).

Cortical TPH activity in young rats was still significantly decreased (68% of control) 7 weeks after treatment with 7.5 mg/kg of fenfluramine. However, there was a significant recovery in enzyme activity when compared to that 24 hours after the last injection. Although both neostriatal and hippocampal TPH activities were reduced at 24 hours (to 66 and 49% of control respectively) recovery was noted by 7 weeks in the neostriatum and by 5 weeks in the hippocampus. After 14 days of treatment with 2.5 mg/kg fenfluramine TPH activity in the cerebral cortex and neostriatum was decreased 24 hours later to 65 and 68% of control respectively; however, hippocampal TPH activity was not significantly different from control. Seven weeks after treatment cortical and neostriatal enzyme activities had recovered. Treatment with 5 mg/kg of d-amphetamine only decreased neostriatal TPH activity 24 hours after the last injection.

These data show that in young rats there is a dose and time dependent recovery of TPH activity in the cerebral cortex, neostriatum and hippocampus following treatment with fenfluramine. This may be important in view of the recent reports suggesting that fenfluramine is useful in the treatment of autism.

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198.7 PROTEIN I: A BIOCHEMICAL MARKER FOR NEURONAL DAMAGE BY TRIMETHYLTIN. G. J. Harry*, J. F. Goodrum*, M. R. Krigman and P. Morell. Biological Sciences Research Center, University of North Carolina, Chapel Hill, NC 27514.

Protein I is a well characterized nervous system specific phosphoprotein. Immunohistochemical studies have localized Protein I to neurons in all regions of the central and peripheral nervous systems where it appears to be concentrated in presynaptic terminals (Bloom et al., 1979; DeCamille et al., 1980). Additional studies have indicated that the developmental appearance of Protein I is a postnatal event (Lohman et al., 1978) which correlates with the morphological development of synaptic structures in the rat (Crain et al., 1973). Because of the neuronal localization of Protein I, measurement of its levels may prove to be a useful biochemical marker for neuronal damage following exposure to a neurotoxicant. Examination of the neurotoxic properties of selected compounds by measurement of Protein I in discrete brain regions may reveal alterations or delays in synaptogenesis in developing animals as well as neuronal loss in adults.

To test the possible use of Protein I measurements as an indicator of neuronal damage, we have measured its levels in brain regions of rats treated with trimethyltin (TMT). Adult Long-Evans rats were dosed with either saline or 4 mg/kg TMT for four consecutive days. This dosing regimen resulted in gross manifestations of neurological damage (tremors and seizures) and necrosis of neurons in the hippocampus. Forty-eight hours following the last dose, animals were killed by decapitation and the brains quickly removed. Each brain was dissected into distinct brain regions and stored at -80°C until assayed. Protein I was extracted with citric acid from each distinct brain region sample and phosphorylated in vitro with exogenous protein kinase and gamma ^{32}P-ATP by an adaptation of the method of Goelz et al. (1981). Radiolabelled proteins were separated by SDS gel electrophoresis. Autoradiographs of each gel were scanned for Protein I and areas under the peaks determined. Animals treated with TMT showed a 38% decrease from control animals in the amount of Protein I in the hippocampus.

A similar dosing regimen was used to examine the effects of TMT on the developing animal. Rat pups were dosed orally with either vehicle or 4 mg/kg TMT on postpartum day 10-12. Animals were killed at day 25 by decapitation; brains removed, dissected into distinct brain regions, and stored at -80°C until assayed. The hippocampus of treated animals showed significant decreased level of Protein I compared to control animals While decreases in Protein I appear to be an indicator of neuronal damage, the nature of that damage requires additional specific analysis.

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198.8 ALUMINUM LOCALIZATION IN THE RABBIT CENTRAL NERVOUS SYSTEM. G.Y. Wen and H.M. Wisniewski* NYS Institute for Basic Research in Developmental Disabilities, Staten Island, N.Y. 10314

Aluminum is a neurotoxic element. Intoxication of aluminum has been implicated in renal dialysis encephalopathy (Alfrey et al., New Eng J Med 294:184, 1976; McDermott et al., Lancet 1:901, 1978). Aluminum has been known to induce neurofibrillary changes in certain species of animals since 1965 (Wisniewski et al., Klatzo et al., J Neuropath Exp Neurol 24:139, 1965; 24:187, 1965). The nature of neurotoxic action of aluminum is still unknown. This study described the specific association of aluminum with certain cellular components in the CNS of the rabbits.

Rabbits were divided into 3 groups and treated as follows: i) intracisterna-magnum injection of 0.1 ml of 1% $AlCl_3$ or 5 mg of aluminum powders, ii) intravenous injection of 5 ml of 16% aluminum lactate, iii) saline injection as control. Animals were sacrificed by intracardiac perfusion. Silver sulfide EM and fluorescent Morin stain were employed in this study.

Results of this investigation indicate that aluminum in a neuron was associated with the nucleic acids. Aluminum was observed in nucleolus, interchromatin granules, rough ER, free ribosomes, euchromatin and heterochromatin. The consistent association of aluminum with the first four r-RNA containing cellular organelles or components and with the last two DNA containing chromatins suggests that the binding of aluminum to both r-RNA and DNA may be the cause of aluminum neurotoxicity. It may interfere with the normal mechanism of protein synthesis of r-RNA and of the transcription or gene modulation of DNA. Aluminum was not observed in the tangle area. Aluminum was also observed in the astrocytic process and in the nuclei of endothelial cells, pericytes and muscle cells of the blood vessels. The detection of aluminum in the pyramidal neurons of cerebral cortex and hippocampus and spinal cord neurons 1 hour after intravenous injection indicates a rapid entry of aluminum from injection site through blood brain barrier, to neurons. Fluorescent Morin stain also indicated that aluminum was associated with nucleolus, nuclear chromatins and certain cytoplasmic areas, but not associated with tangles. Bodian stain of the same section previously stained with fluroescent Morin stain further confirmed the absence of aluminum in the tangle areas of the neurons.

198.9 INTERACTION OF MERCURIALS WITH MYELIN: COMPARISON OF IN VITRO AND IN VIVO RESULTS. D.A. Kirschner* and A.L. Ganser (SPON: H.W. Moser). Deparment of Neuroscience, Children's Hospital and Harvard Medical School, Boston, MA 02115.

We have previously shown (J. Mol. Biol., 1982, 157: 635) that in vitro exposure of peripheral nerve to mercurials results in either labeling of the phosphatidylethanolamine plasmalogen (with $HgCl_2$) or altering of the periodicity and packing of the membranes (with $HgCl_2$ or CH_3HgCl). To test whether these in vitro interactions of mercurials with myelin might explain some of their neurotoxic effects, we carried out structural and chemical measurements on myelin from mice intoxicated with these compounds.

Mercurials were administered to groups of mice by four different routes: i.v. injection; i.p. and s.c. injections; inhalation of Hg^0 vapor; and perorally via drinking water. We monitored the mice for signs of neurotoxicity, measured levels of mercury in tissues using atomic absorption spectrophotometry, examined myelin structure using X-ray diffraction, and localized mercury by an electron microscopic histochemical method that uses sulphide to precipitate mercury in fixed tissue. X-ray diffraction is a particularly sensitive way of detecting structural changes and possible chemical labeling in unfixed, intact myelin.

Neither i.v. injection (20 & 60 mg/kg) nor i.p. and s.c. injections of $HgCl_2$ (1.2 mg/kg/d x 100 d, Mon-Fri) produced neurological symptoms. We found no detectable changes in the X-ray patterns of sciatic and optic nerves, and when these sciatic nerves were treated in vitro with $HgCl_2$, the usual changes occurred.

Daily exposure (for 40 min) to air saturated with Hg^0 vapor produced a decrease in water consumption and weight after 3-5 d, and neurological symptoms after 6 d. X-ray patterns from the sciatic and optic nerves of severely affected animals were normal.

Even after >400 d of $HgCl_2$ perorally at 10-20 mg/kg/d, mice did not develop any neurological symptoms. Mercury was not detected in sciatic or optic nerves, but was present in cerebellum (7 ug/g), liver (20 ug/g) and kidney (37 ug/g). X-ray patterns from the nerves were normal, and sciatic nerve was still reactive in vitro with $HgCl_2$. No electron dense HgS granules were seen in thin-sections. Mice that received CH_3HgCl perorally at 10 mg/kg/d lost weight and developed neurological symptoms within two weeks. Tissue levels of mercury ranged from 28 ug/g in cerebellum and 19 ug/g in sciatic nerve to <1 ug/g in optic nerve. However, X-ray patterns from the nerves were normal, and no HgS granules were observed by electron microscopy.

We conclude that in vivo intoxication with mercurials does not allow sufficient mercurial to diffuse into and interact with the myelin. This was unexpected in view of the reactive sites in the protein and especially in the lipid of myelin. (Supported by NINCDS #14326).

198.10 CLARIFICATION OF APPARENT DESCREPANCIES IN EXPERIMENTAL RESULTS OF LIGHT ENHANCMENT OF THE INHIBITION OF [^{3}H]OUABAIN BINDING TO RAT BRAIN MEMBRANES BY ERYTHROSIN B. Sally M. Anderson. Neurotoxicology Section, NINCDS, NIH, Bethesda, MD 20205.

Erythrosin B has been demonstated to be a potent in vitro inhibitor of: (1) ATP catalysis by Na,K-ATPase, (2) the efflux of ^{86}Rb, and (3) the binding of [^{3}H]ouabain to rat brain membrane tissue homogenates (Lafferman, J. and E.K. Silbergeld, Science, 205: 410, 1979; Silbergeld, E.K., Neuropharmacology, 20: 87, 1981). More recently a light-enhanced increase in the potency of the dye's ability to inhibit [^{3}H]ouabain binding to rat brain was reported by Hnatowich and LaBella (Molecular Pharmacology, 22: 687, 1982). However, a significant difference in the effect of this inhibitory action of erythrosin B measured in the light and dark was not observed by Silbergeld et al. (Silbergeld, E.K., et. al., Life Sciences, 31: 957, 1982). The ostensible conflict between the results of these two studies can be explained by differences in the experimental protocols used by these two groups of investigators. Although there are numerous differences in the experimental protocols used by these two research groups the discriminating variable that explains the seeming contradiction between these two studies is time of incubation of tissue with [^{3}H]ouabain and erythrosin B.

The IC_{50} for erythrosin B inhibition of Type II [^{3}H]ouabain binding (2nM) incubation for 90 min, at 37°C (protected from light) is 2.2 μM; the potency of the dye increases when the incubation mixture is exposed to 350 Lux, of light (IC_{50} = 0.94 μM). The amount of inhibition of [^{3}H]ouabain (2 nM) binding by 5μM erythrosin B increases dramatically from 15 to 90 minutes in light-exposed samples, whereas an increase in inhibition in that time period for light-protected samples is not apparent. Similar results were obtained for Type I [^{3}H]-ouabain binding (2nM). Therefore, the enhanced potency of inhibition of [^{3}H]ouabain binding to rat brain membrane preparations will be discernible only in results from experimental protocols in which the incubation period is greater than thirty minutes. Studies to elucidate of the differences in the inhibitory processes involved in the light-induced vs. light-protected inhibition of [^{3}H]ouabain by erythrosin B are in progress.

198.11 TEMPORAL CHANGES IN DOPAMINERGIC AND SEROTONERGIC FUNCTION CAUSED BY ADMINISTRATION OF TRIMETHYLTIN TO ADULT RATS. Diane L. DeHaven, Martin R. Krigman and Richard B. Mailman. Biological Sciences Research Center and Depts. of Psychiatry, Pharmacology and Pathology, Univ. of North Carolina Sch. of Med., Chapel Hill, NC 27514

Trimethyltin (TMT) is known to cause relatively specific lesions of the hippocampus and pyriform cortex (Am. J. Pathol. 104: 237, 1981), and behavioral changes including seizures, hyperactivity, hyperreactivity and memory deficits (Neurobehav. Toxicol. Teratol. 4: 127; 4:177, 1982). Our earlier studies (Soc. Neurosci. Abstr. 8:562, 1982) have shown that a single dose of TMT caused alterations in 5-HT function in several brain regions 7 days post-dosing. The present experiments followed these changes at 14, 21 and 28 days post treatment. Male Long-Evans rats (ca. 120 days of age) were administered TMT chloride (7 mg/kg, IG) or 0.9% saline, and were sacrificed 14, 21 or 28 days later. Striata, olfactory tubercles, nucleus accumbens, frontal cortex, septum, amygdala/pyriform cortex and hippocampus were dissected and DA, 5-HT and their acidic metabolites DOPAC, HVA and 5-HIAA were quantified by HPLC/EC (J. Chromatog.225: 347, 1981), and the ratios of metabolite to parent amine computed. Hippocampal changes were limited to an increase in 5-HIAA at day 28. A similar change in 5-HIAA was seen in septum at day 28, but there was also an increased 5-HIAA/5-HT ratio and decreased DA in this region at days 14 and 21. In amygdala/pyriform cortex, 5-HT levels were decreased at day 14, and DOPAC and 5-HIAA were increased at day 28. In striatum, 5-HIAA/5-HT ratios were increased at days 14 and 28, whereas DOPAC and HVA were increased only at the latter time. DA concentrations were decreased and 5-HIAA/5-HT ratios were increased at day 21 in olfactory tubercle. In nucleus accumbens, there was a decrease in DA concentration at days 14 and 21, whereas 5-HT was decreased and the 5-HIAA/5-HT ratio increased at day 14. No changes were detected in frontal cortex. From these data, it is clear that a single dose of 7 mg/kg TMT causes profound changes in serotonergic systems that persist, although somewhat attenuated, at least 28 days after treatment. Moreover, there are also less dramatic changes in dopamine systems that appear during the second week post treatment. It is likely that some of these neurochemical perturbations, particularly those in the septum and nucleus accumbens, may be related to the known behavioral effects of TMT intoxication.

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198.12 EFFECTS OF THIAMIN ON THE NEUROTOXICITY OF LEAD. N. D. Woolley-Efigenio*, D. E. Woolley*, M. F. Ferrell* and P. M. Dreyfus*. Depts. of Nutrition, Animal Physiology and Neurology, Univ. of Calif., Davis, CA 95616.

Administration of thiamin has been reported to alleviate the neurotoxic symptoms produced by lead exposure in deer, livestock, and rats. The present studies were undertaken to investigate further the ability of thiamin to counteract the effects of lead exposure by utilizing the developing, postweaning rat. Neurotoxic effects were evaluated using maximal electroshock seizure (MES) patterns. Additional toxic effects evaluated included body growth, food intake, organ weights at autopsy, and tissue levels of lead and certain minerals. Lead exposure was via the drinking water which contained 0.2% lead acetate. In the first study, beginning immediately postweaning at 23 days of age, 3 groups of Sprague-Dawley rats were fed Purina Rodent Chow containing 18 mg thiamin/kg diet. Two groups were exposed to lead; animals in one of these groups received daily injections of thiamin sc as follows: 2 mg thiamin/kg body weight from 24-87 days of age, 20 mg/kg from 88-128 days of age, and then 40 mg/kg to 144 days of age. Lead exposure did not alter body growth, but produced an anticonvulsant effect on the MES as shown by increased duration of flexion and decreased durations of full extension and total tonus. Administration of thiamin reversed most of the effects of lead on the MES. Lead exposure also increased kidney weights and treatment with thiamin increased kidney weights even further. Brain levels of lead were not altered by the thiamin injections. In the second study, specific pathogen free Sprague-Dawley rats were used. Treatment began at 32 days of age and all rats received a semipurified diet containing either 20 or 0.6 mg thiamin/kg diet. Half of the rats in each group received lead in the drinking water. Body growth rate for the low thiamin plus lead group initially fell behind that of the high thiamin plus lead group. At the end of the study, at 99 days of age, rats in both of the low thiamin groups had the same body weights and weighed significantly less than the two high thiamin groups. The high thiamin plus lead group was the heaviest, weighing even more than the controls (high thiamin without lead group). Food intake and body weights followed a similar pattern. Kidney:body ratios were increased in the low thiamin group, and even more so in the low thiamin plus lead exposure group. Lead exposure initially had an anticonvulsant effect in the high thiamin group. Low thiamin itself had a convulsant effect which was worsened by lead. These preliminary results thus indicate that low dietary thiamin levels may exacerbate the toxicity of lead, and that high thiamin levels may ameliorate it. (Supported by NIH grant ES-01503.)

198.13 DIETARY ZINC ALTERS THE NEUROTOXICITY OF LEAD. D. Woolley*, Z. Hasan*, L. Zimmer* and N. Woolley-Efigenio*. Depts. of Animal Physiol. and Nutrition, Univ. of Calif., Davis, CA 95616.

The present study was designed to determine whether or not relatively high levels (100 ppm) of zinc in the diet could counteract and relatively low levels (2-5 ppm) exacerbate some of the neurotoxic effects of lead exposure. Starting immediately post weaning at 22-24 days of age, Sprague-Dawley rats were fed a semipurified diet (Zeigler Brothers, Gardners, PA). Lead exposure was via drinking water which contained 0.2% Pb acetate. To evaluate neurotoxic effects, the duration and phases of the maximal electroshock seizure (MES) pattern were determined at various time points during the lead exposure. In addition, at 135 days of age animals from each group were implanted with electrodes for later study of limbic evoked potentials in unanesthetized rats. Lead exposure slowed body growth whether or not the diets contained high or low levels of zinc. However, rats on the low zinc diet were affected sooner by the lead exposure, perhaps because lead may increase excretion of zinc which then would increase the growth-retarding effects of a low zinc diet. Animals on low zinc showed much more severe MES seizures than did rats on high zinc, as shown by decreased duration of flexion and increased durations of full extension, total extension and total tonus. Lead exposure had little effect on the MES in rats fed the high zinc diet, whereas lead potentiated the effects of low zinc on the MES, so that rats on low zinc plus lead exposure showed the most severe seizures. Thus, the high zinc diet did not protect against the growth-retarding effects of lead but did protect against effects of lead on the MES. Plasma zinc levels were significantly decreased in the low zinc group, and were decreased even more by lead administration. High levels of zinc in the diet reduced accumulation of lead in brain when compared with brain levels of lead in animals on low dietary zinc. Levels of zinc in whole brain did not differ in animals on high or low dietary levels of zinc in the absence of lead treatment. Neither low dietary zinc nor lead exposure altered evoked potentials elicited in CA3 of the hippocampus by stimulation of either the mossy fibers at their origin in the dentate gyrus (DG) or by stimulation of commissural fibers. However, the DG response to stimulation of the olfactory (prepyriform) cortex was potentiated at high (4/sec) rates of stimulation in the low zinc intake plus lead exposure group, when compared with controls. The latter findings agree with the current observation that the low zinc plus lead exposure group showed the most severe MES seizures and with our previous findings that toxicants which increase seizure severity also increase amplitude of the olfactory-evoked DG potential. (Supported by NIH grant ES-01503.)

198.14 BEHAVIOR AND ORGAN WEIGHTS FOLLOWING EARLY CAPSAICIN TREATMENT IN RAT PUPS. E. Murowchick* and J. Diaz (Spon: I. Bernstein). Dept. of Psychology, Univ. of Wash., Seattle, WA, 98195.

Studies in which capsaicin has been given to rat pups have not reported the animals' condition during and immediately after the treatment. Since the blood brain barrier is not well established in neonatal rats, early capsaicin treatment may be exerting central as well as peripheral effects. This experiment examines the developmental effects of neonatal capsaicin administration.

Long-Evans rat pups were assigned by weight to the capsaicin (CAP) group (n=6) or to the vehicle (VEH) group (n=5). The animals in the CAP group were given daily subcutaneous injections beginning on day 2 and ending on day 6 with the following ascending dose regimen: 25,50,100,100,200 mg/kg. The animals in the VEH group were injected with similar volumes of the DMSO vehicle. Beginning on day 8 both groups were tested daily for negative geotaxis. The occurrence of eye opening and incisor eruption was noted. On day 17, each animal was placed in a plastic tube in a warm incubator (28 C) for five minutes and its core temperature was taken. Immediately following this, the animal was placed in a cool incubator (8-9 C) for an additional five minutes and its core temperature was again recorded. On day 18 each animal was sacrificed; its whole brain, cerebellum, liver, kidney and spleen were removed and weighed.

The initial injections of capsaicin were followed by vocalizations, writhing and in some cases convulsions occurring about five minutes after the injection. Following the injections the capsaicin animals gained less weight than the vehicle animals. There were no significant differences between the groups in the ontogeny of negative geotaxis, the occurrence of developmental milestones, in body, liver, kidney or spleen weights at sacrifice. This table summarizes the remaining means:

	BRAIN WT.	CEREB.	WARM TEMP.	COLD TEMP.	DELTA TEMP.
CAP	1.18 g	.116 g *	35.5 C	34.05 C *	-1.48 C **
VEH	1.20 g	.128 g	36.1 C	35.20 C	-.88 C

(* = P<.05; ** = P<.01)

The decreased growth in the CAP group would suggest a potential problem with nutrition and/or mothering. However, since the groups were not different in negative geotaxis ontogeny, nor in milestone occurrence, frank undernutrition is unlikely. The decreased ability in the CAP group to temperature regulate in response to a mild cold challenge indicates that systems other than pain were effected, perhaps some component of the somatosensory system. The decreased cerebellar weight in the CAP group raises the possibility the capsaicin may effect the central nervous system when given neonatally. Measures more sensitive than gross tissue weights may be necessary to address this issue.

198.15 THE EFFECTS OF POSTNATAL LEAD TOXICITY ON CNS CELL DEATH AND MORPHOLOGICAL DEVELOPMENT OF CEREBELLAR PURKINJE CELLS. D. Lorton and W.J. Anderson. Indiana Univ. Sch. Med., Terre Haute Ctr. for Med. Ed., Terre Haute, IN 47809.

Rat pups from pregnant Long-Evans rats were given 600 mg/Kg body weight lead acetate every 24 hrs. beginning one day after birth until cumulative doses of 600, 1200, 1800, 2400, 3000 mg/Kg were administered via stomach intubation. The pups were sacrificed every 24 hrs. after administration of the last dose until 10 days of age and also at 21 and 30 days of age. The brains were prepared for histological examination. Pups which received a cumulative dose of 2400 mg/Kg were sacrificed in the same pattern as stated above and the brains prepared for Golgi analysis. Microscopic examination of various brain regions revealed chromolytic and pyknotic neurons in many brainstem nuclei, cerebral and cerebellar cortex, and other forebrain structures. These alterations had no obvious pattern from one lead exposed brain to the next or with respect to dosage. Examination of camera lucida drawings of Golgi-Cox prepared tissue showed a reduction in the dendritic arborization of Purkinje cells in the cerebellum. In contrast to controls, the dendritic tree frequently did not reach the surface of the molecular layer. There were fewer secondary and tertiary branches on the Purkinje cells. Many of the fine dendritic processes which were present extended from thick dendritic branches. These results are in agreement with previously obtained data in studies involving dietary lead exposure (Patrick, 1979) which have been criticized due to nutritional deficiency observed in the experimental animals. The effects of malnutrition in this study were circumvented by feeding lead acetate directly to the animals by intubation. Thus, the neuronal alterations may be attributed directly to lead exposure or may be the result of secondary vascular insults which occur after postnatal lead exposure with rats.

198.16 TOXIC EFFECTS OF MATERNAL EXPOSURE TO TRICHLOROETHYLENE ON BRAIN GROWTH IN RAT PUPS. D. Wines, R. Pfohl* and D. Taylor*. Department of Zoology, Miami University, Oxford, Ohio 45056.

Trichloroethylene (TCE) is an unsaturated chlorinated hydrocarbon widely used in industry. Studies on adult animals have demonstrated that TCE exerts toxic effects on the CNS. Controversy remains regarding the effects of TCE on the developing nervous system. The purpose of this study is to determine if maternal exposure to TCE results in anomalous brain growth in pups.

Female rats were exposed via drinking water to 2500 ppm TCE from two weeks before breeding until the pups were weaned at 21 days of age. Toxic effects were assayed by comparing wet weights and levels of DNA, RNA, and protein in whole brains and cerebella from control and exposed 21-day old pups. In addition, whole brain volume and length and cerebellar width and vermis length were measured in formalin perfused rats. Nucleic acids were extracted by a modification of the Schmidt & Thannhauser procedure. DNA was determined by the diphenylamine reaction and by a two-wavelength UV absorbance method. RNA and protein were determined by the orcinol and biuret reactions, respectively.

No significant differences in the whole brain or cerebellar volumes, dimensions, or wet weights were noted. Exposure to TCE did, however, result in significant changes in the levels of nucleic acids and proteins. TCE exposure resulted in a 6% decrease ($P<0.01$) in the DNA content (or cell number) per gram of whole brain tissue and a 5% increase ($P<0.01$) in the RNA content per cell. The protein content per gram of cerebellar tissue was 3% less ($P<0.05$) in TCE-exposed pups than in controls. Decreases in DNA and RNA of 3-4% in cerebella from exposed pups approached significance ($P<0.10$).

These data suggest that maternal exposure to TCE produces effects in the developing brain. The cerebellum may be especially sensitive to TCE exposure as has been reported in adult animals (Haglid et al. 1980. Archs. Toxicol. 43: 187-199). These effects may be due to delayed maturation rather than neural damage. Histological studies are underway to investigate these possibilities. Further experiments are also being conducted to determine if these effects persist into adulthood.

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ACTION POTENTIAL AND ION CHANNELS IV

199.1 MONOCLONAL ANTIBODIES AGAINST THE VOLTAGE-DEPENDENT SODIUM CHANNEL FROM RAT SKELETAL MUSCLE. J. Casadei,* L. Lampson, E. Bonilla* and R. Barchi, Depts. of Neurology and Anatomy, Univ. of Penn. Med. Sch., Philadelphia, PA 19104

A panel of monoclonal antibodies that reacts specifically with the saxitoxin (STX)-binding component of the voltage-dependent sodium channel from rat skeletal muscle has been generated. BALB/c mice were hyperimmunized with a partially purified preparation (~1250 pm [^{3}H]-STX/mg protein) of the sodium channel protein from rat sarcolemma (Barchi et al. (1980) PNAS 77:1306-1310). A highly purified fraction, (~2500 pm STX/mg) was injected intravenously four days prior to fusion. Hybridomas were generated by fusion of NS-1 cells with spleen cells from hyperimmunized animals. Hybrids were initially screened by a solid-phase radioimmunoassay in which ^{125}I-labelled sheep anti-mouse F(ab')$_2$ fragments were used to detect antibody bound to the partially purified, immobilized sodium channel protein. Of approximately 2500 hybridoma supernatants tested, 108 gave positive results. A second level of screening was applied to the 57 most reactive cultures by determining the ability of each antibody to specifically immunoprecipitate [^{3}H]-STX binding activity from crude solubilized sarcolemma. Complexes of antibody and sodium channel protein were precipitated by Staphylococcus aureus coated with rabbit anti-mouse immunoglobulins. Of 57 hybridoma supernatants screened, 6 precipitated the sodium channel under these conditions. Of these 6 monoclonal antibodies, 4 are IgG_1 and 2 are IgM. These six hybridomas were subsequently cloned at limiting dilution and grown as ascites tumors. Two of these monoclonal antibodies were further purified from ascites fluid and covalently coupled to CNBr-activated Sepharose 4B. These solid-phase antibodies were shown to precipitate a [^{3}H]-STX binding protein from solubilized sarcolemma in a concentration-dependent manner.

Specific binding of anti-sodium channel monoclonal antibodies could be detected at the surface of skeletal muscle fibers in frozen section using either fluorescein- or peroxidase-labelled second antibody. Using the indirect peroxidase technique with the electron microscope, specific antibody binding was localized to the plasma membrane at the fiber surface and in caveoli. In addition, specific binding was found in the membranes of the T-tubular system. Specific binding of these monoclonal antibodies was also demonstrated in tissue culture both to L-6 and to primary fetal rat muscle.

199.2 BIOCHEMICAL CLASSIFICATION OF MUTANTS AFFECTING THE VOLTAGE-SENSITIVE SODIUM CHANNEL IN DROSOPHILA. L.M. Hall, F.R. Jackson, S.D. Wilson,* and G.R. Strichartz. Genetics Dept., Albert Einstein Coll. of Med., Bronx, N.Y. 10461 and Dept. Anesthesia Res. Labs., Harvard Med. Sch., Boston, MA 02115.

We have found that mutations which cause a reversible, temperature-induced paralysis in the fruitfly Drosophila melanogaster often affect the voltage-sensitive sodium channel which is involved in the propagation of action potentials. These mutants paralyze at 38°C where wild-type are unaffected. ^{3}H-saxitoxin binding to membrane extracts prepared from Drosophila heads has been used to classify mutants. The seizure^{ts-2} (sei^{ts-2}) mutant strain shows a temperature-dependent increase in the dissociation constant (K_D) for saxitoxin binding at 39°C (sei^{ts-2} K_D=6.52± 0.52nM; wild-type K_D=3.74± 0.19nM). The K_D's for mutant and wild-type are the same at 4°C. The total number of saturable binding sites (Bmax) was the same in mutant (127± 14.3 fmol/mg protein) and wild-type (135± 7.8 fmol/mg protein) regardless of assay temperature. The sei^{ts-2} strain also shows a shift in pH sensitivity of ^{3}H-saxitoxin binding (sei^{ts-2} pKa=6.32; wild-type pKa=5.87). These changes in binding parameters suggest the sei^{ts-2} strain has a structural alteration in the saxitoxin receptor. This change appears to be specific for sodium channels because α-bungarotoxin binding to cholinergic receptors in sei^{ts-2} extracts is normal. Another type of mutant-induced change is found in the no-action-potential-temperature-sensitive (nap^{ts}) mutant strain. This strain shows no effects on K_D or on pH sensitivity of toxin binding but does show a dramatic decrease (30-50%) in the number of saturable binding sites. This reduction is independent of assay temperature. The nap strain appears to affect regulation of saxitoxin receptor number. Although we find no biochemical evidence for structural alterations, we cannot rule out structural alterations such as post-translational modifications which might affect the efficacy with which channels are incorporated into membranes without affecting the thermal stability or the structure of the saxitoxin-binding site. A third class of mutant binding defect is illustrated by the temperature-induced paralysis-E (tip-E) strain which shows a temperature-induced decrease in saxitoxin binding sites. This suggests a mutant-induced structural alteration that causes enhanced thermal lability. Preliminary studies on the α-bungarotoxin binding component suggests that this increased thermal lability may not be restricted to sodium channel components but may also be exhibited by other neuronal membrane components as well. This is different from the nap mutation for which the decrease appears to be restricted to sodium channels. Supported by NIH grants NS 16204 to L.M.H. and NS 18467 to G.R.S.

199.3 USE OF GENETIC HETEROZYGOSITY TO DEDUCE THE NATURE OF MUTATIONS AFFECTING THE VOLTAGE-SENSITIVE SODIUM CHANNEL IN DROSOPHILA. F.R. Jackson, S.D. Wilson*, L.M. Hall, and G.R. Strichartz. Genetics Dept., Albert Einstein Coll. Med., Bronx, N.Y. 10461 and Dept. Anesthesia Res. Labs., Harvard Med. Sch., Boston, MA 02115.

Mutations which affect the voltage-sensitive sodium channel may do so by a variety of mechanisms. For example, they might affect channel structure directly by alteration of a gene that encodes a sodium channel polypeptide structural component leading to a change in the primary amino acid sequence of that polypeptide. Alternatively, a structural change could be indirectly induced by mutation of a gene coding for an enzyme involved in the posttranslational modification of a sodium channel subunit. Using ligand binding studies alone it would be difficult to distinguish between these two types of alterations, since either could have effects on channel stability or the affinity of the channel for various ligands. The genetic approach of examining the phenotype of heterozygotes (+/m) and comparing them with homozygous wild-type (+/+) and homozygous mutants (m/m) provides one approach to distinguishing different types of structural alterations. For example, a mutation that directly affects a channel structural gene will be codominant because the heterozygotes (+/m) will contain a mixture of normal and mutant channel subunits. The phenotype of these individuals will be intermediate between the two homozygotes (+/+ and m/m). In contrast, a different situation would be predicted for a mutation affecting a channel processing enzyme. Most such enzymes are actually present in excess. Thus, a mutation affecting such an enzyme will be recessive in heterozygotes (+/m) since one normal copy of the gene encoding the enzyme will result in at least 50% of normal enzyme activity. This will generally be sufficient to produce the modification of all channel subunits, and both (+/+) and (+/m) would be wild-type in phenotype. Only mutant homozygotes (m/m) would show the mutant phenotype.

Using this genetic approach we have characterized three mutants (sei^{ts-2}, nap^{ts}, and tip-E) which were described in the previous abstract. Two of the mutants (nap^{ts} and tip-E) are recessive for both behavioral and saxitoxin-binding phenotypes. The third (sei^{ts-2}) is codominant at the behavioral level; heterozygotes ($+/sei^{ts-2}$) display paralytic behavior that is intermediate to that seen in wild-type (+/+) and homozygous mutants (sei^{ts-2}/sei^{ts-2}). We are presently examining the saxitoxin-binding in $+/sei^{ts-2}$ heterozygotes. The spectrum of saxitoxin-binding defects seen in extracts of sei^{ts-2} homozygotes (see preceding abstract) coupled with the codominant nature of the mutant effects on behavior makes the seizure strain a likely candidate for one having an alteration in a sodium channel structural gene.

199.4 SODIUM CHANNEL MODIFICATION BY BREVETOXIN B. Virginia Scruggs Luke* and Toshio Narahashi (SPON: Charles A. Berry). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Brevetoxin B (BrTX-B) is one of the toxic components isolated from the dinoflagellate Ptychodiscus brevis which causes "red tide". It consists of eleven ether rings connected in a series in the shape of a ladder with a molecular weight of 894. We have studied the effects of BrTX-B on the sodium channels of the squid giant axon.

Squid giant axons were internally perfused at 12°C with K-free standard internal solution containing 50 mM sodium and 275 mM cesium as replacement for potassium. The external artificial seawater (ASW) contained 100 mM sodium, 360 mM tetramethylammonium, 50 mM calcium and 560 mM chloride. The BrTX-B concentration was 10 μM.

Either internal or external application of BrTX-B depolarized the membrane. The depolarization was reversed by applying 1 mM sodium ASW or tetrodotoxin. The kinetics of the tetrodotoxin sensitive sodium current measured under voltage clamp conditions (holding potential -100 mV) showed that a) BrTX-B had little effect on the time to peak, b) slowed the rate of inactivation at potentials more negative than -30 mV but had little effect at more positive potentials, and c) increased the noninactivating steady-state component at all potentials. BrTX-B also had little effect on the sodium tail current following repolarization.

The current-voltage relationship in the presence of BrTX-B showed: a) The activation potential was shifted about 25 mV in the hyperpolarizing direction. b) The peak sodium current was only slightly increased at positive potentials, however, as the potential was made more negative, the increase in peak sodium current amplitude became greater. c) The steady-state current was increased at all potentials. d) There was no change in the reversal potential for either the peak or steady-state current.

In summary, BrTX-B increased both the peak and steady-state sodium current and shifted the sodium conductance voltage dependence in the hyperpolarizing direction. The results are consistent with an interpretation in which BrTX-B at a concentration of 10 μM selectively modifies about 20% of the sodium channels by shifting the voltage dependence of activation in the hyperpolarizing direction and preventing inactivation. BrTX-B samples were provided by Dr. Koji Nakanishi of Columbia University, and the present study was supported by NIH grant NS14144.

199.5 GATE AND PORE PROPERTIES OF SODIUM CHANNELS IN CRAYFISH AXON MEMBRANES TREATED WITH PRONASE AND N-BROMOACETAMIDE. Vincent L. Salgado*, Jay Z. Yeh* and Toshio Narahashi (SPON: Donald H. Harter). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The proteolytic enzyme, pronase, and the protein specific agent, N-bromoacetamide (NBA), both remove the fast sodium inactivation. In the present study, we have found several significant differences between the properties of pronase- and NBA-modified sodium channels.

Crayfish giant axons were internally perfused and voltage clamped in a double sucrose-gap chamber. NBA applied internally in K-free solution at 2-5 mM completely removed the sodium inactivation without increasing leakage current. The sodium inactivation in the membrane outside the "node" region, which had been depolarized by K-free internal solution, was not removed as completely as that in the nodal region, which had been held at -100 mV. This residual inactivation could be further removed by NBA provided that the newly established node was held at the hyperpolarized level. Thus, membrane depolarization, the resultant inactivation, or both protect the h gate from destruction caused by NBA. Pronase treatment at 0.4 mg/ml did not remove sodium inactivation completely. Within 10 min after application at 9°C, 90% of the inactivation was removed, but the remaining 10% was very resistant to further pronase treatment. Any attempts to remove the remaining inactivation such as increasing the concentration of pronase or the duration of treatment resulted in an increase in leakage conductance. After removal of inactivation with pronase, the rising phase of sodium current remained unchanged. With NBA, the rising phase was slowed considerably. With prolonged NBA treatment, the rising phase was slowed even more. This result suggests that NBA modifies more than one site in the channel.

The instantaneous current-voltage (I-V) relation was not linear. The current decreased with hyperpolarization beyond -60 mV, and the rectification was intensified by increasing external Ca concentration. This result is interpreted as Ca block of sodium channels. The I-V relation was not modified by pronase or NBA treatment. Impermeant ions applied internally revealed some differences in the pore properties of pronase- and NBA-treated squid axons (Yeh and Narahashi, Biophys. J. 17, 270a, 1977). Such differences were also evident in crayfish axons. When applied internally, 9-aminoacridine blocked the sodium channel. The apparent K_d values at 0 mV for 9-AA block were 11.3 ± 4.07 μM (mean ± S.D., n=4) and 19.7 ± 4.96 μM (n=6) in NBA-modified and pronase-modified channels, respectively. Supported by NIH grants NS14144 and GM 24866.

199.6 A NEUROTOXIC PEPTIDE FROM A MARINE SNAIL THAT PREFERENTIALLY BLOCKS SODIUM CHANNELS IN SKELETAL MUSCLES. Lynne M. Kerr and Doju Yoshikami. Dept. of Biology, University of Utah, Salt Lake City, Utah 84112.

The venom of the marine snail, Conus geographus, contains several neurotoxic peptides with different targets (c.f. Clark, Olivera & Cruz. Toxicon 19: 691, 1981). We describe here the effects of one of these peptides, designated as μCgTx, which was obtained in pure form from the laboratories of B. M. Olivera and W. R. Gray in our department.

μCgTx (~ < 1 μM) rapidly, totally, and reversibly blocks action potentials (APs) in muscles of frog and mouse (cutaneous pectoralis and diaphragm, respectively). However, endplate potentials (EPPs) in response to nerve stimulation can still be recorded from muscle fibers after toxin has taken effect. This indicates that μCgTx specifically blocks APs in muscles without blocking AP propagation in nerve. μCgTx appears to be the same as one of the toxins (LIII) characterized briefly by Spence et al. (Life Sci. 21: 1759, 1977).

To determine the site of action of μCgTx on muscle, we examined its ability to counteract the effects of the Na^+ channel activator veratridine. Both μCgTx and tetrodotoxin (TTX), a known Na^+ channel blocker, repolarized veratridine-treated frog fibers by about the same amount. This indicates that μCgTx, like TTX, exerts its action by blocking Na^+ channels. Unlike TTX, however, μCgTx acts preferentially on muscle. It appears unlikely that the ineffectiveness of μCgTx on nerve is due to access problems since scorpion toxin, which is a much larger molecule than μCgTx, does affect Na^+ channels in nerve. Furthermore, the motor nerve terminals in frog are relatively bare and APs therein (which are essential for neuromuscular transmission) are also very susceptible to block by TTX (Katz and Miledi J. Physiol. 199: 729, 1968).

In experiments on some, but not all, preparations from frog, the amplitudes of the EPPs were greatly attenuated by μCgTx. This attenuation is not due to postsynaptic effects since miniature EPPs are not affected by μCgTx. It remains to be shown how μCgTx exerts its effect in these instances.

In summary, μCgTx reversibly blocks Na^+ channels in muscles but apparently not in nerve. This suggests that there are differences in the structure or microenvironment of the Na^+ channels in nerve vs. those in muscle.

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199.7 EFFECTS OF SLOW INACTIVATION ON PROPERTIES OF SINGLE Na^+ CHANNELS MEASURED IN THE ABSENCE OF FAST INACTIVATION. Fred N. Quandt, Department of Medical Physiology, University of Calgary, Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Voltage-dependent Na^+ channels in nerve membrane appear to have a "slow" inactivation process which reduces the average current across the membrane over a time course 2 to 3 orders of magnitude slower than the kinetics of the activation, or opening, process. Little is known concerning the mechanism by which slow inactivation operates. Single-channel analysis was utilized to study effects of slow inactivation on the properties of single Na^+ channel currents recorded from patches of differentiated NIE-115 neuroblastoma cells. In order to directly study the slow inactivation process, it was useful to eliminate fast inactivation by the brief application of 2-3mM N-bromoacetamide (NBA) to the internal solution perfusing an inside-out patch of membrane. Effects of NBA were consistant with those obtained by Patlak and Horn (J. Gen. Physiol. 79: 334, 1982) in single Na^+ channel studies employing myotubes. Two effects of NBA were obtained: 1) the decline in the probability of the conducting state during a 50 msec depolarization was eliminated; 2) the average lifetime of the conducting state increased, typically by a factor of three (6°C, -20mV). These results confirm the elimination of fast inactivation by NBA for neuroblastoma cells. Following exposure to NBA, Na^+ channels continue to cycle between conducting and non-conducting states during depolarizations over 16 secs. in duration, to potentials ranging from -40 to -10mV, from a holding potential of -90mV. However, the probability of entry into the conducting state declined from initial conditions over a period of 8 - 15 secs. (10-12°C, -30mV) without any change in the amplitude of the conductance of this state. The rate of decline was faster for greater depolarizations. In the steady-state condition (>2 min.) at any potential, Na^+ channels conduct with only a small probability. In one typical patch which had been exposed to NBA, the total time spent in a conducting state was only 5% of the total observation time (approx. 16 secs.) at -40mV. This proportion decreased with further depolarization (0.5% at -30mV), and openings were not observed at potentials positive to -20mV. These observations are consistant with measurements of Na^+ currents from the whole cell since these potentials lie on the foot of the conductance-voltage curve, yet steady state inactivation (S_∞) is approaching 0. The slow inactivation process of Na^+ channels in neuroblastoma thus appears to reduce the probability of channel opening relatively independent of fast inactivation, without alterations in conductance of the open state.

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199.8 MODIFICATION OF SINGLE SODIUM CHANNELS BY SEA ANEMONE TOXIN ATX-II. S. F. Holloway* and C. H. Wu. Dept. of Pharmacology, Northwestern University, Chicago, IL 60611.

The kinetics of the Na^+ channel modified by the polypeptide toxin, ATX-II, from the sea anemone Anemonia sulcata were studied using the patch clamp technique with N1E-115 neuroblastoma cells. Experiments were performed using the inside-out patch configuration. ATX-II was applied inside the pipette at a concentration of 50-100 nM. The patch potential was stepped to -35 to -25 mV for 180 ms from a holding potential of -85 to -90 mV at 10°C.

ATX-II has been found previously to markedly slow sodium inactivation using the voltage clamp technique. Our results at the single channel level indicate that the most dramatic alteration caused by ATX-II appears to be a prolongation of burst duration of the channel. The normal Na^+ channel exhibited occasional bursting behavior, but very long bursts were consistently observed for the ATX-II modified channel. In our preliminary experiments, the distribution of burst times in ATX-II showed two exponential components with mean burst times of 3-5 ms and 17-26 ms, as compared to a control which showed only a single exponential time constant of 6-8 ms. The second component seen in ATX-II presumably represents those of the modified channel. The mean open time for the same experiments in ATX-II was 4-10 ms, as compared to a control value of 4-6 ms. The closed time during the burst usually was less than 1 ms. Single channel conductance did not appear to be altered by ATX-II.

When many single channel records were combined to give a pseudomacroscopic current, we observed that ATX-II caused a significant prolongation of the Na^+ current. The time constant of inactivation was lengthened to 80 ms, as compared to a control of 40 ms. When 3-second depolarizing pulses were applied, channel openings with long bursts were observed at the end as well as the beginning of the pulse. Interestingly, we also observed subconductance states in normal and, more frequently, in ATX-II treated membranes. It is very likely that the prolonged bursting caused by ATX-II is involved in the prolongation of Na^+ current. One possible explanation for this is that ATX-II modifies the channel so that it returns more frequently from a transient closed state back to the open state.

199.9 EFFECT OF SCORPION TOXIN ON THE MEMBRANE POTENTIAL FLUCTUATION OF NEUROBLASTOMA N1E 115 CELLS. K. Iwasa* and J. Baumgold. (SPON: M. R. Martin). Lab. of Neurobiol., NIMH, Bethesda, MD 20205

Membrane potential fluctuations of neuroblastoma N1E 115 cells cultured with and without 2% dimethyl sulfoxide (DMSO), were examined. 3 M KCl filled microelectrodes (40-60 MΩ) were used for recording the membrane potential and a spectral analyzer (Nicolet 440) was used to obtain power spectra. During electrophysiological recording, the cells were bathed in a medium containing 130 mM NaCl, 5.5 mM KCl, 1.8 mM $CaCl_2$, 1.0 mM $MgCl_2$, 25 mM glucose and 20 mM HEPES buffer (pH 7.3). Membrane potential fluctuations increased in two steps after application of 200 nM scopion toxin (ScTX), purified from Leiurus quinquestriatus venom. The first change took place after about 5 min. and consisted in an enhancement of fluctuation around the 10 Hz region. The second step was an enhancement of fluctuation around 1 Hz which appeared only after 20 min. The first step was less pronounced in cells cultured without DMSO. No significant difference in the second phase change was observed in the cells cultured under the two conditions. When 3μM tetrodotoxin (TTX) was applied to ScTX-treated cells, the fluctuation around 10 Hz was reduced to control levels. The fluctuation around 1 Hz was unaffected by TTX treatment. The resting potential was about -50 mV and -20 mV, respectively, for the cells cultured with and without DMSO. The resting potential was not significantly affected by ScTX or TTX treatment. A peaking of the specta was observed when the fluctuation amplitude around 1 Hz exceeded 1 mV. The peak frequency was 2 Hz in cells cultured in 2% DMSO, and 0.5 Hz in undifferentiated cells cultured without DMSO. The periodic activity of cells cultured in 2% DMSO was suppressed by 3μM TTX as well as by 10 mM $CoCl_2$. External application of 10 mM $CoCl_2$, $MnCl_2$, or $CaCl_2$ were equally effective in reducing the fluctuation around 10 Hz. Although the fluctuation around 1 Hz was not reduced by 10 mM $CaCl_2$, it was reduced by 10 mM $CoCl_2$ or 10 mM $MnCl_2$. When 2 mM $MgCl_2$ and 2 mM EGTA were added to the medium, an enhancement of the 10 Hz component took place together with a reduction of the resting potential. No peaking of the spectra was observed by this treatment. These observations are markedly different from the membrane potential fluctuation of the squid axon [Tasaki & Terakawa, in Cellular Pacemakers, D.O. Carpenter, ed., Wiley, 1982]. We tentatively interpret the fluctuation in the 1 Hz region to be related to a Ca-channel, which seems to be modified by ScTX application. The spectral change around 10 Hz is attributable to Na-channel and a change in RC-(membrane resistance-capacitance coupling) time constant, which is about 50 ms [Moolenaar & Spector, J. Physiol. (Lond.) 278(1978)265, ibid. 292 (1979)297].

199.10 EFFECT OF VERATRIDINE ON SINGLE SODIUM CHANNEL CURRENTS. Mitsunobu Yoshii*, Virginia Scruggs Luke*, and Toshio Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have studied the effect of the steroidal alkaloid, veratridine, on the single Na^+ channel currents in a membrane patch excised from the N1E-115 neuroblastoma cell. Veratridine has long been known to depolarize the membrane and cause repetitive firing in nerve and muscle fibers. We have analyzed the veratridine-modified slow component of sodium current in the squid giant axon (Scruggs and Narahashi, unpublished observation) and found that the conductance of the total modified channel population is about 50% of the control. This conductance decrease could be explained by the following hypotheses: 1) the single channel conductance of the modified open state is reduced to 50% of normal; 2) 50% of the modified channels has a normal conductance and 50% is nonconducting; or 3) combination of 1 and 2. The present experiment is designed to distinguish between these hypotheses.

The gigaohm-seal single channel recording technique was used to record currents from isolated membrane patches (inside-out configuration). Extracellular solution contained (in mM): NaCl(125), KCl(5.5), $CaCl_2$(1.8), $MgCl_2$(0.8), HEPES(20), glucose(25), and sucrose (20). The pH was adjusted to 7.3 by NaOH. Intracellular solution contained (in mM): CsF(150), HEPES(20), and Na-HEPES (1.0). The pH was adjusted to 7.3 by CsOH. Veratridine was added to the external solution contained in the suction pipette at a concentration of 100 μM. Veratridine was dissolved in dimethylsulfoxide (DMSO) and the final concentration of DMSO in test solution was 1%. Control experiments showed no effect of 2% DMSO on single channel currents. Temperature was maintained at 12-14°C.

Amplitude histograms of the single channel currents showed no significant difference with and without veratridine. Mean amplitudes were between 0.90 to 1.05 pA at the membrane potential of -50 to -40 mV. Probability distribution of the lifetime of the open state revealed a double exponential with time constants of approximately 5 and 20 msec at the membrane potential of -50 to -40 mV under the influence of veratridine in contrast to a single exponential with a time constant of approximately 5 msec in control. The ratio of events which comprise the slower decay to the total number of events was estimated to be 0.4 to 0.5. These results support the hypothesis that the veratridine modified sodium channel is in one of two states, i.e. a state with normal conductance and a prolonged mean open time, or a nonconducting state. Supported by NIH grant NS14144.

199.11 TETRODOTOXIN AND SAXITOXIN DISPLACEMENT OF BATRACHOTOXININ-A BENZOATE BOUND TO VOLTAGE-SENSITIVE SODIUM CHANNELS. George B. Brown and Jack A. Johnston*. The Neurosciences Program and the Dept. of Psychiatry, Univ. of Alabama in Birmingham, University Station, Birmingham, AL, 35294.

In one formalism, the voltage-sensitive sodium channel has been characterized as having at least three distinct classes of receptor sites for naturally-occuring neurotoxins. Type I receptors bind saxitoxin (STX) and tetrodotoxin (TTX) which block sodium flux and prevent depolarization. Type II receptors exist for the sodium channel activators batrachotoxin (BTX), veratridine, aconitine and grayanotoxin. The third receptor, type III, binds the polypeptide neurotoxin from sea anemone and certain toxins from scorpion venom which act by inhibition of sodium channel inactivation (see Catterall, Ann. Rev. Pharmacol. Toxicol. 20:15, 1980, for a review). The binding of toxin at type II and III receptors has been shown to be positively cooperative and an allosteric model describing this interaction has been presented (Catterall, J. Biol. Chem. 252:8669, 1977). Type I and type II receptors, however, have been considered to be distinct and non-interacting (Colquhoun et al., J. Physiol. Lond. 227:95, 1972; Catterall, J. Biol. Chem. 250:4053, 1975). We now present evidence for an interaction between type I and type II receptor sites based upon the ability of TTX and STX to displace the tritiated BTX analog, ^{3}H-batrachotoxinin-A benzoate (^{3}H-BTX-B), from its sodium channel site.

Equilibrium binding of ^{3}H-BTX-B to sodium channels in rat brain synaptosomes and microsacs in the presence of scorpion toxin was measured as described previously (Catterall et al., J. Biol. Chem. 256:8922, 1981; Creveling et al., Molec. Pharmacol., in press). At 25°C both TTX and STX inhibited the specific binding of ^{3}H-BTX-B in a concentration-dependent and non-competitive manner. This inhibition was markedly temperature dependent, being negligible at 37°C and maximum at 18°C, the lowest temperature investigated. ^{3}H-BTX-B binding in the absence of TTX and STX was, in contrast, temperature insensitive in the range 37°C-25°C. Scatchard analysis of BTX-B binding isotherms at 25°C in the presence and absence of TTX revealed that the inhibition was due to an approx. 3-fold decrease in the affinity of BTX-B binding with no change in the number of receptor sites (Bmax). Comparison of TTX and STX inhibition of specific ^{3}H-STX and ^{3}H-BTX-B binding produced superposabale curves, thereby relating inhibition of BTX-B binding to a direct effect of STX or TTX binding at type I receptor sites. Another series of experiments demonstrated that TTX and STX did not alter the binding of scorpion toxin to type III receptors. In agreement with other observations, BTX-B did not have an effect on the binding of ^{3}H-STX. We interpret these results to suggest that occupancy of type I receptors by STX or TTX can lead to a conformational perturbation of type II receptors.

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199.12 COMPUTER SIMULATION OF PROPAGATING ACTION POTENTIALS IN MAMMALIAN MYELINATED NERVE FIBERS. R.M. Siegel* and M. Rasminsky. Dept. of Physiology, McGill Univ., and Neurosciences Unit, Montreal General Hospital, Montreal, Quebec, Canada.

Propagation of action potentials in mammalian myelinated nerve fibers was simulated using published data. The dynamics of the nodal sodium and leak currents were given by voltage clamp data from rabbit sciatic nerve myelinated fibers obtained at 14°C (Chiu et al., J. Physiol. 292:149, 1979). Nodal capacity was 2 pF. The Crank-Nicolson implicit integration method (for which code was kindly provided by Drs. M. Hines and J.W. Moore) was used to solve the system of differential equations describing the cable properties of the fiber. Axon diameter was 10 μm, internodal length 1000 μm and axoplasmic resistivity 100 Ω-cm. The conductance and capacitance assumed for each myelin lamella was 2 mSieman/cm^2 and 1 μF/cm^2 respectively (Brismar, Acta Physiol. Scand. 113:161, 1981). The model fiber was 30 internodes in length and conduction velocity was measured between nodes 15 and 16.

At 14°C the nerve impulse propagated at a velocity of 8.4 m/sec and 5.9 m/sec for fibers with 200 and 100 myelin lamellae respectively. Measured maximal conduction velocity of rabbit tibial nerve myelinated fibers at 37°C is 59 m/sec (Thomas et al., J.N.N.P. 44:233, 1981) which corresponds to a conduction velocity of about 12 m/sec at 14°C (Paintal, J. Physiol. 180:20, 1965). We examined the effect on conduction velocity of changes in maximal sodium conductance and leak conductance. Doubling maximal sodium conductance increased conduction velocity to 11.2 and 9.2 m/sec for the 200 and 100 lamellae fibers respectively. Increasing maximal sodium conductance beyond this point led first to minor distortions in the repolarizing phase of the action potential and ultimately to oscillations or failure to return to resting potential. Both of these effects could be eliminated by increasing leak conductance. For example no distortion was seen if a 10-fold increase in maximal sodium conductance was compensated with a 4-fold increase in leak conductance; under these conditions the two model fibers conducted at 12.6 and 12.5 m/sec respectively.

To simulate conduction at 37°C we assumed a Q_{10} of 1.8 (Hodgkin & Huxley, J. Physiol. 117:500, 1952) for sodium activation and inactivation rate constants. Computed conduction velocities for fibers with doubled maximal sodium conductance were 20 and 12.5 m/sec for fibers with 200 and 100 myelin lamellae respectively.

We conclude that a reasonable simulation of conduction at 14°C can be obtained with minimal manipulation of voltage clamp data but that this data cannot safely be extrapolated to 37°C.

199.13 CONDUCTION IMPAIRMENT INDUCED BY LOCALIZED COMPRESSION OF IN VITRO RAT TAIL NERVE DETECTED BY TRAINS OF INCREASING FREQUENCY. D.L. Jewett, C. Walden*, T.C. Chimento*, and J. Morris*. Dept. of Orthopaedic Surgery, Special Studies Unit, Univ. Cal. San Francisco, San Francisco, CA. 94143.

Acute and chronic nerve compression can be detected from disruption of uniform trains of impulses, well before conduction block occurs. The clinical utility of such a method is limited by the need to record multiple runs to determine the highest conduction frequency. A STIF (stimulus train of increasing frequency)can in a single sweep indicate the highest inter-stimulus frequency which can traverse the compressed region, but analysis of the wave form can be in error when there is summation of potentials at short inter-stimulus intervals. This problem might be expected to interfere with clinical use of the STIF method since surface recordings will invole tri-phasic waveforns, some with long lasting slow potentials from the spinal cord. A computerized stimulus and data collection system has been developed which permits reconstruction of the individual waveforms despite other interfering waves. The mehtod improves the accuracy so that the compression can be detected when less than 10% of the fibers are affected, and makes the method more appropriate for clinical use.

Ventral tail nerves of the rat were studied in vitro before and during compression between segments of plastic tubing, controlled within 0.05 mm. A Nova 3 computer generated STIF patterns of supra-maximal stimuli, automatically averaged 10 repetitions, saved the results on disc, and then repeated the sequence over and over, using one less stimulus in the STIF pattern than the previous time. During data analysis the differences between successive data files yielded the isolated waveform of the last waveform of the longer STIF, and these isolated waveforms were then plotted to show the reconstructed waveform.

Variability in the heights of the STIF responses are due to summation with later potentials. Use of the reconstruction algorithm allows accurate evaluation of the effects of compression as compared to before compression. As compression is prolonged or increased, there is an increase in the interstimulus interval first showing a decrement of 10% in the height of the wave. This method thus allows detection of the fibers most affected, at minimal impairment, when the nerve is still conducting. If the compression is sufficiently great to block some of the fibers(first wave amplitude decreased compared with the pre-compression recording) then the frequency block is still apparent, so that this method also applies to those cases where only part of the nerve is functioning.

The accuracy of the reconstructed waveform allows for the use of a template reconstruction from the first wave in clinical use, which would not require multiple STIF averages,as does the technique described here.

199.14 SELECTIVE CONDUCTION BLOCKADE AMONG DIFFERENT FIBER TYPES IN MAMMALIAN NERVES BY LIDOCAINE COMBINED WITH LOW TEMPERATURE. G. Strichartz and M. Zimmermann. II. Physiologisches Institut, Universität Heidelberg, D-6900 Heidelberg, GFR

The susceptibility of nerve impulses conducted by myelinated (Aß-, Aδ-) and non-myelinated (C-) fibers to the local anesthetic lidocaine was investigated in the cat sural and tibial nerves, in vivo. Animals were anesthetized with sodium pentobarbital. A nerve was stimulated and the compound action potentials (APs) were recorded by pairs of extracellular bipolar electrodes. An intervening segment of nerve 8 mm in length was desheathed and enclosed in a plexiglass chamber. The chamber was irrigated with solutions containing different concentrations of lidocaine. The temperature of the perfusing solution was varied from 33 to 15°C. Electrodes, nerve, and perfusion chamber were immersed in a bath of paraffin oil contained within skin flaps of the leg.

With lidocaine concentrations increasing from 0.1 to 1.0 mM, at 33°C, the Aδ-fibers are blocked in the steady-state at the lowest anesthetic concentration, followed by the C-fibers and finally by the Aß-fibers. The extent of conduction block depended on the frequency at which the nerve was stimulated. The amplitudes of the Aß- and Aδ-components of the APs were reduced at the end of a train of 10 stimuli at freqencies above 20 Hz during partial lidocaine blockade, suggesting a use dependent block. In contrast, repetitive stimulation of C-fibers at 5-10 Hz resulted in an increase in the AP amplitude which might be due to either a better synchrony of impulse conduction or to increased impulse currents in individual fibers, whereas it decreased at higher frequencies. The selectivity of the differential block by lidocaine was enhanced when the perfused nerve section was cooled to 25 or 14°C. Although cooling per se reduced the amplitude of the APs and impaired the frequency-following properties of all fiber types, cooling plus anesthetic were markedly synergistic, particularly in the Aδ- and C-fibers when trains of repetitive stimulations were applied at higher frequencies. The increase in amplitude seen in the AP of C-fibers in the presence of lidocaine during repetitive stimulation at 5-10 Hz did not occur in locally cooled nerves. The AP of C-fibers recovered more slowly than the APs of Aß- and Aδ-fibers both when the anesthetized nerve was warmed, and when the anesthetic was subsequently washed out.

These results are of clinical relevance, as a differential block of Aδ- and C-fibers could eliminate pain while maintaining tactile sensation and motor functions.

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199.15 CELL-SPECIFIC ACTIONS OF PROCAINE ON NOCICEPTIVE NEURONS IN THE LEECH. J.Johansen*, J.Yang and A.L.Kleinhaus.Dept. Neurology, Yale U. Sch. of Med., New Haven CT 06510.

Last year we showed that the local anesthetic procaine, exerted opposite actions on the membrane potential of identified neurons in the leech i.e. the Retzius cell and the nociceptive (N cell) situated laterally in the ganglion.

We now report that procaine can further distinguish among subsets of neurons sharing a sensory modality. The effects of procaine on the electrophysiological properties of the leech neurons responding to nociceptive stimuli (N cells) were examined on isolated segmental ganglia of Macrobdella. In the N cell situated laterally in ganglia 7-19, procaine (1-10 mM) produced a dose-dependent depolarization, while in the N cell situated medially it caused a dose-dependent hyperpolarization. In addition, the drug greatly prolonged the action potential of the lateral but not the medial N cell. The procaine-induced depolarization was abolished in Na-free Ringer while the hyperpolarization was unaffected by changes in Cl^-, but was enhanced in low K^+.

In the specialized ganglia 5,6,20 & 21 only two rather than four N-like cells were found. These cells are nociceptive neurons by virtue of their response to noxious stimulation of the skin, the shape of their action potential, and their morphology as revealed by HRP injections. The two N cells in ganglia 5 & 6 responded to procaine like the medial N cell in ganglia 7-19. In addition, they were sensitive to stimulation of the gut wall, which is a unique feature of the medial N cell (Blackshaw et al. J.Physiol.(326),1982). In contrast, the cells in ganglia 20 & 21 responded to procaine as did the lateral N cells.

In the leech nervous system, cells responding to nociceptive stimuli are subdivided into two subsets: a) cells of the lateral type having peripheral receptive fields only and b) cells of the medial type which innervate an additional visceral field. These results agree with the findings of other studies (McKay et al.Neurosci.Abst.8, 1982) using monoclonal antibody techniques. The two subsets also contain conductance mechanisms which respond differently to procaine. The opposite actions exerted by procaine on two subsets of mechanosensory neurons in the leech nervous system could be the basis of this drug's dual (convulsant and anticonvulsant) effects on mammalian nervous systems.

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199.16 BARBITURATES EXHIBIT SIMILAR ACTIONS ON MEMBRANE PROPERTIES BUT OPPOSITE EFFECTS ON FIRING ACTIVITY OF CRAYFISH STRETCH RECEPTOR. K.-S. Tan* and S. H. Roth (SPON: K. E. Cooper), Dept. of Pharmacology & Therapeutics, Faculty of Medicine, University of Calgary, Calgary, Alberta T2N 4N1 CANADA.

We have observed that pentobarbital and phenobarbital produce opposite effects on the extracellular recorded firing activity of the isolated sensory neuron (crayfish stretch receptor). Pentobarbital produced a depression and phenobarbital an excitation of firing frequency. Combination of the two barbiturates at effective concentrations produced an additive effect which could result in firing activity that was similar to control. These results suggest that the sites of action of these two barbiturates are different. Intracellular techniques were utilized to determine whether these two barbiturates produced opposite actions at the membrane level. In general, the effects of the barbiturates on excitable neuronal membrane properties were similar, however, on a molar basis pentobarbital was more effective than phenobarbital. Both barbiturates depolarized the membrane resting potential and decreased the threshold, amplitude and first derivative of the action potential. In addition, the duration of the action potential and membrane resistance were increased. The combination of pentobarbital and phenobarbital resulted in additive effects on membrane properties. We propose pentobarbital to have its major effect on sodium channels, with only a minor effect on potassium channels. This would result in the depression of sodium conductance, a depolarizing shift of the sodium inactivation curve, and a lengthening of the channel inactivation time. In contrast, phenobarbital may activate potassium channels more effectively than sodium channels, resulting in a depression of both conductances, a hyperpolarizing shift of the sodium inactivation curve, and a lengthening of the inactivation time of both channels. Therefore, a differential effect on these ion channels could account for the opposite effects on firing activity.

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199.17 A cAMP ANALOG STIMULATES SINGLE INWARD CHANNEL OPENING FREQUENCY IN THE VENTRAL WHITE CELLS OF PLEUROBRANCHAEA. Daniel J. Green and Rhanor Gillette, Neural and Behavioral Biology Program, Dept. of Physiology and Biophysics, Univ. of Illinois, Urbana, Illinois, 61801.

The ventral white cells (VWCs) of the marine mollusc Pleurobranchaea display minutes-long bursts of progressively broadening action potentials. (Gillette, R., 1982, et al., J. Comp. Physiol. 146: 461-470). These bursts are stimulated by appetitive stimuli applied to the chemosensory apparatus, and drive the rhythmic motor output of feeding. (Gillette, M. et al., J. Neurosci. in press). The driving force for VWC bursting is a slow inward current that can be stimulated in the isolated VWC by cAMP agonists and analogs. (Gillette, R., 1981, Neurosci. Abstr. 7: 113). We examined the effects of the membrane-soluble cAMP analog 8-parachlorophenylthio cAMP (CPT-cAMP) on single channels in the VWCs, using the patch clamp technique. The buccal ganglion was removed and bathed in 0.25% Trypsin for 90 minutes, to provide a clean cell surface for patch clamping. The VWC somata were voltage-clamped with a Dagan single electrode apparatus, and successive patches were made until one or more ion channels were identified. CPT-cAMP (10^{-4} M) was then perfused through the bath. A population of inward current channels was found that is activated concurrent with slow inward current. Channel conductance was typically 20 pS and had a reversal potential between +40mV and +50mV. Bath addition of CPT-cAMP increased channel opening frequency, but did not significantly affect mean open time or conductance. In a typical experiment, the opening frequency increased from a baseline of 1.25±0.19 openings/sec in normal saline, to 2.98±0.32 in CPT-cAMP. Mean open time was 7.21±0.87 msec in normal saline and 8.00±0.68 msec in CPTcAMP. CPT-cAMP had no observable affect on outward channels.

Focal depolarization of the patched membrane did not increase channel frequency, indicating that the channel is not directly voltage dependent. However, depolarization of the whole cell did increase channel opening frequency. This suggests that another factor, such as calcium, may accumulate during depolarization to further increase channel opening frequency. This hypothetical factor would impart the observed voltage dependence of the inward current. Supported by grants PHS 5T32 GM07143 to D.J.G. and NSF BNS 79-18329 to R.G.

199.18 A VOLTAGE SWITCHABLE ION CHANNEL: COLICIN E1, A MODEL FOR NEURONAL CHANNELS? C. Levinthal, M. Cleveland*,R. Fine*, F. Levinthal*, Q.R. Liu*, Department of Biological Sciences, Columbia University, New York, N. Y. 10027

The bacterial toxin colicin E1 kills sensitive cells by forming an ion channel in the plasma membrane. This causes the release of the membrane potential thereby stopping oxidative phosphorylation. The activity of this channel can be demonstrated in a Mondal black lipid membrane in which it can be switched on or off by changing the voltage across the membrane (Schein, et al, 1978. Nature, 376: 159-163). We have initiated studies of this protein as a possible model system for neuronal ion channels.

The sequence of the 522 amino acids in colicin E1 is known from the DNA sequence of the gene which encodes it (Yamada et al. PNAS, 79: 2827-2831). We have isolated a C-terminal fragment, after cyanogen bromide cleavage, which contains 152 amino acids and which has the same channel forming and voltage switching properties as the intact molecule (Cleveland et al. PNAS, June 1983). The C-terminal fragment contains a hydrophobic region of 35 amino acid residues and 41 of the remaining 117 residues are charged at pH7 (22 positive and 19 negative). When the peptide is allowed to contact a planar phospholipid membrane or an oil droplet or a hexane droplet, a strong and irreversible attachment takes place by means of a hairpin structure formed of two alpha helices composed of the hydrophobic region plus additional residues which provide salt-bridges to hold the ends of the hairpin together. When only the hairpin is inserted in the membrane, the channel is assumed to be in the CLOSED state. The time for the voltage induced transition from OPEN to CLOSED or the reverse is of the order of 10's and 100's of seconds. We take this to imply that a major conformational change is involved in the transition.

We have generated a molecular model of the structure of the channel OPEN: we assume that six alpha helices span the membrane to form a barrel-like structure with a lumen in the center. All the charged residues can be accounted for as those which have their charged groups: 1) 14 charges are in the lumen; 2) 9 reach to the outside of the lipid region of the membrane; 3) inter-helical salt-bridges and 4) 14 are facing into the lipid and can form intra-helical salt-bridges since they are of opposite sign and 3 or 4 residues apart.

We are altering the amino acid sequence of this peptide by in vitro, site-specific mutagenesis with the aim of testing this and other molecular models and changing the residues pointing into the lumen in order to alter the ion selectivity and switching properties of the channel.

200.1 VOLTAGE-CLAMP ANALYSIS OF MEMBRANE CURRENTS IN HIPPOCAMPAL CA3 PYRAMIDAL NEURONS. Kerry L. Zbicz* and Forrest F. Weight (SPON: G. Cordingley). Lab. of Preclinical Studies, National Institute on Alcohol Abuse and Alcoholism, Rockville, MD 20952.

Membrane currents were studied in hippocampal neurons in vitro. Thin slices (450 μM) of guinea pig hippocampus were held submersed in flowing, oxygenated artificial CSF and the neurons in the CA3 pyramidal layer were impaled with microelectrodes (15-30 megohms) filled with 3 M KCl. Using the single microelectrode voltage-clamp technique, membrane currents were recorded in response to step changes in membrane voltage. Depolarizing steps from holding potentials negative to -70 mV activated several outward currents, of which at least three could be identified by their kinetics, voltage activation and inactivation characteristics, and their sensitivity to various substances. A fast, transient outward current developed during depolarizing steps to potentials positive to -60 mV. This current was increasingly inactivated as membrane holding potential was changed from -70 mV to more positive voltages. The transient outward current was reduced or abolished by 0.5-2.0 mM 4-aminopyridine (4-AP). A second current, observed during steps positive to -50 mV, activated gradually over a period of 200-500 msec and showed no apparent inactivation with maintained depolarization. This persistant current was reduced or blocked by 20 uM muscarine, 0.5-2 mM Ba^{2+}, and 2-10 mM tetraethylammonium (TEA). A third outward current was activated at potentials positive to -40 mV. This current increased gradually and did not reach a maximum value during depolarizing steps 1 sec in duration. This slow current was reduced by the application of the Ca^{2+}-channel blockers Mn^{2+} (2-5 mM) and Co^{2+} (2mM), indicating that it is a Ca^{2+}-sensitive outward current. In some experiments cesium (Cs^{+}) was applied intracellularly by impaling neurons with microelectrodes filled with 3 M CsCl. In Cs^{+} filled cells, the transient outward current was not observed. The outward current which remained in Cs^{+} filled neurons continued to increase during depolarizing steps 1 sec in duration and was only observed at potentials positive to -40 mV. This outward current was reduced by the application of 2-5 mM Mn^{2+} or 2 mM Co^{2+}. The outward currents observed in hippocampal CA3 neurons resemble outward currents previously described in other neurons, with the fast transient current resembling the A-current first described in molluscan neurons, the persistant outward current resembling the M-current described in frog sympathetic neurons, and the Ca^{2+}-sensitive outward current being similar to Ca^{2+}-activated K^{+} currents described in both vertebrate and invertebrate neurons.

200.2 DELAYED EXCITATION IN NEURONS OF THE NUCLEUS TRACTUS SOLITARIUS STUDIED IN VITRO. M.S. Dekin and P.A. Getting. Dept. of Physiol. and Biophys., Univ. of Iowa, Iowa City, IA 52242.

The ventro-lateral region of the nucleus tractus solitarius (NTS) is thought to be involved in the generation of the respiratory rhythm in mammals. Using extracellular recording techniques in vivo, several classes of respiratory neurons have been characterized in the NTS. The membrane properties of these neurons, however, have not been studied. Using adult guinea pigs, we have developed an in vitro brainstem slice preparation which allows stable intracellular recordings from NTS neurons. Based on electrophysiological criteria we have found four classes of neurons. The most common class exhibits a long delay in the onset of firing when given depolarizing stimuli. This property of delayed excitation was investigated.

NTS neurons with delayed excitation had an average resting potential of -70 mV and an input resistance ranging from 20 to 30 megohms. The membrane time constant, measured by the injection of short hyperpolarizing current pulses, was less than 8 msec. When a depolarizing stimulus was given from rest, the onset of the first spike was delayed 150 to 200 msec from the stimulus onset. The membrane time constant was too short to account for this long delay. The magnitude of the delay was voltage dependent. When a stimulus was given from a membrane potential of -40 mV the delay decreased to 5 msec, while from -90 mV the delay reached a maximum of 400 msec. The magnitude of the delay was also dependent upon the duration of hyperpolarizing prepulses with maximum delays occurring for prepulses of 100 msec or longer. These effects of time and voltage suggest that the delayed excitation was mediated by a transient, outward K^{+} current similar to "A-current" in molluscan neurons (Connor and Stevens, J. Physiol. 213;21,1971).

At -40 mV these neurons responded to depolarization with an initial high firing rate which could reach 100 Hz. Following this initial firing, spike frequency declined to a steady-state level below 30 Hz. When the same stimulus was given from more hyperpolarized potentials, the initial high firing rate was diminished but the steady-state firing level remained nearly constant. The voltage dependence of the reduction in the initial firing rate parallelled that of the delayed excitation suggesting that both these phenomena were mediated by a similar mechanism. As a consequence of delayed excitation these neurons will not always respond in the same manner to a given depolarization or synaptic input. They will, however, display multiple discharge patterns depending upon the membrane potential preceeding the input. (NS15350 and MH15172).

200.3 SYNCHRONOUS BURSTS WITHOUT CHEMICAL TRANSMISSION IN CA2-CA3 AND DENTATE AREAS OF THE HIPPOCAMPUS. R. W. Snow and F. E. Dudek. Dept. of Physiol., Tulane Univ. Sch. of Med., New Orleans, LA 70112.

Synchronous bursts have been reported in the CA1 region of hippocampal slices in which chemical synapses were blocked, but other regions of the hippocampus appeared to be resistant to this activity (Taylor and Dudek, Science 218:810, 1982; Jefferys and Haas, Nature 300:448, 1982). We now report that when divalent cations were lowered further than in previous studies, synchronous activity could occur in any area of the hippocampus. Hippocampi were removed from rats (95-200g), and slices were cut parallel to the alvear fibers. Slices were incubated in flowing saline containing 124 mM NaCl, 26 mM $NaHCO_3$, 5 mM KCl, 1.24 mM KH_2PO_4, 1.3 mM $MgSO_4$, 2.4 mM $CaCl_2$, and 11 mM glucose. After 1 hr, the perfusion medium was replaced with one containing 124 mM NaCl, 26 mM $NaHCO_3$, 5-6.2 mM KCl, 0-1.2 mM $MgCl_2$, 2 mM $MnCl_2$, 0.1-0.5 mM $CaCl_2$, and 11 mM glucose. Synaptic potentials evoked by orthodromic stimulation were blocked after 15-30 min in these solutions. After 20-60 min the tissue became hyperexcitable, and antidromic stimulation could evoke multiple population spikes in any area of the hippocampus. Simultaneous intracellular and adjacent extracellular recordings revealed spikes in pyramidal and granule cells correlated with the extracellular field potentials. Subtraction of the extracellularly recorded potential from the intracellular potential revealed net depolarizations of the neurons during the population spikes. In relatively inexcitable neurons, a subthreshold current promoted firing during the field potentials. Although not each region in each slice generated synchronous bursts, spontaneous synchronous bursts were seen in each area of the hippocampus in some slices. A slow extracellular negativity preceded the spontaneous bursts, suggesting that an increase in extracellular K^{+} occurred. Extensive random firing of neurons marked the beginning of spontaneous bursts and led to population spikes, indicating a recruitment of synchrony. We conclude that each area of the hippocampus can generate synchronous bursts in the absence of chemical transmission, and that electrical field effects contribute to the synchronization. Supported by USPHS Grant NS 16683.

200.4 INHIBITION OF POSTSYNAPTIC FIRING IN THE HIPPOCAMPUS DURING REPETITIVE STIMULATION BLOCKS LONG-TERM POTENTIATION. H. E. Scharfman* and J. M. Sarvey (SPON: A. Williamson). Dept. Pharm., Uniformed Services Univ. of Health Sciences, Bethesda, MD 20814

Long-term potentiation (LTP) in the hippocampus is a long lasting enhancement of synaptic efficacy following repetitive stimulation of a synaptic input. LTP has been proposed to be a model of synaptic plasticity in the central nervous system. Hebb (1949) has suggested that synaptic efficacy increases when one neuron repeatedly causes an action potential to fire in another. Since LTP is a result of enhanced synaptic efficacy, Hebb's postulate could be interpreted to mean that postsynaptic firing is essential during the repetitive stimulation required to produce LTP. Yet it remains to be proven that presynaptic and postsynaptic activity must be coupled during repetitive stimulation to produce LTP. One approach to this problem is to observe whether LTP occurs if the postsynaptic neuron is inhibited during stimulation which otherwise produces LTP.

Extracellular recordings from subfield CA1 were taken from 375um hippocampal slices of the rat. Electrodes were placed in the cell body layer and apical dendritic layer to record the field potential and EPSP, respectively, in response to Schaffer collateral stimulation. Repetitive stimulation of 100Hz for 2 seconds produces post-tetanic potentiation (PTP), which lasts up to 5 minutes, and ranges from 140-200% of control (n=19). LTP is also produced, ranging from 130-210% (n=11). In other slices postsynaptic activity was inhibited by local pressure ejection at the soma of GABA (10mM), pentobarbital (100mM-1M), or tetrodotoxin (1uM). Each agent was able to block the population spike reversibly without affecting the EPSP. When the population spike was blocked during repetitive stimulation, LTP did not occur (n=18). However PTP was not suppressed (n=17). Since tetrodotoxin inhibits postsynaptic activity by an entirely different mechanism from GABA or pentobarbital, the mechanism of inhibition does not appear to be important.

These experiments suggest that postsynaptic firing during repetitive stimulation is necessary for the induction of LTP. Furthermore, it appears that short lasting changes are dependent on different considerations than longer lasting phenomena.

200.5 BLOCKADE OF LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL CA1 REGION BY THE PROTEIN SYNTHESIS INHIBITORS EMETINE AND CYCLOHEXIMIDE. P. K. Stanton* and J. M. Sarvey. Dept. of Pharmacology, Uniformed Services University of the Health Sciences , Bethesda, MD 20814.

Hippocampal long-term potentiation (LTP) is characterized by a long lasting enhancement in the monosynaptically evoked response following high frequency repetitive (tetanic) stimulation. Because LTP is induced by repetitive neuronal activity and has a long duration, it is thought to reflect a form of neuronal plasticity important in memory storage. Researchers have demonstrated increased synthesis of specific protein fractions following tetanic stimulation in the hippocampus. However, it is not known whether protein synthesis is required for, or merely a by-product of, induction of LTP. Therefore,we examined the effects of protein synthesis inhibitors on the induction of LTP. Extracellular monosynaptic potentials evoked in CA1 by stimulation of the Schaffer collateral axons were recorded from transverse slices 375μm thick) of freshly dissected rat hippocampus. A protein synthesis inhibitor, emetine or cycloheximide, was applied by bath perfusion to the slices for more than 30 min prior to tetanic stimulation of the Schaffer collaterals (20/s x 10s). The evoked response amplitude was followed for 30 min after tetanic stimulation, and percent change from baseline amplitude of the population action potential (AP) was used as the measure of enhancement of synaptic efficacy. An increase of greater than 35% at 30 min after tetanic stimulation was used to define LTP, since this was two standard deviations from the mean control baseline amplitudes. In the control slices, tetanic stimulation produced an increase >35% (range 40-1500%) in AP amplitude in 10 out of 20 slices examined. Emetine at 1.5μM prevented the induction of LTP, with 1 of 16 slices showing LTP (χ^2,$p<.05$ compared to controls) while at 15μM, 1 of 12 slices exhibited LTP ($p<.05$). When applied for periods up to two hours, neither concentration of emetine by itself produced a significant change in AP amplitude or any other measure of neuronal excitability. Intracellular recordings indicated that the drug produces no changes in resting membrane potential, input resistance, action potential amplitude or duration (N=5). A second protein synthesis inhibitor, cycloheximide, was applied at 0.35 and 3.5μM. At the lower concentration, LTP was unaffected, with 4 of 6 slices exhibiting LTP. The higher concentration, however, prevented the induction of LTP, with only 2 of 15 slices exhibiting LTP. Neither concentration of cycloheximide by itself produced a significant alteration in neuronal excitability. The data show that increasing concentration of known protein synthesis inhibitors correlates with decreased probability of LTP following repetitive stimulation. These results suggest the necessity for newly synthesized proteins in the induction of LTP.

200.6 The EFFECTS of ORIENTATION of DC or AC EXTRACELLULAR FIELDS on EXCITABILITY in the HIPPOCAMPAL SLICE. A.R. Sheppard, S.M. Bawin, M.D. Mahoney* and W.R. Adey. VA Medical Center and Loma Linda University, Loma Linda, CA 92357.

In previous work we showed short and long-term modulation of the excitability of CA1 neurons by 60 Hz sinusoidal electric fields (20-60 mV/cm, p-p) applied in the solution perfusing the slice and oriented perpendicular to the CA1 cell layer. It was thought that effects might be caused by either the influence of small rapid changes in potential along a dendro-somatic axis or by a more constant potential difference developed by rectification of the sinusoidal current that enters the dendritic branches.

To test for dependence on axial polarizations that would arise from such changes in dendritic potentials, we applied 60 Hz electric fields (10-140 mV/cm, p-p, 10-30s) oriented perpendicular or parallel to the CA1 layer and measured the population spike evoked by test pulses in the Schaffer pathway (SC). As before, we saw long-term potentiation and short-term depression of the evoked potential, but these changes were clearly independent of current orientation.

To determine if CA1 neurons respond to polarizing currents in a direction-sensitive manner in other circumstances, we applied the test pulse during dc pulses (100-170 mV/cm in the bath, 200-400 ms duration). Currents that made the apical dendrites positive with respect to the soma increased potentials evoked in the CA1 cell layer by test pulses in SC, while the opposite field polarity caused decreased responses. Similar results previously seen in the granule cells of the dentate using tissue field gradients of 50-700 mV/cm (Jefferys, 1980) were confirmed in our study. Currents orthogonal to the cell axis had no effect on responses in either CA1 or dentate regions.

These data show that polarization along the cell axis is important for tests during long dc pulses, but the effects produced during and after rapidly varying ac fields are axis-independent and presumably not due to a polarizing effect on CA1 neurons.
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200.7 NATURE OF 7-14 HZ RHYTHMIC HYPERPOLARIZATIONS IN THALAMIC RELAY NEURONS. M. Deschênes*, J.-P. Roy* and M. Steriade (SPON: Y. Lebeux). Lab. Neurophysiol., Dept. Physiol., Laval Univ., School of Med., Québec, Canada, G1K 7P4.

The most striking behavior of thalamic neurons is their membrane potential oscillations during spontaneous spindle waves. Similar oscillations can be triggered by peripheral or cortical stimulation in barbiturized preparations or during slow-wave sleep in behaving animals. It is believed that spontaneous and stimulus-induced oscillations result from the operation of similar mechanisms. Both phenomena are indeed characterized by similar temporal patterns of rhythmic hyperpolarizations interrupted by burst discharges. In order to elucidate synaptic and intrinsic membrane properties underlying these oscillations, intracellular recordings were performed in lateral thalamic nuclei of cats under barbiturate anesthesia. We found that long-lasting rhythmic hyperpolarizations were made of three different components: 1) The early component which was reversed by current and Cl injections and which was also sensitive to picrotoxin was identified as a Cl-dependent IPSP. 2) A depolarizing hump was usually present in the depth of the long-lasting hyperpolarization. This intermediate component was identified as a voltage-dependent dendritic Ca conductance on the basis of recordings and EGTA injections performed in relay cell dendrites. 3) The late phase of hyperpolarization was dissociated from the early IPSP by its differential sensitivity to current and Cl injections and to conditioning tetanic stimulation. This late component was abolished by EGTA and, thus, was interpreted as a Ca-dependent K conductance increase triggered by a dendritic Ca influx.

Activation of intrinsic somatic or dendritic conductances by current pulses never generated rhythmic hyperpolarizations in thalamic relay neurons. Recent observation that thalamocortical cells disconnected from the reticularis thalami nucleus no more oscillate within the spindle range shows that oscillations are imposed on relay neurons by synaptic inputs. It is then proposed that other thalamic neurons (intranuclear interneurons or reticularis thalami neurons) would have pacemaker properties and/or that oscillations would be produced in thalamic cellular pools by feedback interconnections.

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200.8 POLYSYNAPTIC PATHWAYS FROM BRAINSTEM LOCOMOTOR REGIONS TO LUMBAR ALPHA MOTONEURONS. S.J. Shefchyk and L.M. Jordan, Dept. of Physiology, University of Manitoba, Winnipeg, Canada R3E 0W3.

The fact that electrical stimulation of the mesencephalic locomotor region (MLR) can evoke locomotion in the decerebrate cat has been well documented (Shik et. al., Biofizika, 11, 1966), as has the spinal cord's intrinsic ability to produce rhythmic alternating stepping activity when isolated from higher centers (Grillner, Phys. Rev., 55, 1969). Past work done in this laboratory indicates that lumbar spinal alpha motoneurons receive both excitation and inhibition during MLR evoked locomotion. The purpose of this study is to examine the postsynaptic potentials (PSP) evoked in extensor and flexor motoneurons in the lumbar spinal cord from regions in the brainstem which are effective for evoking locomotion.

Fictive locomotion was produced by stimulation of the MLR in decerebrate cats paralyzed with Flaxedil. Intracellular records were obtained using 3M potassium acetate-filled microelectrodes, and motoneurons were identified on the basis of their antidromic-activation from peripheral nerves. Both sides of the brainstem were systematically stimulated (40-120 μA, 10-50 Hz), and records of the PSPs produced in motoneurons as well as the evoked locomotor activity were obtained.

PSPs that were time-locked to the brainstem stimulation during locomotion were observed in hindlimb flexor and extensor motoneurons and had latencies of 4-7 msec. Stimulation of either the ipsilateral or contralateral MLR was effective for PSP production. Records from individual motoneurons during locomotor trials indicated that stimulation of different mesencephalic locomotor sites could produce different PSPs in the cell. The PSPs displayed an increase in amplitude with repetitive stimulation, suggesting that they were polysynaptic. EPSPs, IPSPs, or EPSP/IPSP complexes could be observed, and often the PSPs were modulated in amplitude during the step cycle. In general, EPSPs were maximal during the depolarized phase of the step cycle, and IPSPs were maximal during the hyperpolarized phase. Some EPSPs which were present during the depolarized phase of the membrane potential oscillations associated with the step cycle disappeared during the hyperpolarized phase, when a longer latency IPSP could be observed. Stimulation of a single brainstem location could produce reciprocal effects, with an excitatory or inhibitory PSP occurring in an extensor (LG) motoneuron and a PSP of the opposite sign in an antagonist flexor (TA) motoneuron.

These experiments indicate the existence of polysynaptic pathways to lumbar motoneurons from sites in the brainstem where stimulation produces locomotion. Activity in these pathways is modulated during the step cycle in a manner consistent with the involvement of interposed neurons in the generation of locomotion.

200.9 VOLTAGE SPREAD IN AN INTERNEURON OF THE BARNACLE'S VISUAL SYSTEM. A. E. Stuart, J. H. Hayashi*, and L. A. Oland*. Dept. Physiology, University of North Carolina, Chapel Hill, North Carolina, 27514.

The second-order cell of the barnacle's visual system, the I-cell, offers a unique opportunity to study voltage spread in an interneuron. The cell has two distinct arbors separated by a long thin process that connects them across the commissure of the ganglion and extends to the soma. The photoreceptors (PRs) which synapse upon the I-cell are divided among three ocelli, two lateral and one median, which can be independently stimulated with light or current. Because PRs from each lateral ocellus terminate on a single arbor, it is possible to evoke a response in just one of the I-cell's arbors and study its subsequent spread to the recording site in the soma.

We compared the voltage responses of the I-cell's soma to illumination of the ipsi and contralateral ocelli. Responses elicited in the contralateral arbor were consistently slower in rise time and smaller than those elicited in the ipsilateral arbor.

The efficacy of voltage spread to the soma was tested by comparing the reversal potentials obtained for each lateral input. We illuminated each of the lateral eyes separately while passing hyperpolarizing current into the I-cell's soma. If the cell were isopotential, the ipsi and contralateral responses to light would be expected to reverse at the same potential because the voltage change would be the same in both arbors. However, the ipsilateral response always reversed at a potential substantially less negative than the contralateral response indicating that the cell is not isopotential.

When the ipsilateral ocellus was placed in background light, thus causing a conductance increase in the ipsilateral arbor (Oertel and Stuart, 81), contralateral signals were reduced or eliminated. Thus, as the background light increased, the cell's arbors were further isolated from one another.

To ask whether voltage changes spread well within each arbor, we compared the reversal potentials of median and ipsilateral inputs to the I-cell. Median PRs bifurcate and end on both of the I-cell's arbors. To eliminate potential changes spreading from the contralateral arbor, we cut across the commissure. Median and ipsilateral responses had reversal potentials that differed at most by a few mV. This finding suggests that median and lateral PR synapses are situated at similar electrical distances from the soma, even though anatomical studies show little overlap of these inputs on the arbor (Oland and Stuart, these abst.). Thus, voltages may spread quite well within the individual arbors of the I-cell. Oertel and Stuart, J.Physiol. (1981) 311: 127-46. Supported by USPHS grant EY03347.

200.10 THE ABSENCE OF SYNAPTIC INTERACTION AMONG THE MEDIAN AND LATERAL PHOTORECEPTORS OF THE BARNACLE AND A COMPARISON OF THE RESPONSES OF THE SECOND-ORDER CELL TO THEIR INPUT. L. A. Oland* and A. E. Stuart. (SPON: J. W. Moore). Dept. Physiology, University of North Carolina, Chapel Hill, NC, 27514.

The absense of synaptic interaction among the PRs of a visual system would considerably reduce the complexity of the signals evoked in the second-order cell and permit analysis of the cell's response to input from a single receptor. In the barnacle's visual system, ten photoreceptors (PRs), divided among three ocelli, comprise the barnacle's entire receptor population and converge on the same second-order cell. The PRs within each of the ocelli are electrically independent of each other. That synaptic interaction might occur between receptors of the median and lateral ocelli, was suggested by single cobalt fills of either median or lateral PRs which showed that they arborized in the same region of the ganglion (Hudspeth and Stuart, 77; Oland, et al, 83). When we simultaneously filled median and lateral PRs with cobalt, we found that overlap of the terminals, in one case entirely absent, was limited to the outermost fringes of the receptors' arbors. These results suggested that direct interaction was unlikely. We recorded directly from median or lateral terminals and illuminated the opposite ocellus. Stimulation of one ocellus failed to evoke a response in the PR from the other ocellus, even when the receptor terminal was hyperpolarized or depolarized by 20-30 mV.

The absence of interaction among the PRs of different ocelli allowed us to compare the responses of the second-order cell, the I-cell, to median and lateral inputs. Both inputs generate hyperpolarizing responses that consist of an initial peak that decays to a plateau. The responses are graded with intensity over the same range. However, lateral input always elicits a slower response. Also, following the offset of light over a lateral ocellus, the I-cell simply repolarizes to its dark resting potential or briefly overshoots rest by a few mVs. In contrast, the offset of light over the median ocellus produces a large depolarization often complicated by an oscillation. This difference is especially intriguing for it is at the offset of light that the third-order cell responds.

Because we can evoke responses in the I-cell with inputs from separate ocelli or from single PRs, we have the opportunity to study signal interaction at an interneuron. Hudspeth and Stuart, J. Physiol. (1977) 272:1-23. Oland, et al, J. Neurophysiol. (1983) 49:516-27. Supported by USPHS grant EY03347.

200.11 CELLULAR PHYSIOLOGY OF THE TURTLE CORTEX. B.W. Connors, A.R. Kriegstein* and B.R. Ransom (SPON: K.L. Chow) Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The general cortex of reptiles is a favorable preparation for studying principles of cortical organization because it is relatively simple, consisting of only three layers. We therefore undertook an investigation of the cellular physiology of the cortex of the turtle, Pseudemys scripta. We removed the cortex and placed it ventricular surface upward in a recording chamber where it was bathed in oxygenated saline at 21°C. Intracellular recordings were made with 3 M K acetate or dye-filled microelectrodes.

The cortex contains only two principal cell types, pyramidal cells and stellate cells in a ratio of 37:1. We found that cells could be classified into two groups based upon differences in membrane properties. The most frequently encountered cells had long membrane time constants, mean input resistances (R_N) of 74.5 MΩ, and 5-8 ms action potentials usually with more than one amplitude, possibly indicating more than one spike initiation site. Intracellular staining confirmed these were pyramidal cells. A second cell type was encountered less frequently. These were stellate cells and had mean R_N = 136 MΩ and faster, unitary, action potentials (1.5-2.5 ms).

Stellate cells have aspiny dendrites and local axon ramifications that form flat, symmetrical synapses onto pyramidal neurons. Smith et al. (J. Comp. Neurol. 190:445, 1980) have hypothesized that these are inhibitory interneurons. Degeneration studies have shown that thalamic afferents form 6 times as many contacts onto each stellate cell as onto each pyramidal cell. Each afferent volley should therefore be a powerful activator of inhibitory interneurons. We studied synaptic responses to local stimulation in the molecular layer of the cortex. Pyramidal cell responses were characterized by inhiibtory postsynaptic potentials (IPSPs) lasting from 150-1000 ms. IPSPs were associated with a large conductance increase and had reversal potentials 8 mV negative to the mean resting potential of -63 mV. IPSPs appeared to be Cl^- dependent since they were inverted by intracellular Cl^- injection. Focal GABA application produced a hyperpolarizing response with the same reversal potential as the IPSP. Stellate cells, in contrast, responded to local stimulation with excitatory PSPs, sometimes followed by IPSPs.

These results suggest that cell form and function are related in this primitive cortex. In addition, they provide physiological support for the role of the aspiny stellate cell as an inhibitory interneuron in cortex and confirm the prediction that afferent stimulation produces profound inhibition of pyramidal neurons.

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200.12 INTERACTIVE COMPUTER-GRAPHICAL REPRESENTATION OF CALCULATED VOLTAGES OVER THREE-DIMENSIONAL ARBORIZATIONS OF INDIVIDUAL NEURONS. S. Senft* (SPON: S. Rothman). Dept. of Anatomy & Neurobiology and McDonnell Center for Studies of Higher Brain Function, Washington Univ. Sch. Med., St. Louis, MO 63110

A number of procedures exist for quantifying and displaying the three-dimensional structure of single neurons (e.g., Wann et al., 1973). While the function of neurons undoubtedly depends on dynamic ionic, metabolic, and structural components distributed heterogeneously over their processes, there are few direct ways to visualize these spatio-temporal distributions in relation to a cell's form.

However, the expected value of certain electrical quantities at arbitrary locations on dendritic trees may be calculated, based on biophysical assumptions and data collected at point locations on the neuronal tree (e.g., Koch et al., 1983).

As a step towards more accurate conceptualization about subcellular neural physiology in toto, we have constructed an interactive computer-graphic system capable of representing the steady-state distribution of voltages using color and depth cues on complex neuronal dendritic trees.

The standing influence of excitatory and inhibitory synaptic inputs may be assessed over a range of assumed electrical parameters. Examples, showing the effects of inputs at different spatial locations on the three-dimensional dendritic arbors of several different neurons, will be displayed. (Supported by NIH Grant 17763.)

200.13 EXTRACELLULAR STIMULATION ANALYZED BY COMPARTMENTAL MODELS

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An exact theory of extracellular stimulation of neurons is unavailable, except for the simplest axons in restricted situations, and difficult empirical measurements are seldom attempted. But it is fairly simple to compute the potential along variously-shaped cells that are represented by resistively-linked, parallel-RC compartments. For present purposes, compartments were assigned a position in 3-space, and the neurons assumed to be electrically linear below threshold, unmyelinated, internally 1-dimensional, without resistance to external current flow, and surrounded by a homogeneous and isotropic, infinite-volume conductor. Electrodes were modelled as superimposed sinks and sources, always placed further from the cell than any intercompartmental distance; thus a bipolar concentric electrode was a central sink equalled by 100 encircling sources. A battery (Φ_i) between each ith compartment's external port and ground represented the stimulus, with $\Phi = I_0/(4\pi SR)$ (R, distance to electrode; S, external conductance; I_0, stimulus current). The time-dependent, simultaneous, linear, first-order, differential equations thus developed were given anatomically and physiologically realistic parameters and solved by the Runge-Kutta method.

The following are among observations and relationships which may be instructive to the practical user of electrical brain stimulation. First, membrane polarization tends to be larger at constrictions and terminations; this might explain the sometimes greater efficacy of anodal stimulation, for example in the case of pyramidal cells excited from the neocortical surface. Second, especially in thinner processes, charge redistribution can cause the potential to increase after the stimulus is turned off. Third, as suspected intuitively, concentric bipolar compared to monopolar stimulation is preferred for restricting activation to nearby membrane, although comparatively more current is needed. Fourth, the threshold (I_t) of uniform axons varies as the inverse of diameter. Fifth, in stimulation of uniform axons with a monopolar electrode $I_t \propto R$, and with a concentric bipolar electrode $I_t \propto R^3$ (at any constant electrode angle). In monopolar stimulation of spherical somatodendritic regions of volume D and radius A with regular "3/2 power law" branching, assuming a central trigger zone, I_t is inversely proportional to $\int\Phi/R$ within 10% error; that is, outside the sphere $I_t \propto 3R/A^3$, inside $I_t \propto 2(A^2-R^2/3)^{-1}$.

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200.14 CURRENT-VOLTAGE RELATIONSHIPS OF PERICRUCIATE CORTICAL NEURONS OF AWAKE CATS USING A COMPUTER ASSISTED METHOD. N. E. Berthier and C. D. Woody. Depts. of Anatomy and Psychiatry, UCLA Med. Ctr., Los Angeles, Ca. 90024.

Current pulses ranging from -4nA to +4nA and of 30-500 msec duration were injected into 17 pericruciate neurons in order to investigate their current-voltage relationships. An active bridge circuit was used to inject the current pulses. It was important that the circuit be accurately balanced so that the measurements would not be distorted by changing electrode resistance. Takeuchi, Kondoh, & Kohno (J. Neurosci. Methods, 4:365-372, 1981) devised an electronic circuit to balance the bridge circuit automatically, but their circuit had the drawback of requiring several synchronizing inputs. We used a similiar algorithm that required only that the electrode time constant be much shorter than the cell time constant, and implemented it on PDP-11/44 computer. Current monitor and intracellular voltage records were A/D converted and analyzed (using a threshold detection method) to determine the time of current pulse onset and offset. The intracellular voltage record was sampled just before pulse onset and just after charging of the electrode (i.e., about 50-100 μsec after pulse onset), the difference in the two measurements being the magnitude of bridge imbalance. This difference was then subtracted from the voltage trace for the duration of the current step, and the data D/A converted and displayed oscillographically.

The slope of the IV plot in the hyperpolarizing region was taken as the best estimate of R_m. It averaged 9.1 mΩ across the 17 neurons. The cells had a mean resting potential of 62 mV, and a mean action potential height of 59.6 mV. A rectification ratio was computed by dividing the average chord resistance of the depolarizing region by the hyperpolarizing resistance. Three patterns emerged: 1) nearly linear, where the ratio of resistances was slightly less than unity (mean ratio = .85, seen in 35% of the cells), 2) "anomalous rectification", where the apparent resistance in the depolarizing region was greater than in the hyperpolarizing region (mean ratio = 1.28, 23% of the cells), and 3) "delayed rectification" where the apparent resistance in the depolarizing region was much less than in the hyperpolarizing region (mean ratio = .55, seen in 41% of the cells). The resting potentials for group 1 averaged 61 mV, group 2 averaged 58 mV, and group 3 averaged 64 mV. Rectification was not detectable in the range of ± 0.5 nA current injection in 94% of the cells. (Supported by AFOSR F49620-83-C-0077 and HD 05958.)

200.15 SYNAPSES BETWEEN HIPPOCAMPAL NEURONS IN CULTURE. Matthew P. Frosch, Deborah M. Barnes and Marc A. Dichter, Dept. of Neuroscience, Children's Hospital, Boston, MA 02115.

Neurons were obtained from the hippocampal regions of 20-21 day gestation rat embryos and were maintained in primary culture for up to 14 weeks. Cells were dissociated mechanically and plated onto collagen and poly-L-lysine coverslips (500,000 cells per 35 mm dish). Trituration, plating and growth medium (Eagles MEM) was supplemented with 3.5% rat serum, 5% horse serum, 3.75 mg/ml glucose and 0.3 mM glutamine. Growth was at 37°C in 5% CO_2; medium was changed three times weekly. Cytosine arabinoside (10^{-5}M) was added to inhibit further mitoses in confluent cultures (7-12 days).

Physiological studies of synaptic connections were made at various ages in culture. Spontaneous excitatory and/or inhibitory post-synaptic potentials (psp's) were noted in the majority of neurons sampled in cultures between 1 and 11 weeks in vitro. However, connections between pairs of neurons within one microscopic field (350 um diameter) were not found at times up to 3 weeks but between 3 and 11 weeks, such 'local' connections were seen in 60-90% of sampled pairs. Such connections were uni- or bi-directional with inhibitory synapses predominating. These connections were assumed to be monosynaptic based on their ability to follow at rates up to 50-100 Hz and fixed, short latencies. Few failures of transmission were seen. No electrical connections were found in any of the cultures examined for synaptic coupling; however, occasional dye coupling was observed in an eight day culture using intracellular 6-carboxyfluorescein injection (a single electrode experiment).

The properties of synaptic transmission under conditions of repetitive stimulation were examined. All connections could be driven at rates up to 50 Hz, with summation of events observed. When inhibitory connections were repeatedly stimulated no diminution in psp size was observed. Tetanization led to a clamping of the resting potential of the post-synaptic cell with little diminution over several seconds. During train stimulation, at interspike intervals sufficient to avoid summation of psp's, it was possible to see early potentiation of some connections, both inhibitory and excitatory. Additionally, post-tetanic potentiation was seen for synaptic connections of both polarity.

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200.16 ANALYSIS OF VOLTAGE-SENSITIVE IONIC MECHANISMS MEDIATING INTRINSIC ACTIVITY OF CULTURED PURKINJE NEURONS. D.L. Gruol, The A.V. Davis Center for Behavioral Neurobiology The Salk Institute, P O. Box 85800, San Diego, CA 92138

Cerebellar Purkinje neurons (PNs) in vivo are characterized by a unique firing pattern which is generated by a combination of events including simple spikes, complex spikes and a series of pauses. Recent data from a cerebellar model system, cultured fetal rat cerebellar neurons, suggests that both synaptic and intrinsic components contribute to the pattern of activity characteristic of PNs (Gruol, Brain Res. 263, 1983). In the present studies, three types of electrophysiological recordings were used to investigate further the intrinsic mechanisms which generate the electrical activity of PNs: extracellular unit recording, intracellular voltage recording and single channel recording using the patch clamp technique. PNs were identified on a morphological basis and by immunohistochemical techniques (Franklin and Gruol, this volume). A variety of firing patterns were observed in extracellular recordings from the PNs. Two common patterns correlated well with two types of intrinsically generated, voltage-sensitive activity observed in intracellular recordings: (1) a regular simple spike firing pattern and (2) a regular pattern of large membrane depolarizations which evoked 1 or 2 spikes. The differences in the types of intrinsically generated activity of PNs presumably results from differences in the type, location or regulation of the voltage-sensitive ionic mechanisms mediating the activity. As a first step in testing this hypothesis, the technique of single channel recordings was applied to identify the voltage-sensitive ionic mechanisms present in PNs and to characterize the regional distribution (soma vs dendrite) of these mechanisms. Single channel recordings from cell-attached patches revealed a variety of channel activities. Two channel types mediating outward current events were of particular interest because their large size and frequency of detection indicated that they play a major role in the activity of PNs. Both channel types were voltage-sensitive, activated by membrane depolarizations, largely K+ selective and observed in both the somal and dendritic membranes. One channel type had a single channel conductance of approximately 35 picosiemens (pS) and the second approximately 100 pS. The characteristics of the larger channel suggest it may be a Ca++ activated K+ channel, a possibility that is presently being tested. A major role of K+ currents in generating or regulating the pattern of activity characteristic of PNs was further supported by intracellular studies with K+ channel blockers. (supported by NIAAA 03504)

200.17 VOLTAGE CLAMPING OF PURKINJE CELLS IN VITRO: A STUDY IN GUINEA PIG CEREBELLAR SLICES. M. Sugimori and R. Llinás. Dept. of Physiol. & Biophys., New York Univ Med. Ctr., 550 First Ave., New York, NY 10016.

The ionic current activated by depolarization steps applied to somata of Purkinje cells was studied under voltage-clamp conditions. Voltage clamping was obtained with a single patch microelectrode attached to a currentometric amplifier configuration. The electrode was applied directly against the body of the cell after the slice was given a short exposure to trypsin. Following a high resistance seal of the electrode against the surface membrane, negative pressure was utilized to break the membrane in front of the electrode tip which produces a stable intracellular recording paradigm with the input resistance of the electrode being as low as half a megohm. Under these conditions voltage steps as fast as 25-50 μsec can be achieved. In order to simplify the interpretation of the voltage clamp data, the axons and the dendrites of the cells are moved leaving then a spherical isolated somatic Purkinje cell recording. Under these conditions depolarization of the somata generates a fast graded inward current carried by sodium followed by a large outward potassium current. When this later current is blocked by intracellular cesium and tetraethylammonium, the initial sodium current is followed by a slow inward current carried by the non-inactivating sodium conductance (Llinás & Sugimori, J. Physiol. 305: 171, 1980; Sugimori & Llinás, Soc. Neurosci. Abst. 7: 76, 1981). This slow inward current is blocked by tetrodotoxin and absent when sodium is removed from the extracellular bath. When the dendritic tree or part of it is left intact prior to clamp, a sizeable calcium current can be observed which is blocked by cobalt or cadmium. In the case where well isolated somata were voltage clamped, such I_{Ca} was not observed. Supported by USPHS grant NS13742 from NINCDS.

200.18 INTRACELLULARLY RECORDED ACTION POTENTIALS IN DORSAL ROOT AFFERENT FIBRES PRODUCED BY ANTIDROMIC ACTIVATION OF MOTONEURONES. F.J. Alvarez-Leefmans and H. Cruzblanca*. Department of Neuroscience, CINVESTAV del IPN. Apartado Postal 14-740, México 07000, D.F.

Orthodromic electrical coupling between some afferent fibres and motoneurone dendrites is well established in amphibian spinal cord. Electrical coupling also occurs in the antidromic direction. Shapovalov and Shiriaev (Exp. Brain Res. 33: 299, 1978) showed that ventral root (VR) volleys produce action potentials in sensory fibres. However, this could only be demonstrated in the presence of TEA or 4AP, which might have unwanted direct pharmacological actions, complicating the interpretation of their findings. Here we report preliminary data showing that such an antidromic electrical coupling can be demonstrated lowering the temperature below 10°C in the absence of pharmacological agents other than Co^{2+}.

Experiments were performed in isolated and hemisected spinal cords of frogs, superfused with Ringer (5-10°C) in which Ca^{2+} was replaced by 2 mM Co^{2+}. Suction electrodes were used for stimulating and/or recording from dorsal roots (DR) or VR from segment IX. Intracellular recordings (3 M-KCl microelectrodes 60-90 MΩ), were obtained from sensory fibres at their point of entry into the spinal cord. In 10 out of 13 cases VR stimuli produced the temperature dependent depolarizing DR potential (VR-DRP-II) (Cruzblanca, H. & Alvarez-Leefmans, F.J. Soc. Neurosci. Abst. 8: 912, 1982) which was postulated as resulting from firing of some DR afferent fibres. 2 out of 10 of these fibres fired by VR stimuli, with latencies corresponding to those of the VR-DRP-II (7.4 and 15 ms). The low stimulus strenght used, and the latencies found, rule out any possibility of artefactual spread of the stimulating current to the DR. All the fibres were fired by orthodromic DR stimuli. The mean resting potential was 45.3 mV (S.E.$\pm$ 3.1 mV). The amplitude of the action potential was 63 $\pm$ 4.9 mV (mean $\pm$ S.E.). The shape of these action potentials was similar to that described for the node of Ranvier. The low proportion of fibres fired antidromically is expected since only a small number of axons make monosynaptic contacts with motoneurones. Increasing the temperature to 15-17°C and adding 5mM TEA produced antidromic firing of some sensory fibres (Cf. Shapovalov op.cit). These results resemble those found in the cat spinal cord (Decima, E.E. & Goldberg, L, J. Brain Res. 57: 1, 1973).

200.19 SYNAPTIC TRANSMISSION FROM A SINGLE SACCULAR FIBER ONTO THE GOLDFISH MAUTHNER CELL. Jen-Wei Lin and Donald S. Faber. Div. Neurobiology; Dept. Physiology; SUNYAB, Buffalo, NY 14214.

In goldfish, the Mauthner cells (M-cells) mediate the startle response evoked by auditory and visual stimuli. The primary auditory afferents (the saccular nerve) impinge on the M-cell distal lateral dendrite as club endings and endbulbs (Lin, Faber and Wood, Brain Res., in press). Both gap junctions and chemical synapses are morphologically identified in a single club ending. Previous physiological studies have shown that electrical stimulation of the saccular nerve evokes a strong electrotonic and a weak chemical excitatory postsynaptic potentials (epsp) in the distal lateral dendrite which is consistent with the notion that club endings mediate both modes of transmission. In order to further evaluate this hypothesis and to determine the relative contribution of individual afferents to the M-cell auditory responses, we have employed simultaneous pre- and postsynaptic intracellular recordings. Presynaptically, the large myelinated fibers in the anterior part of the saccular nerve were impaled at the point where they enter the medulla. The postsynaptic recording site was in the M-cell, about 300um lateral to the axon hillock. In 18 experiments, an impulse evoked in an afferent by current injection produced a short latency (<0.1ms) electrotonic psp which was typically about 1 mV (range 0.1-1.5mV) and could follow stimulation frequencies up to 100 Hz. If we assume that the presynaptic spike amplitude at the club ending terminal is 80 mV, then the orthodromic coupling coefficient for impulses is about 0.019. When the M-cell axon hillock-initial segment was activated antidromically, the resulting 10 to 15mV spike potential in the lateral dendrite was synchronous with a depolarization of about 1 mV in the saccular fiber. Therefore, the electrotonic coupling between club endings and the M-cell appears to be bidirectional.

Identification of the chemically mediated epsp evoked by single fibers was more difficult because the responses were quite small and exhibited marked fluctuations. Small depolarizations (<150 uV) following the electrotonic psp could be detected at low stimulation frequencies; these responses disappeared with high frequency stimulation.

Generally, the stimulation of the entire saccular nerve fires the M-cell at the peak of a prominent chemical epsp, rather than electrotonic psp. The major source of this chemical epsp is presently being investigated by stimulating discrete areas of the saccular nerve. (Supported by NIH grant NS15335).

201.1 ATP-DEPENDENT CALCIUM TRANSPORT INTO NATIVE DISC MEMBRANES ISOLATED FROM BOVINE ROD OUTER SEGMENTS. K.L. Puckett* and S.M. Goldin (SPON: J.R. Glowa) Dept. of Pharmacology, Harvard Medical School, Boston, MA 02115

An ATP-dependent Ca^{++} transport activity has been found to be present in preparations of native disc membranes isolated from bovine retinal rod outer segments (ROS).

Osmotically active discs were isolated from bovine ROS by hypoosomotic shock and subsequent floatation on a 5% Ficoll 400 solution. Quantitative analysis of the rhodopsin content was performed spectrophotometrically for each sample by bleaching and regeneration of rhodopsin in the presence of 1% digitonin. Ca^{++} transport assays were performed by separation of intradiscal from extradiscal $^{45}Ca^{++}$ on cation exchange resin both in the presence and absence of 2 mM Mg^{++}ATP to determine ATP-dependent Ca^{++} uptake. After 5% Ficoll floatation the discs were further purified by separation into two distinct bands on a linear 5-20% Ficoll gradient.

The "rhodopsin" band, containing 90% of total protein in the disc preparation and all the bleachable and regenerable rhodopsin, was further characterized. This band was demonstrated by electron microscopy to have morphology characteristic of retinal discs. Also associated with this band was an ATP-dependent Ca^{++} uptake activity: ATP stimulated Ca^{++} uptake 4 to 8 fold, with a net uptake of $\sim$0.1-0.2 nmoles Ca^{++}/mg protein/min. In addition 65-80% of the ATP-dependent accumulated $^{45}Ca^{++}$ was released upon incubation with the Ca^{++} ionophore A23187.

To determine whether the association of this Ca^{++} transport activity with the rhodopsin band was merely coincidental, we exploited the osmotic properties of these sealed disc membranes. The sedimentation properties of the rhodopsin band upon centrifugation on a second 2-15% Ficoll gradient ($\rho \simeq 1.007$ to 1.052 g/ml) varied with the composition of the gradient solution. In several separate gradient solutions of differing osmotic and ionic strengths, the observed migration position of the rhodopsin band varied from $\rho \simeq 1.02$ to 1.03 g/ml. On each gradient, the Ca^{++} uptake activity always precisely co-migrated with the rhodopsin containing membranes. The different sedimentation patterns observed on each gradient were attributed to differences in the osmotic strength between gradients and the resultant shrinking and swelling of the discs.

Therefore, these results strongly suggest that the ATP-dependent Ca^{++} transport activity characterized here is associated with intact disc membranes. In addition, the amount of Ca^{++} uptake activity was sufficient, in principle, to account for a role of this Ca^{++} "pump" in the regulation of cytosolic Ca^{++} levels in the rod cells in vivo. Supported by the NIH (NS16475). The McKnight Foundation. and The Searle Scholars Program, to SMG.

201.2 EFFECT OF 2-DEOXY-D-GLUCOSE ON CAROTID BODY (C.B.) CHEMORECEPTORS. A.Obeso*, L.Almaraz*, and C.González. Dpt. of Physiol. Fac. de Med. Valladolid, Spain.

The biochemical and/or biophysical steps through which low levels of arterial pO2 promote and increase of chemoreceptors activity are presently unknown. Among the theories of sensory transduction in this receptor perhaps the most commonly accepted is the metabolic theory; according to it, hypoxia will promote a decrease in ATP/ADP quotient which ultimately will trigger the release of a transmitter from type I cells leading to increase of sensory impulses in the carotid sinus nerve (c.s.n.). This theory rest on the observations that any metabolic poison which decreases the above quotient excites the arterial chemoreceptors. Nevertheless the theory as a whole has been criticized because the metabolic poisons so far used have a very wide spectrum of actions. Here we present data in support of the metabolic theory by the use of 2-deoxy-glucose.

C.bs were excised from adult cats and prepared to record in vitro. In a first group of experiments the c.bs were superfused with modified Tyrode containing 5 mM glucose or 5 mM Na-pyruvate; the responsiveness of the preparation (activity in the c.s.n.) to different types of stimuli were assessed with either solution. In a second group of experiments the c.bs were superfused with pyruvate containing Tyrode saturated with 100% O_2 and dose-response curves for 2-deoxy-glucose vs. activity in the c.s.n. were obtained. In a final group of experiments the effects of 2-deoxy-glucose on the release of ^{3}H-DA and the activity in the c.s.n. were monitored simultaneously in c.bs. previously incubated with ^{3}H-Tyrosine.

Our main findings were: The responsiveness of the c.b to hypoxia, acidosis, Ach, Na -cyanide and DA was the same with glucose or pyruvate. The results suggest the independence of the chemoexcitation from the glycolisis. 2-Deoxy-glucose excites the c.b. chemoreceptors; the threshold concentration was 0.25 mM, the maximum effect was obtained with 4 mM and the dose-response curves were hiperbolic in shape. 2-Deoxy-glucose promoted a dose-dependent release of ^{3}H-DA. The data support the metabolic theory on the basis of the known actions of 2-deoxy-glucose on celular ATP levels. In addition a linkeage between ATP levels and the release of DA seems evident in our data.

This work was supported by a Grant from the C.A.I.C.T

201.3 **PDE AND PDE ACTIVATION: EFFECTS ON ROD MEMBRANE POTENTIAL.** **B. Oakley II[1], J.W. Clack*[2], and P.J. Stein*[2]. [1]Dept. of Elect. Eng. and Bioeng. Program, Univ. of Illinois, Urbana, IL, [2]Dept. of Ophthalmology, Yale Univ. Sch. Med., New Haven, CT**

In an attempt to determine if the activation of the cGMP phosphodiesterase (PDE) in the rod outer segment (ROS) plays a role in the transduction process, we recorded membrane potential, V_m, intracellularly from red rods in the isolated retina of the toad, Bufo marinus, using double barrel micropipettes. V_m was monitored with one barrel while a protein solution was pressure-injected into the ROS through the other barrel. The injected solution contained 22 mM MOPS (pH 7.2), 4 mM DTT, 15% glycerol, and 1-7 μM of various proteins isolated from toad ROS. Injections of pure GTP-binding protein (termed G) that was binding a hydrolysis-resistant analog of GTP, p(NH)ppG, (together termed G*) and thus was able to activate PDE, or injections of (partially-purified) active PDE itself, resulted in reversible hyperpolarizations of the ROS membrane as large as 13 mV. A mixture of G* and PDE was especially effective and produced prolonged hyperpolarizations. Either of these proteins caused cGMP hydrolysis (as determined by a ^{3}H-cGMP assay) when added to dark (unbleached) ROS membrane suspensions. Active PDE hydrolyzed cGMP in the absence of membranes as well. Injections of proteins that did not significantly cause hydrolysis of cGMP when assayed did not significantly (<1 mV) affect V_m when injected. These proteins included G binding GDP, boiled PDE, and boiled G*. These results are consistent with the hypothesis that the activation of PDE and the subsequent hydrolysis of cGMP are steps in the transduction process.

The hydrolysis of cGMP yields 5'-GMP and H^+. Others have suggested that the excitatory signal is not the loss of cGMP but that it is the production of H^+. To test this idea, we have injected H^+ (acetic acid in 100 mM K^+ acetate$^-$) into ROS. Injections of H^+ at pH 4.2 did not significantly affect V_m, while injections at pH 2.0 caused reversible depolarizations of the ROS membrane. These results argue against a simple model, in which the excitatory signal is carried solely by the H^+ that are produced by cGMP hydrolysis. It seems that if protons are involved in the transduction process, other biochemical reactions also are required.

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201.4 CAN LOUDNESS GROW NORMALLY IN COCHLEAR-IMPAIRED EARS WITH SLOPING HIGH-FREQUENCY LOSSES? Rhona P. Hellman. Communication Sciences Laboratory, Boston Univ., Boston, MA 02215.

Loudness growth was measured by the psychophysical procedures of magnitude estimation and production in three listeners with steep bilateral high-frequency losses of cochlear origin. Within the normal-hearing region, two test frequencies, a minimum of one octave apart, were studied. Individual loudness functions were obtained by combining the results of magnitude estimation with those of magnitude production. Results disclose that for each listener the loudness function is steeper at the lower test frequency than at the cutoff frequency, beyond which thresholds decrease by more than 40 dB/octave. On the average, the loudness growth rate decreases by 17% at the cutoff frequency. However, the loudness growth range is the same as in normal hearing. In good agreement with previous loudness measurements in normal ears masked by an adjacent high-pass noise (Hellman, R.P., J. Acoust. Soc. Am., 63:1114, 1978), the loudness of tones as high as 3200 Hz continues to grow, but at a slower rate, over a stimulus range of at least 100 dB. Since very little residual hearing above the cutoff frequency was available, further evidence that the loudness growth range does not require the recruitment of neural units at frequencies above the stimulus or test frequency is provided.

201.5 G-PROTEIN IN VERTEBRATE PHOTORECEPTORS: BEHAVIOR DURING DARK-ADAPTATION. N. J. Mangini*, D. R. Pepperberg, W. Baehr* and M. L. Applebury*. Department of Biological Sciences, Purdue Univ., West Lafayette, IN 47907.

The light-induced increase in affinity of ROS G-protein (GTP-binding protein, consisting of subunits α, β, and γ) for the disc membranes is reversible in the dark (1,2). To examine the possibility that the state of binding of G-protein influences the photic responsiveness of the receptors during dark-adaptation (3), we analyzed the time course of recovery from the light-induced state of relatively tight binding of G to the discs. The analysis utilized the facts that (i) hypotonic buffer elutes G from dark-adapted membranes but not from bleached membranes, and (ii) hypotonic buffer containing 40 μM GTP (hypotonic-GTP) elutes G even from bleached membranes (1,2). Isolated retinas of the toad, B. marinus, were subjected to a standard bleach (∿65%; 10-min irradiation). After incubation (for T min) in darkness at 20° C, retinas were chilled (0° C) and homogenized. The ROS were isolated by sucrose flotation and subjected to initial washing with isotonic buffer. Levels of G eluted by hypotonic and subsequent hypotonic-GTP washes of the ROS were quantitated by SDS-PAGE and densitometry of identified G bands. Relative binding of G to the membranes was analyzed as the ratio of (G eluting in the hypotonic-GTP wash) ÷ (total G eluting in the hypotonic plus hypotonic-GTP washes).

Bleaching induces an immediate increase in the affinity of G for the disc membranes; for freshly bleached (T = 0 min) vs. unilluminated retinas, the fraction of G bound was >80% vs. <15%, respectively. With increasing time of incubation in darkness, the fraction of G eluted in the hypotonic-GTP wash declined gradually and that eluted in the hypotonic wash increased. For T = 90 min, the fraction of G in the hypotonic-GTP wash (≈15%) indicated an essentially complete reversal of the bleaching-induced binding to the disc membranes. The period in darkness required for half-completion of the recovery was ≥30 min. Throughout the period of T = 0-90 min, total G eluted in the hypotonic plus hypotonic-GTP washes was roughly constant. These observations will be discussed in relation to the time course of dark-adaptation in toad photoreceptors.

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(1) Kühn, Nature (1980) 283:587.
(2) Baehr, Morita, Swanson and Applebury, J. Biol. Chem. (1982) 257:6452.
(3) Clack, Oakley and Pepperberg, Proc. Natl. Acad. Sci. USA (1982) 79:2690.

201.6 ELECTROPHYSIOLOGICAL INVESTIGATION OF DROSOPHILA MUTANTS DEFECTIVE IN PRESUMPTIVE RHODOPSIN STRUCTURAL GENE. E. C. Johnson* and W. L. Pak (SPON: G. S. Wasserman). Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Recent work in our laboratory has shown that the ninaE gene on the third chromosome is very likely to be the structural gene for the majority class of opsin (R1-6 opsin) in the Drosophila compound eye (Scavarda et al., 1983). Mutations recovered to date from this locus severely reduce the rhodopsin content in R1-6 photoreceptors but not in R7/8 photoreceptors. We have examined several parameters of light-evoked photoreceptor responses of two allelic mutants of ninaE, ninaE^{P332} and ninaE^{P334}, by means of intracellular recording techniques.

V-log I curves, which plot the amplitude of the receptor potential against log intensity, are shifted toward the direction of high intensity by about 3 and 6 log units in P332 and P334, respectively, in comparison to those of wild type. The receptor potential of invertebrate photoreceptors consists of small voltage fluctuations, known as "bumps," each of which is thought to be generated by the absorption of a single photon. From the amount of light needed to generate a single bump, we estimate the amount of rhodopsin in R1-6 photoreceptors of P332 and P334 to be approximately 10^{-3} and 10^{-6} of the wild-type level respectively. Since in flies the receptor threshold is thought to be linearly related to the amount of visual pigment, these estimates agree with the threshold measurements based on V-log I curves. Power spectra obtained from wild type and P332 display nearly the same shape changes with increasing stimulus intensity, suggesting that both P332 and wild type follow the same course of adaptation. The bump rate varies linearly with stimulus intensity over a wide range of intensities for both wild type and P332 with the P332 curve shifted toward the high intensity direction by a few log units. For a given level of depolarization, the noise amplitude is larger in P332 than in dark adapted wild type and larger still in P334 (>2X), suggesting that the amplitude of individual bumps are substantially larger in the mutants. The increase in bump size in the mutants with a drastically reduced level of rhodopsin is difficult to understand in terms of the existing models of bump production.

201.7 THE DIRECT RESPONSE OF THE FROG IRIS TO LIGHT IS INHIBITED BY cAMP. L. Barr and K. Hunsicker*. Dept. of Physiology and Biophysics. University of Illinois, Urbana, IL 61801.

The pupillary sphincter smooth muscle cells of the amphibian iris contain rhodopsin and contract when the rhodopsin is bleached by light. Therefore there is a response sequence in these cells which starts with a sensory response system like that of a retinal rod but ends by triggering smooth muscle cell's contractile apparatus. Preparations of sphincter pupillae were dissected free and cleaned of the iridial stroma. The resulting rings were hung in vitro in order to measure the time course of the tension responses. Responses as small as 0.1 mg were recorded. We investigated the sequence of events between the absorption of light by the rhodopsin and the generation of active tension. As we surmised from the persistence of the iridial response to light in Ca^{++} free or isotonic K_2SO_4 bathing solutions, neither TTX nor Verapamil (i.e., blockade of voltage dependent Na^+ channels or Ca^{++} channels) prevent the photoresponse of the iris. The photoresponse is unaltered by even 10^{-4} M 8-bromo cGMP. On the other hand, as could be surmised from the excitation-coupling sequences seen in other smooth muscles, IBMX and 8BcAMP inhibit tension development in the sphincter pupillae. The calmodulin blocker, TFP, also blocks the iridial photoresponse. Our working hypothesis is, therefore, that light activated rhodopsin causes an increase in free intracellular calcium which combines with calmodulin to activate the contractile apparatus without the necessity of a transmembrane potential change or an intervening change in cGMP.

201.8 EFFECTS OF CALCIUM ON OUTER SEGMENT MEMBRANE CURRENT OF TOAD RODS. G. Matthews. Dept. Neurobiology, SUNY, Stony Brook, NY 11794

External calcium concentration is known to have strong effects on the light response and dark potential of vertebrate photoreceptors. To examine more directly the effect of calcium on the light-sensitive conductance, the outer segment membrane current of an isolated rod was recorded with a suction electrode (Baylor et al., 1979, J. Physiol., 288, 589) in solutions containing different calcium concentrations. In these experiments, calcium concentration was changed by moving the suction electrode and recorded cell from one solution to another. The inner segment of the rod was held within the suction electrode and the outer segment was exposed to the bathing fluid. When calcium concentration was lowered from 1.0 to 0.1 mM, the dark current and the flash sensitivity (peak amplitude of a linear flash-response divided by flash photon density) increased 2-3 fold. The time-to-peak of dim-flash responses increased slightly in low calcium, and the relation between flash sensitivity and time-to-peak was similar to that observed during background desensitization (Baylor et al., 1980, J. Physiol., 309, 591). When calcium was decreased still further, however, this relation did not hold, and in fact at 10^{-8} M calcium the sensitivity declined by as much as 3-4 orders of magnitude, as observed previously in experiments using intracellular recording by Bastian & Fain (1982, J. Physiol. 330, 307). Addition of the divalent cation ionophore A23187 to normal saline at 0.2 μM reduced the dark current and flash sensitivity without significant effects on response kinetics. The desensitizing effect of very low external calcium could be counteracted by background illumination, as reported earlier by Bastian & Fain. This sensitizing effect of illumination was not associated with an appreciable increase in the dark current and could be produced by backgrounds that themselves produced no detectable response. The desensitization in very low calcium and the sensitizing effect of light could be explained at least in part if the outer segment conductance is very high in 10^{-8} M calcium, shunting the dark current. In experiments in which half of the outer segment was drawn inside the suction electrode, some evidence for such a shunt was the observation of a response of reversed polarity when light was flashed on the portion of the outer segment inside the electrode. This suggests that current from the base of the outer segment, which would normally flow entirely inside the electrode and thus not be recorded, was divided between the inner segment and the abnormally low resistance of the outer segment membrane. (Supported by USPHS Grant EY03821 and by the Alfred P. Sloan Foundation.)

201.9 LINEARITY AND TIME-INVARIANCE OF TURTLE CONE PHOTORECEPTORS. Scott J. Daly* and Richard A. Normann, Department of Bioengineering, University of Utah, Salt Lake City, Utah 84112.

Baylor and Hodgkin (J.Physiol., 234:163, 1973) have shown that turtle cone photoreceptors manifest linearity for dim flashes which evoke low amplitude responses. They also suggested that the turtle cone is time invariant in this linear region based on their observation that the integral of the cone impulse response was equal in amplitude and kinetics to the cone step response. From these principles of linearity and time invariance, we have modelled the dependence of cone quantal sensitivity on stimulus duration. For short duration pulses, cone sensitivity should be constant; the response is a function only of the number of quanta in the pulse. For long duration pulses, cone sensitivity should be inversely proportional to pulse duration. Plotting log sensitivity versus log pulse duration, the short and long pulse regions approach asymptotes which intersect at a time which characterizes the temporal properties of the cone. The model indicates that this asymptotic intersection time should equal the area under the impulse response divided by the response amplitude (the normalized impulse response).

We used standard intracellular recordings from turtle cone photoreceptors to verify the above predictions. We measured the dependence of cone sensitivity on pulse duration. The experimental data showed the predicted asymptotic behavior. However, the intersection time was three times less than the normalized impulse response, and consequently, the asymptotic intersection time measured was three times lower than predicted. An additional anomaly appeared in the data. We predicted that the ratio of the area under any linear range response to the number of quanta in the stimulus should be constant. Our measurements indicate that this ratio is only constant for short duration stimuli, and decreases monotonically for longer duration stimuli.

These results suggest that the time invariant aspect of the linear range needs more investigation. Moreover, light adaptation may occur even in the "linear range" of the cone.

201.10 BRANCHING OF CENTRAL PROCESSES OF DORSAL ROOT GANGLION CELLS IN CATS. K. Chung and R.E. Coggeshall. Departments of Anatomy and Physiology and Biophysics and the Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas.

Dorsal root ganglion cells are described as pseudounipolar neurons with a single central process that enters the spinal cord via a dorsal root. The major evidence supporting the concept that each dorsal root ganglion cell has a single central process are the counts showing equality of axon and ganglion cell numbers. Recently, however it has been shown that the axonal counts are inaccurate because of the limited resolution of the light microscope, and when the counts were repeated in the rat, there was a surplus of axons to ganglion cells. The present report extends this work to the cat.

Dorsal root ganglion cells were counted in serial light microscopic sections and dorsal root axons from the same segment were counted in the electron microscope. Our preliminary data are shown in tabular form below.

SEGMENT	AXONS	CELLS	SEGMENT	AXONS	CELLS
S1	18,963	8,777	S2	13,674	8,769
S1	11,858	11,920	S2	13,645	8,881
S1	19,242	12,311	S2	17,128	8,405
S1	22,474	16,982	S2	20,236	12,478
S1	18,148	14,458	S2	16,041	10,508

Note that there is an excess of dorsal root axons as compared to ganglion cells and that the ratio is approximately 1.5/1. We interpret these data as indicating that the central processes of some, perhaps many, dorsal root ganglion cells in the cat are branched. It will be important to determine if different branches have different destinations and if certain functional catagories of dorsal root ganglion cells have branched central axons whereas others do not. Partially supported by grants NS10161, 17039, 11255 and 07377 from NIH and an award from the Moody Foundation of Texas.

201.11 INHIBITION OF G-PROTEIN FUNCTION BY MONOCLONAL ANTIBODY IN LIGHT- AND HORMONE-SENSITIVE CELLS. Heidi E. Hamm, Joseph S. Takahashi and M. Deric Bownds. Lab of Molecular Biology and Dept. of Zoology, Univ. Wis., Madison, WI 53706, and Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

In an effort to find a specific blocker of the light-activated cGMP pathway in frog rod outer segments, a series of monoclonal antibodies to the G-protein were tested for functional effects on the cGMP cascade. One out of the seven purified antibodies tested completely blocked light-induced phosphodiesterase (PDE) activity of permeabilized rod outer segments at low and intermediate light levels; at saturating light levels the antibody caused a significant reduction in PDE activation compared to controls incubated with nonspecific antibody (commercially available IgG or IgG purified from control NS1 ascites fluid). The antibody also inhibited light-induced guanyl nucleotide binding, thus it appears to turn off the cGMP pathway by blocking its first step. The antibody inhibition was stoichiometric, i.e., it required only one antibody per G-protein in the mixture for inhibition, and the effect was complete by 10 min of incubation. An unexpected second effect of the antibody is to block the phosphorylation of two small proteins, Components I and II, that have previously been shown to be controlled by light and cGMP; the other six antibodies had no effect on any phosphorylation. Addition of cGMP to the mixture did not reverse antibody inhibition of Components I and II phosphorylation; this indicated that the antibody was not having its effect simply by changing cGMP levels in the outer segment. One possibility is that Components I and II normally control earlier steps of the pathway, such as G-protein or PDE activity.

In view of the possible homology between G-proteins in light- and hormone-sensitive systems, the effect of the inhibitory G-protein antibody on beta-adrenergic stimulated adenylate cyclase was studied. In rat pineal membranes, the antibody inhibited basal, as well as isoproterenol-, fluoride- and guanine nucleotide-stimulated adenylate cyclase (approx. 50% inhibition in all cases), while nonspecific antibody had no effect. Protein blotting (Western) analysis shows a protein band present in rat pineal membranes that binds G-protein antibody at approx. MW 40,000 and another minor band at approx. MW 52,000. These results are further evidence for a structural and functional homology between G-proteins in different tissues.

202.1 MEASUREMENT OF ELECTRICAL COUPLING BETWEEN RECEPTORS OF THE SALAMANDER RETINA. Martin Wilson, David Attwell* and Samuel Wu. Department of Zoology, University of California, Davis, CA 95616, Department of Physiology, University College London and Cullen Eye Institute, Houston, TX 77030.

Electrical coupling between receptors has been measured using pairs of intracellular microelectrodes under visual control. These data have been combined with voltage-clamp data obtained previously from isolated rods and cones (Attwell and Wilson, J. Physiol. Lond., 309:287, 1980; Attwell, Werblin and Wilson, J. Physiol. Lond., 328:259, 1982) to provide a quantitative model of the receptor network. The use of the model to predict deviations from spectral univariance in retinal receptors is described in another abstract (Wu, Attwell and Wilson, Neurosci. Abst. 1983).

Injection of -1nA current into a rod elicited a hyperpolarization of about 20 mV in an adjacent rod and 4 mV in an adjacent cone. More distant receptors showed smaller responses. Injection of -1nA into a cone elicited a hyperpolarization of about 4 mV in a neighboring rod but only 0.5 mV in a neighboring cone. No voltage change was detected in half of a double cone when -1nA was injected into the other half.

Assuming that every rod is directly coupled, via ohmic junctions, to 4 neighboring rods and 4 neighboring cones, and that cones are not directly coupled to each other, our results imply a coupling resistance of 300 MΩ between adjacent rods and 5,000 MΩ between a rod and an adjacent cone.

Because of their weak coupling to rods we have omitted cones from a consideration of signal processing by the rod network. Our model predicts that spatial resolution by the rod network is time-dependent and that the temporal frequency response of the rod network decreases below 5 Hz.

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202.2 AN ANALYSIS OF SPECTRAL INTERACTIONS BETWEEN RODS AND CONES OF THE TIGER SALAMANDER RETINA. Samuel M. Wu, David Attwell* and Martin Wilson. Cullen Eye Institute, Baylor College of Medicine, Houston, Tx.; Dept. of Physiology, University College London, England; Dept. of Zoology, University of California, Davis, Ca.

Photoreceptors are considered to be univariant if their voltage responses depend on the number of photons absorbed, and not on the wavelength (Naka & Rushton, J. Physiol. 185:556,1966). Receptors in the intact retina showed deviations from univariance (e.g. Fourtes, Schwartz & Simon, J. Physiol. 234:199,1973). In this paper, we report experiments which re-examine these deviations and determine whether they can be explained by photoreceptor coupling.

Light responses from rods, single cones and double cones isolated from the tiger salamander retina were recorded. Stimuli of various wavelength were adjusted in intensity until they produced voltage responses of equal peak amplitudes. Results obtained demonstrated that univariance is obeyed by these isolated photoreceptors.

Receptors in the intact retina, however, showed significant deviations from univariance: (1) Green-sensitive rods: For low response amplitudes, 700nm light gave a faster response onset than 520nm light. For large response amplitudes, 520nm light gave a faster response onset than 700nm light, and at the termination of a 1sec light step 700nm light produced a transient depolarization which 520nm light did not. (2) Single and double cones: For low response amplitudes, 520nm light gave a faster response onset than 700nm light. For all response amplitudes, after a 1sec step of 520nm light the response of cones showed a long hyperpolarized tail which was not produced by 700nm light.

Green-sensitive rods have a peak spectral sensitivity around 520nm, single and double cones have theirs around 620nm. Using a quantitative model of the photoreceptor network described in the previous presentation (Wilson, Attwell & Wu, Neurosciences abstract 1983), we found from computation that most of these deviations can be explained by electrical coupling between rods and cones.

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202.3 INTER-PHOTORECEPTOR CONTACTS IN THE TURTLE RETINA: COLOR CODING. Jill Jones* and Helga Kolb. Department of Physiology, University of Utah, Salt Lake City, Utah 84108.

Synaptic contacts between photoreceptors in the OPL of the turtle retina have been studied by light and electron microscopy. Neighboring cone pedicles, and rod and cone pedicles make a combination of narrow and wide gap junctions at apposed surfaces (Lasansky, 1971). Photoreceptor telodendria end or give off en passant small projections that become central elements in cone invaginations but the contact may only be with the horizontal cell lateral element (Lasansky, 1971). Telodendria also swell into large vesicle filled profiles that lie in the neuropil of the OPL or enter photoreceptor synaptic complexes, where they engage in wide cleft junctions with photoreceptors and occasionally with bipolar cell dendrites.

Light microscopic examination of Golgi stained photoreceptors indicate some cone specific connections of their telodendria. Blue single cones go to green single cones, red members of the double cones and red single cones. Both green and red single cones project to neighboring red single cones, green single cones and both members of the double cones but each appears to send more projections to its own color type. Green and red members of the double cone have extensive short projections to each other. The green member also has telodendria to red, green and blue single cones and to both members of other double cones, while the red member goes to a green single cone type. Rods have very long ranging and multibranched telodendria that project to all the cone types but not to other rods. However, their wandering telodendria could be making gap junctions with other rod telodendria in the neuropil of the OPL. Further electron microscopy of the Golgi stained photoreceptors may elucidate the exact nature of these photoreceptor contacts.

202.4 GAIN, ABSOLUTE SENSITIVITY AND SIGNAL/NOISE IN BUFO MARINUS RETINA. D. R. Copenhagen and T. Reuter*, University of California, San Francisco, CA 94143 and University of Helsinki, SF-00100, Helsinki 10, Finland.

Responses to flashes and steps of light were recorded intracellularly from rods, horizontal cells and spiking cells and extracellularly from ganglion cells. All recordings were made under identical conditions in dark-adapted eyecups. Maximum flash sensitivities (response amplitude [mV]/photoisomerization per rod [Rh*]) were found to be 0.9 mV/Rh* in rods and 6.5 mV/Rh* in horizontal cells. Flash sensitivities in the rarely encountered bipolar cells (n = 4) appeared to exceed those of the horizontal cells by 1.5 to 2 times, while spiking cells exhibited flash sensitivities up to 100 mV/Rh*. Thus, rod signals are amplified as they are transmitted through the retina. The overall amplification exceeds that at the rod to second order neuron synapses, indicating that the gain is distributed sequentially at synapses along the rod pathway.

Threshold responses in ganglion cells could be elicited with stimuli producing 0.006 to 0.02 Rh*. At these flash levels, the average hyperpolarization in each rod ranged from ~ 5 to 20 μV.

Analysis of signal/noise ratios at absolute threshold, which considered integration times and receptive field sizes of each type of neuron, indicated that detection of light is limited primarily by spontaneous thermal isomerizations of rhodopsin molecules. Thus, noise processes associated with the random action of synaptic transmitters degrade detection minimally. Furthermore, the enhancement of signal/noise, which enables ganglion cells to reliably detect threshold stimuli, can be accounted for on the basis of simple spatial summation in which the noise generated in rods is summed as $\sqrt{n}$ and light signals as n.

202.5 TEMPORAL TRANSFER PROPERTIES OF INTRARETINAL PATHWAYS IN TURTLE: EFFECTS OF TEMPERATURE, SPOT SIZE, AND INTENSITY. A.R. Adolph. Eye Research Institute, Boston, MA

Horizontal cell(HC) response linearity and transfer properties are a function of stimulus diameter and intensity, as well as temperature. At a fixed temperature and stimulus intensity, transfer function low-pass cut-off frequency or low-frequency peak breadth decrease with decreasing spot size. With fixed temperature and stimulus size, nonlinearity(the number and amplitudes of second-order distortion products) decreases as a function of light intensity; with fixed temperature and light intensity, nonlinearity decreases with stimulus diameter. For light intensities which do not saturate the response dynamic range, nonlinearity is negligible. Dopamine(DA) modifies HC transfer properties and flash responses. Flash response to a large spot, after DA, resembles the pre-DA response of the same HC to a small spot. An HC having a smooth, broadly peaked low-pass transfer function to a 5mm spot, and a monotonic low-pass transfer function to a 500u spot, before DA, has a monotonic low-pass transfer function to the large spot, after DA. Large spot flash response, after DA, resembles control small spot response. These effects of DA may be due to its uncoupling action on HC intercellular junctions. Flash response of a hyperpolarizing bipolar cell(HPBC), at a fixed temperature to a 1mm spot is larger than its response to a 5mm spot (less surround inhibition). There is a slight decrease in transfer function low-pass cut-off frequency for the smaller spot compared to the larger spot. For constant spot size, flash response exhibits slower on-and off-set as temperature decreases. Temporal characteristics of the flash response of a sustained amacrine cell are temperature dependent: onset, offset, and amplitude of full-field flash response slow and decrease as temperature decreases. Transfer function is also temperature dependent: The broadly peaked, low-pass shape changes to a monotonic, low-pass shape as temperature is reduced. Generally, cells having relatively sustained flash responses(receptors, horizontals, bipolars, sustained amacrines) differ in their transfer properties from cells like transient amacrine or ganglion cells. Transfer functions of "sustained-type" cells are narrowly peaked at low frequencies with monotonic, low-pass, high-frequency shapes. In contrast, transfer functions of "transient-type" cells are attenuated at low-frequencies, with broad, mid-range peak and monotonic low-pass, high-frequency shape.

202.6 COMPUTER MODEL OF CONE PATHWAYS IN FROG'S RETINA. Y. Lee* and M. Arbib. COINS Dept. and the Center for Systems Neuroscience, Univ. of Mass., Amherst, MA 01003.

There is a rich set of physiological and anatomical data that describes the functions and the underlying structures of the vertebrate retina. But our partial understanding of the neural mechanisms of the retina, together with the differences in the species, levels and methodologies make it almost impossible to integrate essential data into a self-consistent framework. Choosing a species to model cuts down much of the diversities and incompatibilities of the available data. The frog retina is chosen because of the complexity in its response characteristics among other reasons.

Cone pathways were chosen to be modelled first to reduce the initial modelling efforts without ignoring the salient features of the output characteristics of the frog's retina. A computer model at the cellular level can provide a coherent and, eventually, comprehensive conceptual framework to work/think with. The retina is viewed as multiple layers of computing elements which process local visual information in a parallel manner. Overall synaptic organization of the retina follows that of Dowling (1976), where a spatial analysis of visual input is initially performed in the outer plexiform layer and a temporal analysis is added in the inner plexiform layer. Major portions of light/dark adaptations are carried out at the photoreceptor level in the model. Cell densities, e.g. 36 single cones per degree^2 at the photoreceptor level, as well as receptive field (RF) sizes are represented following the anatomical data. The center/surround RFs are represented as a 2-dimensional difference of Gaussians. To explain the ethological data quantitatively, the blurring of the retinal image caused by the frog's optical apparatus is included in the model as well as the explicit relationship between the stimulus size, distance and the image size.

Initial results show the simulation of the model agrees qualitatively with the well-known frog's retinal ganglion cell responses.

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202.7 A MODEL OF THE CAT RETINAL AII AMACRINE CELL: ELECTROTONIC RECTIFICATION VIA A LOBULAR APPENDAGE. J.K. Stevens, J.R. Jacobs Playfair Neuroscience Unit, Toronto Western Hospital, University of Toronto, Toronto CANADA.

The AII amacrine cell of the cat retina likely conveys rod signals to ganglion cells (Nelson, J. Neurophys.47:928-947 1982). The narrow (40-60 um dia.) dendritic field of the AII arborises in sublamina b of the IPL, and receives synaptic inputs from rod bipolars and makes gap junctions with invaginating cone bipolars. A unique feature of this neuron is a number of lobular appendages connecting with the soma or proximal dendritic tree through a 1 to 3 micron long neck, as little as 0.2 microns in diameter, and terminating in a swelling 1 to 6 microns in diameter. Lobular appendages are limited to sublamina a of the IPL, receiving inputs from other amacrines and contacting cone bipolars and ganglion cells (Famiglietti and Kolb, Br. Res.84:293-300 1975).

Three complete reconstructions of AII amacrine cells were made from series of electron micrographs of ultra-thin, transversely sectioned cat retina, using a computer assisted reconstruction system (Stevens, Br Res Rev2:265-293 1980). Quantitative data of cell morphology obtained from these reconstructions were used to create a steady state electrotonic model of AII amacrine response to synaptic inputs. With sustained synaptic inputs on the lobular appendage, the membrane potential saturates to local maximum; however, the thin neck minimizes the potential change in the soma or the dendritic tree. In contrast, inputs to the dendrites or to the soma create large changes within the soma and dendrites as well as in the lobular appendage. Thus, the lobular appendage neck effectively acts as a rectifer allowing signals to pass from soma to the lobular appendage, but attenuating signals passing from the lobular appendage to the soma. (Supported by MRC of Canada grant MA7345 to JKS.).

202.8 ON GRATING RESPONSES OF VISUAL CELL SUBUNITS INTERACTING BY MULTIPLICATIVE LATERAL INHIBITION. R,B,Pinter Departments of Electrical Engineering and Zoology,University of Washington,Seattle,98195.

The ability of a visual cell to discriminate between narrow and wide field stimuli can be explained on the basis of nonlinear multiplicative lateral inhibition (Pinter,J.theor.Biol.100,525;1983). Further, the numerical values and distribution of this lateral inhibition are not critical to this and other nonlinear response properties,such as latency shift with mean illumination shift.

The nonlinear pathway of Y retinal ganglion cells consists of many nonlinear subunits(Victor and Shapley,1979,J.Gen.Physiol.74, 671)of rectifying character,possibly bipolar cells.Koch et al. (1982,Phil.Trans.R.Soc.Lond.B 298,227)showed the possibility of nearly independent electrical subunits of Y cells. It was suggested that the ganglion cell's activity observed in soma potential and spike rate might reflect a linear summation of the electrical subunits' activity.That the nonlinear subunits' activity might be the input to the electrical subunits is a reasonable possibility. This would explain the adding up of subunit activity for the Y cell.

The rectifying character of Y cell response arises not necessarily from a mathematically specific but cellularly unspecified rectifier,but possibly from multiplicative lateral inhibition between subunit cells.This interaction between subunit cells in the Y cell receptive field clearly explains the difference in relative amounts of fundamental and second harmonic response for the temporally modulated stationary bar and peak spatial phase grating versus the null spatial phase grating.This is simply a result of the multiplicative lateral inhibition system's fundamental response polarity dependence on polarity of the input fundamental,and the second harmonic response polarity independence.Thus an array of closely spaced subunits stimulated by a centered null phase stationary grating possesses equal amounts of positive and negative fundamental frequency response, and these add up to zero in the linear summation of subunit activity.The summation of second harmonic and DC component responses is unaffected by polarity of the input fundamental frequency, and therefore remains constant with respect to stationary grating position, or bar versus null grating stimulation.The dependence of second harmonic response on contrast, and the independence of DC response on contrast, are also explained by multiplicative lateral inhibition.

A distinctly different problem is presented by the response of Y cells to drifting gratings. There is not as much second harmonic response as for stationary gratings, but low spatial frequencies and moderate temporal frequencies of the drifting grating cause large second harmonics. This property is also demonstrated by multiplicative lateral inhibition.

202.9 LINEAR AND NONLINEAR RESPONSES OF BRISK-TRANSIENT (Y) GANGLION CELLS OF THE CAT RETINA VARY WITH GRATING ORIENTATION IN DIFFERENT WAYS. L.N. Thibos* and W.R. Levick. Physiology Dept., J.C.S.M.R., Australian National University, Canberra, AUSTRALIA 2601.

Y-cells display two types of behaviour, linear and nonlinear, which can be isolated for study by a low-frequency drifting grating and by a high-frequency alternating grating, respectively. Previous experiments have revealed that responses of brisk-transient Y-cells to drifting gratings often depend strongly upon stimulus orientation. We asked whether the same might also be true for alternating gratings and, if so, whether the characteristics of such orientation bias are the same or different from those found for drifting gratings.

Data was collected from 5 adult cats anaesthetized for surgical procedures with 1-2% halothane and maintained during data collection on 70:28.5:1.5 mixture of nitrous oxide, oxygen and carbon dioxide. The gas mixture was supplemented during inactive stages of the experiment by 30 mg/kg.hr of urethane. Eye movements were controlled by intravenous infusion of neuromuscular blocking agents and the animal was artificially ventilated. Extracellular recordings were made from the cell bodies of cells with tungsten-in-glass microelectrodes. Experimental procedure was to collect histograms of nerve action potentials for gratings of fixed contrast (50%), mean luminance (82 cd/m^2) and temporal frequency of modulation (2 Hz). Grating orientation and spatial frequency were the stimulus variables. Histograms were subjected to Fourier analysis to determine the magnitude of responses.

Some cells were found to have a significant degree of orientation bias for both drifting and alternating gratings. For these cells the preferred orientations for the two types of grating stimulus tended to be the same but substantial differences were frequently observed. Other cells were biassed for only one or other of these stimuli and still other cells were not biassed for either stimulus.

Orientation bias for drifting gratings has been shown previously to have a radial pattern of symmetry which is centred about the area centralis. A systematic preference for radially-oriented gratings was evident also for alternating gratings in present experiments. Furthermore, there was an additional preference for circumferentially-oriented gratings when the stimulus was alternating.

Results indicate that the axes of symmetry of the receptive field components responsible for linear and nonlinear behaviour in brisk-transient Y-cells are not necessarily the same.

202.10 MODIFIED KELLY'S MODEL FOR HUMAN VISUAL SYSTEM PREDICTS RESPONSES FROM TURTLE HORIZONTAL CELLS. Richard L.Chappell, Masanori Sakuranaga* and Ken-Ichi Naka. Hunter College of the City University of New York, New York, NY 10021, USA; Nippon Medical School, Bunkyoku, Tokyo 113, Japan; and National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan.

Recordings were made from horizontal cells in the retina of the red-eared turtle, Pseudemys scripta elegans. The stimulus used was a white-noise modulated (white) light whose mean (and the depth of modulation in proportion) was varied by interposing neutral density filters between the light source and the retina. Power spectra were computed from the responses produced by white-noise stimuli of various mean irradiances. Kelly (1971. J. Opt. Soc. Amer. 61:537) described human photopic flicker data in terms of a theoretical model, a diffusion process followed by an inhibition stage. Human photopic flicker data (the incremental threshold function) and turtle horizontal cell response shared the following features: 1) response power spectra or photic flicker threshold function was constant-gain lowpass at low mean but became bandpass at high mean, 2) sensitivity decreased roughly in Weber-Fechner fashion, and 3) the power spectra measured at various means had a single asymptote. Kelly's model was modified into a diffusion stage with a negative feedback stage to reflect functional structure of the retina. The modified model predicted the impulse response (first order kernel) from turtle horizontal cells, as well as Kelly's original human data. As the turtle horizontal cells respond linearly over a large range of potential excursion (more than 20 mV), Kelly's diffusion model is an adequate description of the turtle horizontal cell's dynamic response.

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SYNAPTOGENESIS I

203.1 DEVELOPMENT OF ACTIVE ZONES AT NEUROMUSCULAR JUNCTIONS IN THE TADPOLE. C.-P. Ko. Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

The active zone is a unique specialization at the presynaptic membrane and is believed to be the site of transmitter release. To study the development of this important structure, cutaneous pectoris muscles in the tadpole and adult bullfrog (Rana catesbeiana) are freeze-fractured. As seen in Rana pipiens, the active zones in the adult bullfrog have two double rows of large intramembrane particles, which are located perpendicular to the long axis of the nerve terminal and precisely aligned with junctional folds. During early development in the tadpole, active zone particles are observed before junctional folds can be identified. Most of these particles have not yet organized and are scattered all over the terminal membrane. Some particles are beginning to show the adult organization of two double rows, but they are often short and randomly oriented and located. This process differs from regenerating terminals in the adult, where even the early clusters of active zone particles are found only opposite to junctional folds. As development progresses in the tadpole, junctional folds are seen and just opposite to them are active zones with normal organization and orientation.

Cross-fracture shows that developing nerve terminals with scattered active zone particles already contain synaptic vesicles. When the nerve is stimulated in dilute fixative, dimples, which may represent openings of synaptic vesicles, are seen at developing active zones regardless of their stage of organization.

At the premetamorphic stages, some junctions have two or more nerve endings lying side by side in the same junctional gutter. The orientation and location of developing active zones at these multiple terminals are also associated with the appearance of junctional folds. After metamorphosis, the incidence of multiple innervation decreases but is still observed in young adults. Freeze-fracture of a small bullfrog (body length 2 in.) shows that some junctions still have two terminals in the same gutter. One is large and has mature active zones. The other is small and has short or disorganized active zones. It is possible that this small terminal is in the process of being eliminated.

This study suggests that the localization of developing active zones may relate to the maturation of junctional folds. Also, developing nerve terminals, are already functional before active zone particles organize into the adult pattern. Furthermore, this preparation provides an opportunity to study changes at active zones and other intramembrane structures during synapse elimination.

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203.2 ULTRASTRUCTURAL SPECIALIZATIONS AT NEWLY FORMED ACETYLCHOLINE RECEPTOR AGGREGATES ON CULTURED MYOTUBES. A. Olek*, A. Ling*, and M.P. Daniels. Laboratory of Biochemical Genetics, NHLBI, N.I.H., Bethesda, MD. 20205

Ultrastructural specializations have been described at regions of high acetylcholine receptor (AChR) site density at the neuromuscular junction and on cultured myotubes. To help understand the formation and function of these specializations, we have examined the ultrastructure of identified myotube regions in cultures fixed during the initial formation of AChR aggregates. Using video image intensification, the distribution of tetramethyl rhodamine-α-bungarotoxin labelled AChR was observed sequentially from 0-6h after the addition of embryonic brain extract (EBX). AChR aggregates at various stages of development were mapped for later examination of thin sections with the electron microscope. In some experiments the precision of mapping was confirmed using horseradish peroxidase-α-bungarotoxin as a marker at the ultrastructural level. Distinct specializations were present within mapped AChR aggregate sites by 4-6h after addition of EBX. They were characterized by the enriched presence of basal lamina and a specialized cytoplasmic structure, within 50-100 nm of the plasma membrane, which largely excluded membranous organelles and myofibrils. The submembrane structure was composed of filamentous (4-10 nm) and particulate material and was most densely packed between 20-50 nm from the sarcolemma, leaving a relatively electron lucent zone immediately under the membrane. The cell surface in these regions was frequently scalloped and sometimes deeply folded.

The proportion of the plasma membrane subtended by this distinct cytoplasmic structure was estimated in random sections from control myotubes and myotubes treated 4h with EBX. In treated cells~7% of the top half of the myotube membrane was subtended by specialized structure, and the mean length of a specialized region was 0.9 μm. In control cells~3% of the top cell membrane was so occupied, and the mean length of a specialized region was 0.2 μm. These values agree reasonably well with the increase (from~1-10%) in the area occupied by AChR aggregates on the myotube surface found after 4h exposure to EBX.

The results indicate that distinct ultrastructural specializations are associated with newly formed AChR aggregates and are assembled at least in part during the early formation of the aggregates.

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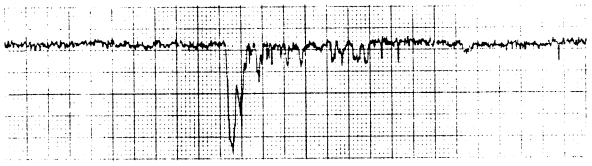
203.3 DIFFERENTIATION OF POSTSYNATPIC SPECIALIZATIONS ON EMBRYONIC MUSCLE CELLS DEVELOPING IN VIVO WITHOUT MOTOR INNERVATION. G.S. Sohal and R.W. Wrenn*. Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.

The postsynaptic specializations at the mature vertebrate neuromuscular junction include synaptic folds, postsynaptic densities, basal lamina, clusters of acetylcholine receptors and acetylcholinesterase. The mechanism by which postsynaptic specializations develop during the course of embryonic development is unknown. The present study tested the hypothesis that interaction of developing motor nerve fibers with the muscle cells triggers development of postsynaptic specializations on muscle cells developing in vivo. Superior oblique muscle of the eye in duck embryos was used as a convenient model to determine if postsynaptic specializations characteristic of mature neuromuscular junctions develop in the absence of motor innervation. Superior oblique muscle was made aneural by permanent destruction of trochlear motor neurons in the midbrain. A relatively large area of the dorsal midbrain was cauterized on embryonic day 7 which is three days before the trochlear nerve fibers normally project their axons into the muscle. Absence of innervation was verified with light and electron microscopy of the muscle. Electron microscopy revealed that synaptic folds, postsynaptic densities, basal lamina and associated acetylcholinesterase developed in the absence of motor innervation. Acetylcholinesterase reaction product was often associated with the synaptic folds and basal lamina-like material. Biochemical assay indicated that the high molecular weight form of acetylcholinesterase appeared on muscle cells developing without innervation. $I^{125}\alpha$-bungarotoxin autoradiography indicated that clusters of acetylcholine receptors appeared in aneural muscle. These observations indicate that the presence of motor nerve fibers is not necessary for the differentiation of postsynaptic elements of the neuromuscular junction. Thus, the mechanism for the differentiation of the postsynaptic specializations must be preprogrammed in the developing muscle cell.

203.4 SPONTANEOUS RELEASE OF ACETYLCHOLINE FROM GROWTH CONES OF EMBRYONIC NEURITES. S. H. Young and M-m. Poo, Department of Physiology and Biophysics, Univ. of Calif., Irvine, CA 92717.

A patch of outside-out embryonic Xenopus muscle membrane formed with gigohm seal at the tip of a micropipette was used as a probe for the presence of channel-inducing substances. Spontaneous single channel events appeared when the probe was positioned near the growth cones of 66% of Xenopus embryonic neurons in culture (total neurites tested 15). No activity was detected along the neurites or near the soma of any of the neurons. Most of the single channel events have the characteristics of acetylcholine (ACh) induced channels in mean open time, single channel conductance, reversal potential, as well as susceptibility to blockage by α-bungarotoxin. This suggests that these neurites release ACh from growth cones. The release of ACh occurred in bursts. Some bursts were characterised by rapid rise of multiple channel current followed by slow decline in number of active channels in a "staircase" pattern (see figure). Prolonged release activity was found to be composed of a sequence of staircase bursts. In addition, activation of other as yet un-identified channels were also observed as the membrane patch approached the growth cone. Release of ACh and other substances from growth cones may occur as a by-product of new membrane incorporation during the process of neurite extension; it may also be used by the growth cone to signal its presence to the environment. This work is supported by grants from NIH (NS-17558-01A1) and NSF (BNS 80-12348). S.H.Y. is supported by a fellowship from Muscular Dystrophy Association of America.



203.5 RAPID INDUCTION OF ACETYLCHOLINE RELEASE FROM THE NEURONAL SOMA BY MUSCLE CONTACT. I. Chow and M-m. Poo. Dept. of Physiology and Biophysics, University of California, Irvine, CA 92717.

Spherical muscle cells were manipulated into contact with the soma of neurons in 2-day old Xenopus co-cultures, and changes in the muscle cell membrane potential were monitored by using intracellular microelectrode. Miniature acetylcholine potentials (MAPs) were detected within 10 min of contact in 55% of the soma-muscle cell pairs (N=65). An additional 10% of the cell pairs showed MAPs in the subsequent 20 min of contact. The average MAP amplitude in these cell pairs was 3.8 ± 0.2 mV (± SE) and the average frequency was 0.06 ± 0.02 events/s (± SE), whereas the average miniature end plate potential (MEPP) amplitude at already established neurite-muscle junctions was 6.1 ±1.0 mV (± SE) and the frequency was 0.35 ± 0.05 events/s (± SE). The average MAP rise time in the induced soma-muscle cell pairs, 9.7 ± 0.6 ms (± SE), was longer than the MEPP rise time found at the already established junctions, 4.7 ± 0.4 ms (± SE). No MAPs were detected when α-bungarotoxin pretreated muscle cells were pushed into contact with identified cholinergic neuronal somas. Interestingly, no MAPs were detected within 30 min of contact between a muscle cell and the soma of a neuron which had already established functional contact with other muscle cells (10 cell pairs tested). These results indicate that neuronal soma can be induced to release acetylcholine by direct contact with a muscle cell. Furthermore, the prior functional contact with other muscle cells apparently led to cellular modulation of the neuron which prevented this muscle cell-induced acetylcholine release from the soma.

This work is supported by grants from NIH (NS-17558-01A1) and NSF (BNS 80-12348).

203.6 NON-QUANTAL RELEASE OF ACETYLCHOLINE AT EMBRYONIC NEUROMUSCULAR SYNAPSES IN CULTURE. Y-a. Sun* and M-m. Poo. Dept. of Physiology and Biophysics, Uni. of Calif. Irvine, CA. 92717

Local application of tubocurarine (1.5 mM) at neuromuscular synapses in Xenopus embryonic nerve-muscle culture resulted in a transient hyperpolarization of muscle membrane potential. Miniature endplate potentials (MEPPs) were abolished during the hyperpolarization and recovered after the return of resting membrane potential. The average peak amplitude of hyperpolarization was 5.8 ± 0.7 mV (N=19, S.E.). The peak amplitude was independent of the frequency of MEPPs, and decreased when curare was applied at regions away from the synapse. Prolonged application of curare led to sustained hyperpolarization of the membrane potential. Ejection of recording medium with similar pressure was without effect. Local ejection of curare on non-innervated muscle cells resulted in a transient depolarization of the membrane potential, consistent with the previous reports of agonist action of curare in embryonic muscle cells. Bi-phasic response (depolarization followed by hyperpolarization) was observed in newly-formed synapses upon local curare application, suggesting a transition of curare action on muscle cells from that of an agonist to an antagonist after innervation. Taken together, our results indicate that innervated embryonic muscle cells were maintained at a depolarized state relative to that of a non-innervated muscle cell by a steady spontaneous release of acetylcholine (ACh) from the nerve terminal. We estimated that the total amount of ACh released in this non-quantal mode exceeds that of the quantal release underlying the normal spontaneous MEPPs by about 100-fold. This work was supported by a grant from NIH (NS-17558-01A1).

203.7 RELATIONSHIP BETWEEN SYNAPTIC VESICLES AND MINIATURE ENDPLATE POTENTIALS IN ADULT AND NEONATAL MOUSE DIAPHRAGM. R. Hanna* and M.E. Kriebel*, (Spon: J. Frank), CESF and Upstate Medical Center, SUNY, Syracuse, NY 13210.

Miniature endplate potential (MEPP) amplitude distributions of adult mouse diaphragm junctions show two classes. The larger MEPPs form a bell-shaped distribution (bell-MEPPs or classical MEPPs) with a variance of 20-30%. The smaller MEPPs form a right-hand skew-distribution composing 1-10% of the MEPPs with a mode 1/10th (s-MEPP class) that of the bell-MEPPs. One day old junctions generate mainly s-MEPPs and the MEPP distribution gradually changes to mainly bell-MEPPs during the first 3 weeks of post-natal development. The purpose of this study was to compare the synaptic vesicles and synaptic clefts of one to three-day-old junctions to those of adults. We found no differences between neonates and adults in regards to synaptic vesicle diameter (52 nm O.D.) distributions nor in synaptic cleft widths. Synaptic vesicle diameters were similar through the terminal. Day old junctions showed no or shallow postsynaptic folds and fewer synaptic vesicles. Junctions were essentially developed by the 3rd day although one-quarter of the MEPPs were of the skew-class. Therefore, skew-MEPPs must originate from synaptic vesicles of the same size as those generating bell-MEPPs because the diameter of a vesicle required to generate s-MEPPs would only be 30 nm O.D. (assuming that vesicles are simple containers). We found that distributions of adult synaptic vesicle volumes show a marked right-hand skew. Assuming that vesicles contain free ACh and that there is a simple relationship between ACh released and MEPP amplitude, the distribution of vesicle volumes would not generate the observed bell-shaped distribution of MEPPs but a right-hand skew distribution. Moreover, the wide dispersion of vesicle volumes is much greater than the standard deviation of bell-MEPP amplitudes. Since the distribution of MEPPs does not match distributions of vesicles, we conclude that within the constraints of the vesicle hypothesis of transmitter release that skew- and bell-MEPPs are from the same vesicles and that vesicle volume does not determine the amount of ACh released.

203.8 **APPEARANCE OF ACETYLCHOLINE CHEMOSENSITIVITY DURING SYNAPTOGENESIS IN FROG SYMPATHETIC GANGLIA.** **P.M. Dunn* and L.M. Marshall* (SPON: J.D. Greenspan) Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27514.**

In the frog, synapse formation in the lumbar sympathetic ganglia occurs during the late metamorphic stages of larval development (Marshall, Soc. Neurosci. Abstr. 8:866, 1982). We have now studied the development of nicotinic acetylcholine sensitivity in relation to the functional innervation of the sympathetic ganglia of the bullfrog (Rana catesbiana) in vivo.

Intracellular recordings were made from principal neurons in the 9th and 10th paravertebral ganglia of adult and larval (stages XV - XXV; Taylor & Kollros, Anat. Rec. 94:7, 1946) frogs. Recordings from developing neurons were quite stable, with resting potentials (range -40 to -90 mV) equal to or greater than those of adult neurons. The level of synaptic development was assessed from the response to preganglionic nerve stimulation or the presence of spontaneous miniature potentials. Chemosensitivity was examined by recording the response to iontophoretic application of acetylcholine from a micropipette 10 μm above the cell surface (in the presence of 0.5 μM atropine).

At the earliest stages studied (XV - XVII), about 10% of the neurons showed no response to preganglionic nerve stimulation and were insensitive to applied acetylcholine. By the completion of metamorphosis (stage XXV) all neurons examined were functionally innervated.

These results suggest that in frog sympathetic ganglia, nicotinic acetylcholine sensitivity is not acquired until the onset of synaptic transmission. Experiments are now in progress using light and electron microscopy to correlate the morphological development of synaptic contacts with the appearance of acetylcholine sensitivity.

(Supported by NIH grant NS 17203)

203.9 INNERVATION OF MYOFIBERS BY CHICK CILIARY GANGLION NEURONS AND RAT PC12 PHEOCHROMOCYTOMA CELLS IN CULTURE. J.C. Norris, N.R. Ways* and B.G. Wallace, Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

We have compared the interaction of various cholinergic neurons with myofibers in culture with the aim of determining if all cholinergic cells make similar synaptic contacts. Embryonic chick ciliary ganglion neurons and nerve growth factor primed rat PC12 pheochromocytoma cells were dissociated mechanically and plated onto 5 day old cultures of myotubes derived from dissociated embryonic chick skeletal muscles. Intracellular recordings were made from the myotubes after one or two days of coculture and the frequency, amplitude, and time course of synaptic potentials were monitored. Both cell types gave rise to depolarizing synaptic potentials which were abolished by curare or α-bungarotoxin. The synaptic potentials evoked in the myotubes by ciliary ganglion cells varied in amplitude from 0.15 to 12.4 mV and from 2 to 109 msec in time to peak. Similarly, those arising from PC12 cells ranged from 0.17 to 12.7 mV and 3 to 173 msec rise time. The distribution of amplitudes for the two cell types was similar, the mean +/- SEM amplitude of the synaptic potentials was 1.2 +/- 0.04 mV (N=1058) for ciliary ganglion neurons and 1.4 +/- 0.04 mV (N=1235) for PC12 cells. The distribution of rise times of the synaptic potentials evoked by each cell type was unimodal and skewed, with rapidly rising events being more frequent. However, synaptic potentials evoked by PC12 cells were slower and had a broader distribution of times to peak. For potentials arising from ciliary ganglion neurons the mode of the distribution was 3.5 msec, for potentials evoked by PC12 cells the mode was 12.5 msec. Thus, although both PC12 cells and ciliary ganglion neurons gave rise to depolarizing synaptic potentials in chick myotubes, the longer time to peak for PC12 evoked potentials suggests that these two types of neurons differ in the time course of acetylcholine release or in the way in which their neurites interact with myotubes in culture.

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203.10 DEVELOPMENT OF MUSCARINIC RECEPTOR BINDING IN THE RAT OLFACTORY BULB M.A. Gremillion, T.H. Large and W.L. Klein Dept. of Neurobio. and Physio. Northwestern Univ. Evanston, Ill 60201

Rat olfactory bulb has no intrinsic cholinergic neurons but receives extensive cholinergic innervation from the horizontal nucleus of the diagonal band soon after birth. This system provides us with a simple, isolatable model of cholinergic innervation and synaptogenesis. Appearance of muscarinic receptors (MAChRs) and development of binding properties were studied in three developmental ages of rat; embryonic, neonatal and adult.

MAChRs were assayed with 3H-Scopolamine; maximum specific binding in the adult was 1647 fm/mg membrane protein with a dissociation constant of .49 nM. Carbachol competition against 3H-NMS reflected complex binding typical of the MAChR in other tissues. The IC50 of carbachol blockade was approximately 300 uM and a Hill coefficient of .31. Complex agonist binding may indicate the presence of either separate classes of receptor or could result from receptor coupling to effector proteins. GppNp, an analog of GTP which uncouples the receptor from adenylate cyclase, did not drastically affect the shape or affinity of the carbachol competition curve, primarily reducing the ability of carbachol to bind at low doses. In preliminary experiments, carbachol binding to solubilized receptors had a Hill coefficient of .68. This result suggests that separate classes of receptors may be present in the olfactory bulb or alternatively, the receptor is tightly coupled to effector proteins following solubilization. Binding to solubilized receptors was unchanged by GppNp. MAChR levels in neonate bulb were 237 fm/mg membrane protein. Carbachol competition curve in neonates was essentially identical to adult with a Hill of .38. MAChR were present in the embryonic bulb as early as day 19, well before the onset of cholinergic innervation and synaptogenesis. Specific binding was 192 fm/mg membrane protein; carbachol competition was identical to adult. The results suggest that while MAChR levels develop in parallel with cholinergic innervation and synapse formation, MAChR binding properties develop much earlier.

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203.11 REGULATION OF SYNAPTIC DEVELOPMENT: A ROLE FOR INSULIN. D.G. Puro and E. Agardh*. LAB. of VISION RESEARCH, NATIONAL EYE INSTITUTE, NIH, BETHESDA, MD 20205.

Certain regions of the brain, including the retina, contain receptors for insulin (Havrankova et al., 1978). However, the function of these insulin receptors is unclear. One hypothesis is that insulin may have a role in neural development. To help test this hypothesis, we used a cell culture system to examine the effect of insulin on the developmental step in which a presynaptic neuron acquires the ability to transmit excitatory information across a synapse.

Previously, we have shown that cholinergic neurons dissociated from the perinatal rat form functional synapses in culture with rat striated muscle cells. Myotubes are useful as postsynaptic targets since their membranes have areas with a high density of cholinergic receptors and because their response to acetylcholine has been studied both in vivo and in culture. Also, their relatively large size permits prolonged intracellular monitoring of postsynaptic responses.

This retina-muscle cell culture system is well suited for studies of the regulation of specific steps in the development of a synapse. Early in the functional maturation of retina-muscle synapses, there is a period in which the release of acetylcholine occurs spontaneously, but cannot be evoked by excitatory stimulation. This non-transmitting stage is followed by the emergence of transmitter release that can be stimulus-evoked. Here, we report that insulin precociously induced the onset of evocable transmission at retina-muscle synapses. This effect of insulin was dependent on concentration. The half-maximally effective concentration for bovine insulin was 1 nM. A study of the time-course for this insulin-induced effect revealed a 2 to 4 hr interval between the onset of hormonal exposure and the induction of evocable synaptic transmission. The effect of insulin on synaptic transmission was long lasting. Evocable acetylcholine release could be detected for at least 30 hr after a 6 hr pulse of insulin. Experiments have demonstrated that only the retinal cells, not the myotubes, need to be exposed to insulin in order for this precocious induction of synaptic maturation to occur. Other findings suggest that this hormonal induction involves the maturation of mechanisms linking neuronal depolarization with transmitter release.

The results indicate that insulin can regulate the timing of the developmental step in which cholinergic neurons of the rat retina become capable of releasing acetylcholine at synapses in response to excitatory stimulation.

203.12 ENHANCED SURVIVAL OF APPARENT PRESYNAPTIC ELEMENTS BY AN ANTI-MITOTIC DRUG. Richard W. Burry, Department of Anatomy, College of Medicine, The Ohio State University, Columbus, Ohio 43210

In cell cultures of the rat cerebellar cortex neurites grow on polylysine coated beads form apparent presynaptic elements with the beads in the position of the postsynaptic element. The number of apparent presynaptic elements increases to 5 days incubation and then declines. Morphological observations suggest that the non-neuronal cells grow up onto the bead and phagocytose the apparent presynaptic elements (Burry, Brain Research, 247 (1982) 1-16). To test this idea, non-neuronal cell proliferation was inhibited with an antimitotic drug and survival of apparent presynaptic elements was measured.

Dispersed cell cultures of the rat cerebellum were exposed to $5X10^{-6}M$ cytosine arabinoside (Ara-C) from 5 to 7 days in vitro. Polylysine coated sepharose 4B beads were added at 7 days in vitro.

First, cultures were evaluated to determine the antimitotic drug affected the growth of neurites up onto the large sepharose beads. With a procedure using tetanus toxin, anti-tetanis toxin, and rhodamine labeled secondary anti-body, the neurites on the beads with observed in the fluorscence microscope. Quantitation of the neurites on the beads was done on micrographs focused on their largest diameter. It was found that the percent of labeled bead surface was similar in both non-treated and Ara-C treated cultures at incubations to 14 days. Thus the growth of neurites on the beads was not affected by the antimitotic drug.

Next cultures were evaluated to determine if the inhibition of non-neuronal cell proliferation sustained the apparent presynaptic elements. Cultures with or without Ara-C pretreatment were prepared for electron microscopy. The number of apparent presynaptic elements in the cultures with Ara-C declined from a high at 1 day incubation to a low value at 14 days incubation. The cultures treated with Ara-C showed high numbers of apparent presynaptic elements at all times through 14 days incubation. Thus Ara-C enhanced the survival of apparent presynaptic elements in cultures incubated with beads through 14 days.

These results suggest that the antimitotic drug Ara-C can enhance survival of apparent presynaptic elements on polylysine coated beads, but not affect the growth of neurites onto the polylysine coated beads. Thus the phagocytosis of apparent presynaptic elements by non-neuronal cells may be an explaination for the loss of apparent presynaptic elements.

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203.13 ULTRASTRUCTURE OF THE POSTSYNAPTIC MEMBRANE AND ASSOCIATED STRUCTURES IN DEVELOPING MOUSE OLFACTORY BULB. R.S. Cohen* and H.-M. Hwang* (SPON: L. Benevento). Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60612.

The prenatal development of axodendritic synapses in the glomerulus of mouse olfactory bulb using serial thin sections has been shown. Emphasis was placed on the development of the postsynaptic membrane, the postsynaptic density (PSD) and its associated cytoskeletal network. The fine structure of mouse olfactory bulb seen in the present study is in agreement with previous studies in adult (Pinching and Powell, J. Cell Sci., 9:347, 1971) and developing (Hinds and Hinds, J. Comp. Neurol. 169:41, 1976) animals. Synaptogenesis, in this system, can occur in either of two ways. One involves the formation of a symmetric phase of synapse where there is an equal amount of dense material on the pre- and postsynaptic membranes, followed by a final asymmetric phase, where there is a greater amount of dense material underneath the postsynaptic membrane. The second involves the formation of an unpaired postsynaptic density, i.e., dense material appears on a postsynaptic membrane which lacks its presynaptic partner. Also, in agreement with Hinds and Hinds (J. Neurocytol., 1:169, 1972), a dendritic cytoskeleton was found appearing in two forms, an irregular arrangement of microfilaments and a more regular arrangement, which in the present study, appeared as microfilament bundles. A close association between the cytoskeletal material immediately beneath the postsynaptic density and microfilament bundles was seen. Fine filamentous material seemed to connect the microfilament bundle to the dendritic growth cone membrane and to the postsyaptic density, thereby, representing possible attatchment sites of the microfilament bundle to the membrane and PSD. Based on serial thin section analysis a working model for the formation of spine-like processes, called spine-like branchlets in the olfactory bulb, is proposed. This model involves the irregular microfilament arrays immediately underneath the postsynaptic density, the microfilament bundles, and the association between them, as well as the association between the microfilament bundles and adjacent dendritic growth cone plasma membrane. The microfilament bundles may contract away from the membrane, and because of its apparent attatchment to the membrane, may cause the dendritic growth cone membrane to invaginate, thus representing an initial stage in the formation of a spine-like process. Supported by NIH grant NS 15889.

203.14 FREEZE-FRACTURE ANALYSIS OF THE POSTSYNAPTIC MEMBRANES OF AXODENDRITIC SYNAPSES IN ADULT AND PRENATAL MOUSE OLFACTORY BULB. H.-M. Hwang* and R.S. Cohen* (SPON: M. Rezak). Dept. of Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60612.

The fine structure of the postsynaptic membranes in adult and developing glomeruli of mouse olfactory bulb was revealed by freeze-fracture of this tissue. Many aggregations of particles, similar to those characterized as postsynaptic sites in other nervous tissues (Sandri et al., Prog. Brain Res., 46:1, 1977) appeared on fractured faces of the neurites. Aggregations of intramembranous particles (IMPs) appeared on the extracellular leaflet (E-face) of the postsynaptic processes, i.e., dendrites, rather than on the protoplasmic leaflet (P-face). These IMP aggregates have various sizes and shapes and contain different sizes of particles ranging in size from approximately less than 7 nm to larger than 20 nm. Some of the IMP aggregates appeared to have a particle-free area in the middle of the aggregate which may correspond to areas of postsynaptic membrane overlying perforations in postsynaptic densities (PSDs) seen in serial thin sections of these synapses. Prenatal dendritic processes showed small clusters or single rows of various sized particles. With increasing age, these aggregates increased in size and contained different sized particles, the two most prominent being smaller than 7 nm and between 7-11 nm. As development proceeded, the aggregates also became irregularly shaped and occasionally, a small perforation was seen; these may correspond to the perforations seen in adult IMP aggregates, but they were not detectable in serial thin sections of prenatal tissue. Computer analysis of the area of IMP aggregates and density of intramembrane particles, revealed that the overall IMP density increased with age. The density of one of the predominent particle sizes, 7-11 nm, remained unchanged throughout prenatal life; a similar density was also seen in the adult. Because of its constant density during prenatal life and in the adult, it is thought that this size particle may be involved in stabilization of initial synaptic contacts and of mature synapses, rather than represent receptors or ion channels which would be expected to increase during development. Particles smaller than 7 nm increased prenatally, decreased at birth, then increased in the adult. This size may represent two different molecules, one important in synaptogenesis, the other, an important component of adult synapses. Supported by NIH grant 15889.

203.15 PRIMORDIAL SYNAPTIC STRUCTURES IN OLFACTORY CORTEX OF EARLY POSTNATAL RATS. R.A.E. Bakay, D.D. Kunkel* and L.E. Westrum (Spon:R.W. Rodieck). Depts. of Neurological Surgery and Biological Structure, Univ. of Washington, Seattle, WA 98195 and Dept. of Neurosurgery, Emory Univ., Atlanta, GA 30322.

Early postnatal synaptogenesis has been examined in many areas of the rodent brain with emphasis being placed on mature synapses. Immature synapses (i.e. with a few vesicles aggregated against membrane specializations of two apposing profiles) and partial synaptic structures are recognizable and common in postnatal development of rat pyriform cortex. We have studied these primordial synaptic structures and compared them with the emergence of mature synapses from postnatal ages 1-30 days. Two major categories of primordial synaptic elements are identifiable and their morphology is based on the presence or absence of pre- and/or postsynaptic membrane specialization(s), presence and position of synaptic vesicles and single versus partial or multiple appositions of profiles. The categories are defined as: (1) single apposition - only two completely apposing profiles, variations include: a) pre- and postsynaptic membrane specializations, b) postsynaptic membrane specialization only, c) presynaptic membrane specialization only; the above have a few or no associated vesicles; (2) partial or multiple apposition(s) - present onto a single postsynaptic membrane specialization with a few or without vesicles. Primordial synaptic elements of category 1 are predominant in early postnatal ages. Their frequencies (number/100μm^2) from birth to 7 days doubles to about 2/100μm^2, remains relatively the same until 14 days and then rapidly declines to less than half by 30 days. Category 2 develops slower with the highest level reached by 21 days, after which a slow decline is observed. A shift in frequency from single apposing profiles to partial or multiple synaptic appositions is observed. The numbers of primordial synaptic elements parallels our counts of mature synapses between 1 and 21 days after which mature synapses increase and primordials decrease. At 30 days, the frequency of primordial synaptic structures returns to a level near that seen at birth. The presence of primordial elements of immature synapses at 30 days of age suggests the probability of synapse formation and possible competitive synaptogenesis as late as 30 days.

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203.16 DEVELOPMENT OF SYNAPTIC TRANSMISSION IN A MODEL CULTURE SYSTEM: REGULATION BY DOPAMINE. H.H. Yeh, B-A. Battelle and D.G. Puro. Lab. Vision Res., National Eye Institute, NIH, Bethesda, MD 20205.

We have been using a culture system consisting of retinal and muscle cells to study the maturation of synaptic function. In our experimental model, cholinergic neurons dissociated from perinatal rat retinas form, in time, functional synapses with rat striated muscles cells. In the initial stages of synaptic development, neurotransmitter release occurs spontaneously at the retina-muscle synapses but cannot be evoked by excitatory stimulation. This non-transmitting phase then progresses to a transmitting phase in which excitation of the presynaptic cholinergic retinal neurons with iontophoretically applied glutamate evokes neuronal release of acetylcholine onto the postsynaptic muscle cells.

We have previously shown that the induction of this stimulus-dependent cholinergic transmission in our culture system is a regulated step. Specifically, we have found that the cAMP analogs, 8-bromo-cAMP or dibutyryl cAMP, precociously induced stimulus-evoked release of acetylcholine from embryonic cholinergic neurons of the rat retina. In this study, we considered the possibility that agents which stimulate cAMP generation may influence the maturation of cholinergic transmission. Here, we present dopamine as a candidate for regulating the development of synaptic function.

The results of our electrophysiological experiments indicate that, within 2 hr after changing from control medium to medium containing 1mM 8-bromo-cAMP or 0.25-0.5μM dopamine, stimulus-evoked acetylcholine release could be induced at synapses newly formed by embryonic cholinergic retinal neurons. In the presence of 8-bromo-cAMP, glutamate-evoked synaptic activity could be detected in 44% of the innervated muscle cells which had no evocable synaptic input prior to exposure to the cAMP analog. Similarly, dopamine induced evocable cholinergic transmission in 47% of the cases tested. Pre-incubation of cultures with 1.0μM haloperidol, a dopamine receptor antagonist, blocked the inductive effect of dopamine but not that of 8-bromo-cAMP. Using techniques of local microperfusion, the inductive effects of 8-bromo-cAMP or dopamine could be detected within minutes. Preliminary biochemical studies indicate an elevation of cAMP levels following stimulation of retinal cell cultures with 0.25μM dopamine. Additional experiments have detected dopamine synthetic activity. Chronic exposure of cultures to 0.5μM haloperidol delayed the time course for the development of cholinergic transmission.

In summary, dopamine exerts a marked inductive effect on the emergence of cholinergic transmission in our cell culture system. Electrophysiological data are correlated with biochemical measures of dopamine-stimulated cAMP levels and dopamine synthetic activity to suggest that the catecholamine, by activating cAMP, may play a role in the maturation of synaptic function.

203.17 FURTHER STUDIES ON DEVELOPMENTAL CHANGES IN SYNAPTIC MEMBRANE FLUIDITY. B.A. Hitzemann*, R.J. Hitzemann and R.A. Harris (spon. D.L. Garver) Departments Psychiatry and Pharmacology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267 and V.A. Hospital and Department of Pharmacology, University of Missouri, Columbia, Missouri 65212.

Previously we demonstrated that during development there is a decrease in synaptic membrane fluidity as monitored by changes in the steady-state flourescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Hitzemann and Johnson, Neurochem. Res. 8:121, 1983). The DPH polarization increased 15% from day 7 to the adult. This change compares to the 7% decrease in DPH polarization caused by a high (200 mM) concentration of ethanol (Harris and Schroeder, Mol. Pharmacol. 20: 128, 1981). The developmental changes in DPH polarization persisted in bilayers prepared from lipid extracts of the synaptic membranes, suggesting that the changes in the intact membranes are in part related to developmental changes in lipid composition. In the present study we have compared three flourescent probes to assess the changes in membrane order that occur during development. DPH was used as a probe of the hydrophobic core of the membrane (Blitterswijk et al., Biochem Biophys. Acta 644:323, 1981). 1-(4-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH) was used as a probe of the outer portion of the membrane (Prendergast et al., Biochem. 20:733, 1981). Cis-parinarate (CP) was used as a probe of the middle region of the acyl chains (Schroeder, Eur. J. Biochem. 112:293, 1980). Synaptic membranes were prepared from 7 day, 14 day and adult rat cortex as described elsewhere (Hitzemann, Neurochem. Res. 6:935, 1981). Confirming our previous observation DPH polarization showed a significant developmental increase from .187 (day 7) to .198 (14 day) to .243 (adult). In contrast TMA-DPH showed no developmental changes. Polarization values were .301 (day 7), .300 (day 14) and .309 (adult). CP gave an intermediate developmental response. The polarization values were .215 (day 7), .215 (day 14) and .250 (adult). Overall these results show that it is the hydrophobic core rather than the membrane surface which shows the greatest developmental regulation of membrane order. Data will be presented documenting the polarization changes over a more extended time course (days 3 to 35 and adult) and the results from a flourescence lifetime study of DPH in these membranes. Supported in part by grants MH-37377, DA 02855 and the Veterans Administration.

203.18 cAMP-DEPENDENT CHANGES IN GLYCOPROTEINS OF NG108-15 CELLS ASSOCIATED WITH SYNAPTOGENESIS. Karl E. Krueger*, Mark J. Miller†*, and Marshall Nirenberg. Laboratory of Biochemical Genetics, NHLBI, and †Laboratory of Carcinogen Metabolism, NCI, NIH, Bethesda, MD 20205.

Treatment of NG108-15 neuroblastoma-glioma hybrid cells with PGE_1 activates adenylate cyclase and gradually shifts the cells to a more differentiated state which enables them to form synapses with cultured striated myotubes. Since many of the newly expressed neuronal properties may be dependent on intrinsic membrane glycoproteins, the effects of PGE_1 on the glycoproteins of NG108-15 cells were investigated.

Logarithmically dividing, control NG108-15 cells or cells treated with 10 μM PGE_1 for 7 days were incubated for 18 hours with ^{35}S-methionine. ^{35}S-Protein soluble in 1% Triton X-100 was fractionated by chromatography on wheat germ agglutinin-, Lens culinaris agglutinin-, and Ricinus communis agglutinin$_{II}$-agarose columns. ^{35}S-Glycoproteins were eluted with 0.5 M N-acetylglucosamine, 0.2 M α-methylmannoside, or 0.2 M lactose, respectively, and subjected to two-dimensional polyacrylamide gel electrophoresis and autoradiography. Autoradiograms were analyzed and compared with a computerized system similar to that described by Vo et al, (Anal. Biochem. 112; 258-271, 1981).

In most cases, different species of ^{35}S-glycoproteins were eluted from each lectin column. Most ^{35}S-glycoproteins were expressed by both control and PGE_1-treated cells. However, some ^{35}S-glycoproteins were expressed by PGE_1 treated cells, but not by control cells. Other species of ^{35}S-glycoproteins were found that were expressed only by control cells. Apparent shifts in the isoelectric points of some species of glycoproteins also were found. In addition, large quantitative changes in the radioactivities of some species of ^{35}S-glycoproteins resulted from treatment of cells with PGE_1. In all, approximately 10-15% of the total ^{35}S-glycoproteins detected were altered in cells treated with PGE_1 for 7 days. These effects were not detected when cells were incubated with 10 μM PGE_1 for 20 minutes.

These results show that prolonged treatment of NG108-15 cells with PGE_1 affects many glycoproteins, and suggest that elevation of cAMP levels of cells results in changes in the post-translational modifications of glycoproteins and/or changes in gene expression.

203.19 MONOCLONAL ANTIBODIES WITH REGIONAL SPECIFICITY IN THE NERVOUS SYSTEM. Gerald B. Grunwald, David Trisler*, and Marshall Nirenberg. Laboratory of Biochemical Genetics, NHLBI, National Institutes of Health, Bethesda, MD 20205.

Our objective is to use monoclonal antibodies to identify molecules involved in synaptogenesis or synaptic function. Monoclonal antibodies directed against 14 day embryo chick neural retina were assayed by indirect immunofluorescence for binding to frozen, unfixed sections of retina from 7, 10, 14 and 19 day embryos and adults, and to other neural and non-neural tissues. Molecules recognized by antibody 13H9 were found on the surface of most, or all, retina cells at each age examined. The antibody markedly agglutinated cells. Retina proteins were solubilized and separated by SDS-PAGE; the antibody bound to a single band of protein with an Mr of approximately 180,000. The antigen was not detected elsewhere in the nervous system (cerebral lobe, optic lobe, cerebellum, spinal cord, dorsal root ganglia and peripheral nerve), or in kidney, or striated, smooth, or cardiac muscle. However, antigen was detected on hepatocytes and erythrocytes. Antibody 107H11 bound to photoreceptor cell soma of 14 day and older embryos and adults, but not to 7 or 10 day retina. Antibody also bound to some cells in the optic tectum, but not to other neural and non-neural tissues tested. Antibodies 18B8, 66E1 and 92A2 recognize punctate antigens in the outer synaptic layer of 19 day embryo retina which were not found in adult retina. In adult retina, antigen 18B8 was not detected; antigen 66E1 was localized in punctate structures in some cell soma in the inner nuclear layer; and antigen 92A2 was found in structures which resemble ellipsoids of photoreceptor inner segments. 18B8 bound to a discrete fiber tract in 19 day embryo spinal cord, probably the ascending spino-cerebellar tract, and to punctate structures associated with plasma membranes of muscle, kidney and liver, possibly neuronal processes. Antigen 18B8 also was detected in cerebellum, but not in optic or cerebral lobes. Antibody 66E1 bound to smooth muscle cells in blood vessels, and 92A2 to erythrocyte plasma membranes. In neither case was binding detected in other tissues tested. Antigen 115C11 was first detected in 14 day embryo retina associated with fibers traversing the width of the retina, while in 19 day embryo and adult retina the antigen was restricted to the synaptic layers. Antigen also was present in microvilli surrounding the lumen of kidney proximal tubules and in A bands of striated muscle. Antibody 93A7 bound to a punctate antigen restricted to the inner and outer synaptic layers of 19 day embryo retina. In adult retina, the antigen was found in photoreceptor and neuron soma at the margins of the outer synaptic layer. The antigen was not found in other neural tissues, but was observed on plasma membranes of smooth, cardiac, and striated muscle cells.

Antibody 113F4 recognizes an antigen restricted to the outer synaptic layer of retina from 14 day or older embryos and adults. Little antigen was detected in cerebellum, smooth muscle, or kidney. Antigen was not detected in cerebral lobe, optic lobe, or liver. Antibody 114E10 recognizes an antigen in 7 day embryo retina in broad bands at the inner and outer margins of the retina. Four bands of antigen were found in 10 day embryo retina associated with the interfaces between layers of cell soma and processes in synaptic layers. Antigen also was associated with ganglion neuron soma and with other principle neurons such as Purkinje neurons of the cerebellum and large neurons of dorsal root ganglia.

Thus, antibodies were obtained to cell surface molecules which distinguish retina neurons from other neurons, to molecules expressed only by some retina neurons, and to molecules restricted to the synaptic layers of retina.

203.20 IMMUNOHISTOCHEMICAL LOCALIZATION OF SYNAPTIC VESICLE ANTIGENS IN DEVELOPING CAT CORTEX. J.J.M. Chun and C.J. Shatz. Department of Neurobiology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

As part of ongoing studies of synaptogenesis during mammalian CNS development, we have begun to use 3 separate antibodies (Ab's) that recognize synaptic vesicle antigens. These are: sera 30 and 48 from Reichardt and Matthew (J. Cell Biol., 91:257, 1981) and Protein I antibody from Greengard and De Camilli (PNAS 76:5977, 1979). In adult tissue prepared for immunohistochemistry, these Ab's selectively label synaptic regions. We therefore studied the distribution of these Ab's during pre- and postnatal development of the cat's brain. Tissue from 17 animals aged adult, postnatal day 65 (P65), P21, P14, P7, P1, embryonic day 61 (E61), E50, and E48 was prepared and reacted using HRP-DAB immunohistochemistry.

In adult cats, staining patterns and intensities for all 3 Ab's are indistinguishable. Highest density staining is always localized to known synaptic regions, e.g., the cerebral cortex is intensely stained as are thalamic nuclei and basal ganglia. White matter (WM) is virtually unstained. In embryonic material (E48-P1), regional staining is distinctly different. For example, there is almost no staining within the cortex itself but rather intense stain is present immediately below in the incipient WM, and also above in future layer 1. Within the basal ganglia, irregularly shaped islands of staining are seen. Within the lateral geniculate nucleus, most stain is located within and adjacent to the optic tract. These staining patterns were again seen with all three Ab's.

The intensity of cortical staining gradually increases, and WM staining decreases, during the immediate postnatal period (P1-P21) until by P65, the pattern of staining becomes adult-like. Layer 1 staining intensity remains relatively constant during this period. Preliminary microdensitometric scans of cortex and WM from E48 to adult have confirmed quantitatively this shift in the pattern of cortical staining during development. Thus the ratio of staining density in WM to that in cortex declines from E48 until adult values are reached. In contrast, control sections of adult cerebellum reacted at each age varied in density by only about 10%.

These findings show that some components of mature synaptic regions are present in embryonic cat brain as early as E48. This could reflect the accumulation of synaptic machinery within the growing axons for later use. However, in many mammals both layer 1 and the region directly below the cortex are known to contain synapses during development, suggesting that the staining seen here indeed signals the presence of synapses.

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203.21 SYNAPTIC CONNECTIONS BETWEEN EMBRYONIC AMPHIBIAN SPINAL NEURONS IN CELL CULTURE. Leslie P. Henderson & Nicholas C. Spitzer. Biology Department, UCSD, La Jolla, CA 92093.

The development of several membrane properties of Rohon-Beard (RB) neurons in vivo has been described, and the development of the action potential and neurotransmitter sensitivity in spinal cord neurons grown in dissociated cell culture is similar to that occurring in the animal. The population of cultured neurons appears to include primarily motoneurons (MNs) and RB cells: the former are hyperpolarized by GABA and glycine and depolarized by glutamate; the latter are depolarized by GABA or by GABA and glycine (Spitzer & Bixby, 1982). Although these cells do not respond to a variety of other classical neurotransmitters, a small number of MNs are depolarized by aspartate. RB cells in vivo have also been shown to be electrically coupled to one another in a voltage-dependent, non-rectifying manner until the early tailbud stage of the embryo. We wanted to determine if coupling and stage-dependent uncoupling occur in culture as they do in the spinal cord. Further, if chemical synapses were formed by RB cells, application of pharmacological blockers might provide clues as to the identity of their neurotransmitter(s).

The neural plate region was dissected from embryos of Xenopus laevis and dissociated cells were plated in a manner that increased the probability of neuroneuronal contacts. Cultures were maintained for periods up to 48 hrs, and intracellular recordings were made from pairs of cells during continuous perfusion with physiological saline. Neurotransmitters were bath applied at concentrations of 100 μM to identify neurons.

Electrical junctions were formed by both RB cells and MNs in vitro. Coupling was observed as early as 8 hrs after plating. In some, but not all cases, coupling exhibited rectification. No voltage-dependent uncoupling has been observed. Coupling was not found between all pairs of cells in apparent contact with one another.

With a lower frequency, neurons were also seen to make chemical connections and joint electrical and chemical contacts with other neurons. Action potentials in one cell could elicit long duration psp's (up to 0.5 sec) in other cells. These potentials were abolished reversibly by 10 mM Mg/1.8 mM Ca saline. In most cases, psp's were depolarizing in sign. Since MNs are believed to be purely cholinergic, and no cells in these cultures show sensitivity to ACh at the times studied, we believe that the presynaptic neurons are not MNs and are likely to be RB neurons. In some cases, the postsynaptic cell was identified as a MN. To determine the putative neurotransmitter(s) of RB cells, experiments were made in which the effects on synaptic potentials of picrotoxin and strychnine, which block GABA and glycine responses respectively, were examined. Supported by the Giannini Foundation and the NIH.

203.22 SYNAPTOGENESIS IN THE PRIMARY VISUAL CORTEX: QUANTITATIVE ANALYSIS IN PRE- AND POSTNATAL RHESUS MONKEYS. J.P. Bourgeois* and P. Rakic, Sec. Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

The upper bank of the calcarine fissure of primary visual cortex (area 17) was analyzed by electron microscopy (EM) in 17 rhesus monkeys, ranging in age from the 65th embryonic day (E65) to 17 years of age. Two EM probes spanning the cortical thickness were made for each age. The number of synapses/100um^2 of neuropile was determined in each probe on photomontages consisting of consecutive overlapping micrographs. On E65, immature synaptic contacts were observed within the marginal zone (prospective layer I) and in the subplate and intermediate zones below the developing cortical plate, which at this age contains only prospective layers V and VI. By E112, when all visual cortical neurons have been generated and attained their final positions, synapses are present throughout the full span of the cortex with high density in layers I,IV and V and only occasional synapses recorded in layers II and upper III. Synaptic density increases exponentially during the second half of gestation and by the time of birth (E165) the mean density of synaptic contacts is equivalent to that found in the adult visual cortex. The density of synapses nevertheless continues to increase until the third postnatal month (P96), forms a "plateau" until the seventh month (P222) and then decreases slowly towards puberty where it reaches adult level. The time-dependent changes in synaptic densities for symmetric and assymetric synaptic contacts situated on cell soma, dendritic shafts and spines will be described for each cortical layer. For example, two successive peaks were observed in synaptic density (around P20 and around P96) in layer I, which is known to receive multiple afferent inputs; and only one peak was observed in layer IVC (around P96) which receives its input mainly from one source, the lateral geniculate body. The highest rate of increase in synaptic density in layer IVC is observed between E112 and P61. The rapid accretion of synapses in this layer coincides with the highest rate of neuritic degeneration, as revealed by the frequency of myelin-like membrane whorls, and darkened vesicular, autophagic-like profiles in the neuropile. Moreover, the observed increase in synaptic density in layer IVC and the peak of neuropile degeneration coincide with the time of segregation of ocular dominance columns (Rakic, 1976; Hubel and Wiesel, 1977), but it may be significant that the fastest increase in the synaptic density in this layer precedes rather than follows segregation of terminals subserving the two eyes. [Supported by grant EY02593]

203.23 ULTRASTRUCTURAL CHANGES IN THE RAT SUPERIOR COLLICULUS ATTRIBUTED TO DEPRIVATION. M.R. Gurski* and N.M. Kettlewell. Dept. of Physiology & Biophysics and The Neurosciences Program, University of Alabama in Birmingham, Birmingham, AL 35294 and Dept. of Psychology, University of Montana, Missoula, MT 59801.

Normal ultrastructural development of the superior colliculus in the albino rat occurs in three distinct stages. In stage 1 there is an initial proliferation of optic synapses followed by the proliferation of intrinsic synapses. Stage 2 is characterized by continued optic synapse development, as well as by multiple and serial synaptic formation. The third stage is typified by another increase in the formation of intrinsic collicular synapses (Lund, R.D. and Lund, J.S., Brain Research, 42:1-20, 1972).

The purpose of this study was to assess neuronal plasticity in the superior colliculus during third stage development. This was accomplished by evaluating ultrastructural changes in the superior colliculus as a function of various environmental conditions: dark rearing (Group SD), low and high intensity light homogeneous environments (Groups LH and HH), and low and high intensity light patterned environments (Groups LP and HP). With the exception of the SD group, all groups were on a 12 hour light/dark cycle. The data analysis consisted of planned comparisons between the dark-reared group and the four other groups and a 2 X 2 factorial design between light and pattern. The functional organization of the superior colliculus was assessed by the following dependent measurements: percentage of spheroid vesicled type terminals, percentage of flat vesicled type terminals and density of the synaptic junctions. Length of the synaptic junction and the number of vesicles per terminal were used to assess terminal integrity.

With respect to Groups SD, LH, and LP, results indicate that light in and of itself produces an intermediate level of development in the superior colliculus; however, for normal development to occur, a patterned environment is needed. The groups that developed in an intense light field were in most ways similar to the SD group. Indications are that as the light level increases, this has an adverse effect on collicular development and possibly collicular function. A light microscopy analysis of the retinas revealed the retinas to be normal under the experimental conditions utilized.

203.24 MUSCARINIC RESPONSES IN THE AVIAN RETINA DEVELOP PRIOR TO SYNAPTOGENESIS T.H. Large, N.J. Cho*and W.L. Klein Dept. of Neurobio. and Physiology, Northwestern University, Evanston, Ill. 60201

Muscarinic responses are produced through coupling of the activated receptor with separate effector proteins. To determine if the muscarinic system develops prior to synapse formation, we have investigated changes in receptor binding and identified a muscarinic mediated phosphatidylinositol response (PI) in the developing avian retina.

Reproducible differences were observed between the agonist binding curves of membranes from retinas prior to synapse formation (9 days in ovo) and following synapse formation (4 weeks post hatching). Carbachol competition against 3H-Scopolamine binding in adult membranes displayed a Hill coefficient of .32, indicative of heterogeneous binding properties. In comparison, carbachol competition in embryonic membranes was steeper, Hill = .58, but still complex. In contrast, the Hill coefficient of atropine competition was equal to 1, consistent with the notion that agonists, but not antagonists, induce complex binding through receptor-effector coupling. To determine if heterogeneous agonist binding was due to protein interactions or separate classes of receptors, retina membranes were solubilized with 2% digitonin. Solubilization converted receptors from both age groups into a homogeneous population with low affinity. However, the possibility remains that only one class of receptor was solubilized from the membrane.

The early appearance of receptor coupling was further investigated by assaying cultured retina cells for a muscarinic mediated PI response. In monolayer cultures, at a developmental age just prior to synapse formation, PI turnover was stimulated 416% by 1 mM carbachol. Receptor coupling to adenylate cyclase (AC) was investigated with GppNp, an analog of GTP which uncouples AC. GppNp (100 uM) decreased the agonist affinity of both embryonic and adult receptors but increased the Hill coefficient to a greater extent in embryo (Hill = .82) than adult (Hill = .47) Solubilized receptors were unaffected by GppNp.

The results suggest that prior to synapse formation, the MAChR is coupled to two different effector systems, AC and PI response. This is consistent with the heterogeneous agonist binding in embryonic membranes and suggests why GppNp fails to produce a completely homogeneous receptor population. Differences in agonist binding between adult and embryo may result from changes in receptor environment during synapse formation, changes in coupling or modification of the receptor protein. The role of the PI response and diacyglycerol stimulated protein kinase C activity in synaptogenesis is currently being investigated. (Supported by NIH grant NS 15299 to WLK)

REGENERATION: CENTRAL I

204.1 GLIAL CELLS SECRETE SPECIFIC PROTEINS DURING REGENERATION OF THE GOLDFISH OPTIC NERVE WHICH MAY PLAY A ROLE IN AXON GROWTH. Michael A. Deaton* and John A. Freeman. Department of Anatomy, Vanderbilt University, Nashville,Tennessee 37232.

Molecular interactions between neurons and their surrounding glial cells following axonal injury are likely to play a significant role in neuronal regeneration. In order to determine whether glial cells synthesize selective proteins which are secreted following axonal injury, we examined changes in protein synthesis by glial cells in the goldfish optic nerve using one- and two-dimensional gel electrophoresis coupled with fluorography. To study glial proteins, we either crushed one nerve intraorbitally or enucleated one eye, waited ten days, and then incubated the glial cell-containing nerve sheath in 35S-methionine for 2.5 hours. We found 3 newly synthesized proteins (MW 16, 30 and 42K daltons) that are released into the medium, and thus appear to be secreted by glial cells. All three proteins are acidic (pI < 5.5 pH) and display charge microheterogeneity on IEF gels, typical of glycoproteins. These proteins are not secreted by tectal cells following these manipulations, or by normal optic nerve glial cells; they can, however, be detected at very low levels in nerves that have been lesioned as short a time as 3 hours prior to incubation. Thus, there appears to be a rapid inducution of their synthetic mechanism following axonal injury. The signal that triggers the synthesis of these glial proteins is unknown, but since they are synthesized in the absence of nerve fibers, it is possible that the latter might normally release a suppressive factor. The 30K protein exhibits the greatest synthesis, and binds to the membrane fraction obtained from regenerating optic nerve. Binding occurs in the presence of a four-fold excess of unlabeled glial-secreted protein, indicating that a specific 30K receptor may be present on the regenerating axon membranes. A specific glial protein/axon receptor binding would suggest that glial proteins whose synthesis is triggered after nerve injury might interact with and influence the subsequent behavior of the injured axons. It is possible that glial cell proteins released during nerve regeration play a role in the induction or in the guidance of axon growth.

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204.2 REGENERATION OF TRANSECTED AXONS ACROSS A SPINAL GAP IN LAMPREYS. John W. Pendleton* and Melvin J. Cohen, Dept. of Biology, Yale University, New Haven CT 06511.

Regeneration of central neurons can be studied in larva of the lamprey, Petromyzon marinus, a primitive vertebrate in which identified neurons, including the giant Muller and Mauthner cells, regenerate across a spinal transection. In this study, we describe a lamprey preparation in which axons and their growth cones from regenerating giant neurons are exposed as they cross a gap in the central nervous system made by removing a section of spinal cord.

Larval lampreys were anesthetized and 1 to 4 mm of spinal cord was removed through a dorsal incision. After ten to 200 day postoperative intervals, the animals were re-anesthetized, and a few ul of 5-10% horseradish peroxidase (HRP) in lamprey saline was extracellularly injected into a new transection site either in or close to the gap. Twelve hours to five days after the HRP injection, the preparations were processed with standard histochemical methods, and examined as cleared whole mounts under a light microscope.

HRP injections placed 5-10 mm anterior to the gap selectively stained the regenerating giant axons within the gap. Ten to 30 days after the initial surgery, some Muller and Mauthner axons approached the proximal margin of the cut cord and then sent one or more processes into the gap. Regenerating axons exposed in the gap have have swollen "growth cone"-like endings. At later stages, axons crossed the gap and entered the distal spinal segment; SEM observations show that the growing axons follow a base of presumably mesenchymal tissue across the surface of the notochord. The cellular elements in the gap are directly exposed to the extracelluar environment of the spinal canal. Using Nomarski optics, regenerating axons and their growth cones can be clearly observed within the gap in the living animal. Fibers in the gap showed a strong tendency to fasciculate; this fasciculation tends to disappear once the distal edge of the gap is reached by the growing fibers.

Injection of HRP directly into the gap selectively labels cells that have regenerated into the gap. Cells and axons labeled by intra-gap HRP injections include descending giant reticulospinal axons, lateral interneurons, giant interneurons,and edge cells located close to the gap. Thus it is possible to identify which cells contribute to regeneration and the recovery of function in the larval lamprey. The spinal gap preparation provides a system for spinal trauma study with ready access to relatively exposed regenerating vertebrate central neurons and their growth cones. (Supported by NIH Spinal Trauma Grant NS 10174).

204.3 THREE FACTORS AFFECTING THE GROWTH OF BRAIN STEM AND NEOCORTICAL TRANSPLANTS. C.M. Chanaud* and G.D. Das*. (SPON: J. Altman). Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

It is well established that embryonic neural tissues, when transplanted in the brain of neonatal or adult rat hosts, survive, grow and become integrated with the host brain parenchyma. This study examines the affect of several factors on the growth of both brain stem and neocortical transplants. These factors are: 1) initial volume of transplanted tissue, 2) freezing of the embryonic tissue, and 3) embryonic age of the neural tissue (16-day versus 21-day tissue).

Donor embryos were obtained from pregnant Wistar-Albino rats on day 16 and 21 of gestation. Brain stem or neocortical tissue was dissected from these embryos and tissues with initial volumes of 3.5, 7.0, 10.5 and 14.0 mm^3 were transplanted as fresh tissues into the vermis of the 21-day-old host animals. Frozen neural tissues were frozen in advance for 2-3 months in lactated Ringer's solution containing 10% DMSO. Prior to transplantation, the frozen tissue was washed, rapidly thawed, and transplanted. Thirty days after transplantation, host animals were sacrificed. Brain sections were stained with cresyl violet and Bodian stains. Volumetric analysis of each transplant was performed.

With brain stem tissue, there was an optimal initial volume of tissue that resulted in a maximum final volume of the transplant. With neocortical tissue, regardless of the initial volume, the neocortical transplant grew large. Transplants of frozen 16-day and 21-day neocortical and 16-day brain stem tissues survived, whereas, frozen 21-day brain stem tissue did not. All fresh tissues survived and yielded larger, more highly integrated transplants than frozen tissues. Transplants of 16-day neocortical or brain stem tissue yielded larger, more integrated transplants than did the 21-day tissue.

The transplants composed of neuroepithelial cells, e.g. neocortical transplants of 16-day embryonic tissue, grew large, became integrated with the host brain and contained well-differentiated neurons. In contrast, transplants composed of partially differentiated neuroblasts, e.g. brain stem transplants of 21-day embryonic tissue, did not grow well, became extraparenchymal, and contained atrophying and hyperchromatic neurons. Under identical conditions, neocortical transplants achieved a greater final volume than brain stem transplants.

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204.4 OPTIC TRACT REGENERATION IN THE ADULT RAT: PHYSIOLOGY. A. Foerster, M. Holmes* and J. Diamond. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

The location of the superior colliculus (SC), of pentobarbital-anesthetized adult male Long Evans rats, with respect to a permanent landmark, a graphite-filled crossline on the skull, was determined by transcortical penetrations, 0.5 mm apart (except for the most medial 0.5 mm underlying the sagittal sinus),with tungsten-in-glass microelectrodes which recorded multiunit receptive fields (MRFs); these were evoked usually with a black edge moving just outside a transparent hemisphere, r=34 cm, centered upon the eye. Eye movements were monitored by retesting of sites. The optic disc location was projected on the hemisphere with a reversible ophthalmoscope. A lesion was made with a cutting device aimed at the optic tract (see accompanying Poster by Diamond, Foerster and Visheau). Immediate remapping revealed either (1) absence of visual response, (2) a well-defined scotoma or (3) no change, indicating respectively a (1) complete, (2) partial or (3) "control" lesion of the tract. The animals were allowed to recover for periods of 1-10 wk when a third mapping was done, and on occasion a fourth. So far 8/9 rats have shown a return of visual function in the silenced SC, evidenced by responses from regions of the retina which were ineffective immediately after the lesion. Although somewhat bizarre, an approximately topographic retinal projection was present by 3 wk. In the same SC "recovered" MRFs varied in vigor, excitability and fatiguability; they ranged from nearly normal size (about 10°) to ones responding over the entire visual field. The large MRFs often had one or more foci; single foci, not always in the MRF centers, could be near the correct retinal projection site. The orderliness of maps, and the restriction of field sizes, appeared to "improve" with time after the lesion. Interestingly, after a partial cut, the spared normally-responsive portions of the SC later might acquire new sizes and foci of their MRFs. None of these changes occurred in control SC remappings.

The observed topography of "recovered" maps is not explained by collateral sprouting of a discrete portion of a topographically organized tract that might have escaped lesion. Perhaps spared, grossly aberrant, "silent" optic fibers sprout to become effective. Conceivably, the recovered MRFs might be driven by normally undetectable but now strengthened SC inputs from brain regions served by the portion of the tract proximal to the lesion. However, in the light of the anatomical findings (see accompanying Poster) we suggest that the observed return of visual function is mediated by regenerated fibers which tended to select their normal region of the SC and compete for it, with progressively increasing success.

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204.5 OPTIC TRACT REGENERATION IN ADULT RAT: ANATOMY J. Diamond, A. Foerster and B. Visheau*. Dept. of Neurosciences, McMaster University, Hamilton, Ontario L8N 3Z5.

Lesions were made in the postchiasmatic optic tract (OT) of anesthetized adult male Long-Evans rats, with cutting devices each consisting of 1-4 mm portion of razor blade, or 90 μm diameter wire, supported between two vertical (also 90 μm) wires, lowered by a micromanipulator until it reached the floor of the skull; the two protruding support wires were cemented to the calvarium, and the excess lengths cut off. The animals, some of which were used in the experiments described in the accompanying Poster (Foerster, Holmes and Diamond), were sacrificed immediately or at times up to 5 months; after fixation of the brains the implanted device was removed via the ventral surface. In horizontal sections the two support wire channels appeared as two holes which marked the limits of the original cut. Such devices make precisely predictable lesions around which severed tracts appear to regenerate (Foerster, 1982; J. Comp. Neurol. 210: 335). As in the earlier studies, silver-stained brains revealed (unless the lesion had been too extensive) a progressive reorganization of severed optic axons to form a "detour" that curved around the lesion and back into the distal tract. The retinal origin of these axons was confirmed by TMB-staining after intraocular injection of HRP. Two days after the lesion, HRP-filled axon stumps faced the retinal side of the cut, but at later times HRP-filled optic axons were observed to have extended around the cut, and into the distal tract and SC. (We are further examining the SC and the OT at various times following OT lesion for degenerating boutons, immature boutons, growth cones, etc.). In a typical animal whose OT was almost totally cut, the third electrophysiological mapping, done after 3 weeks, revealed a recovery of visual responsiveness within the initially blinded region of the SC (see accompanying Poster); following intraocular HRP the recovered region of the SC was shown by TEM examination to contain, among the numerous normal non-retinal nerve endings and synapses of the stratum griseum superficiale, less frequent endings that contained HRP reaction product, generally had characteristically pale mitochondria, and in many instances made synaptic contacts with dendritic profiles. These endings sometimes had fine structural appearances suggestive of immaturity. Often degenerating myelinated axons were seen in the same regions. We suggest that the endings containing the HRP reaction product were those of optic axons that had regenerated after the initial cut, and that they were responsible for the observed recovery of visual function in the SC.

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204.6 TARGET REGULATION OF THE CELL BODY REACTION DURING REGENERATION OF GOLDFISH RETINAL GANGLION CELLS. D.W. Burmeister*, G.W. Perry*, and B. Grafstein, Dept. Physiology, Cornell University Medical College, New York, N.Y. 10021

Axonal regeneration of retinal ganglion cells in the goldfish is accompanied by increases in the size of the cell body and in the synthesis and axonal transport of proteins. In order to investigate the role of the target tissue in regulating these changes we have examined the effect of removing the major axonal target, the contralateral optic tectum.

During regeneration following intraorbital optic nerve crush, the cell body reaction was more prolonged in tectum-ablated fish than in tectum-intact (sham operated) fish. Under both conditions cell size and ^{3}H-proline incorporation showed the same initial rise. In the tectum-intact animals both values gradually returned to near normal levels after 10 to 12 weeks, whilst in the tectum-ablated animals both values remained elevated. The presence of the contralateral optic tectum affected the survival of the regenerating cells. Regeneration with an intact tectum resulted in a small decrease in the overall incidence of ganglion cells across the retina. In the absence of a target tectum there was a further decrease in cell incidence although over 80% of the ganglion cells still remained after 12 weeks. By 12 weeks at least some of the axons had innervated the ipsilateral tectum. However, when both tecta were ablated the cell body changes were the same as when only the contralateral tectum was removed.

When rapidly transported proteins, labeled by intraocular injection of ^{3}H-proline, were separated by two-dimensional gel electrophoresis, the analysis of 20 of these proteins showed increases in labeling during regeneration, but two of them changed more than the others. One, which constituted the largest fraction of rapidly transported protein in normal nerves, had a molecular weight of about 150 kD and was spread across a pI range of 4.9 to 5.3, a pattern characteristic of many glycoproteins. The second, with a molecular weight of about 45 kD and pI about 4.6, increased more than 50-fold over normal to become the most prevalent transported protein in regenerating nerves. Labeling of the 20 rapidly transported proteins was the same in tectum-ablated and tectum-intact fish at 3 weeks. At 5,8, and 12 weeks, however, when re-establishment of connections is occuring in normally regenerating nerves, differences were seen, particularly in the two proteins mentioned above.

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204.7 REGENERATION OF SENSORY AND MOTOR AXONS INTO THE SPINAL CORD OF THE ADULT FROG. A STUDY USING DORSAL TO DORSAL AND VENTRAL TO DORSAL ROOT ANASTOMOSES. F.J. Liuzzi and R.J. Lasek, Dept. of Anatomy, Case Western Reserve Univ., Cleveland, OH 44106.

Anterograde injury-filling with HRP was used to demonstrate the extent and distribution of central (dorsal root axons) or peripheral (motoneuron axons) when dorsal or ventral roots were grafted into the proximal stumps of cut lumbar dorsal roots in Rana pipiens.

Contrary to the results reported in the cat (Messenger and Kingsley, 1982), cut dorsal root axons do regenerate into the cord with large myelinated axons entering the dorsal funiculus and small diameter unmyelinated axons entering a small discrete bundle in the dorsolateral funiculus, which is homologous to Lissauer's tract in mammals. Not only do the regenerating dorsal root axons travel in the spinal white, they also send collaterals into the spinal gray where at the light microscopic level they appear to reproduce the normal segmental distribution as has previously been shown with HRP (Liuzzi, Beattie, and Bresnahan, 1982).

Motoneuron axons within ventral roots grafted to cut dorsal roots, will also regenerate into the frog lumbar cord as has been previously demonstrated in the cat (Messenger and Kingsley, 1982). Many of these axons form a discrete bundle in the DLF suggesting, as Katz and Lasek (1981) have proposed, that this region is a substrate pathway which, in the case of the Rana pipien, persists in the adult, thereby allowing axons to grow and fasciculate. Other large diameter motoneuron axons are seen within the dorsal funiculus. A few of these axons give rise to collaterals, which, with long-term survival, are observed to enter the dorsal gray. Here, at the gray-white interface, they immediately bifurcate and subsequently arborize within the dorsal horn having numerous varicosities in close apposition to dorsal horn neuronal somata suggestive of synaptic terminals.

On occasion, sensory, and more often, motor axons are observed with quite large swellings along their lengths as well as at their apparent ends. Some of these swellings are up to 12.5 um in diameter and may represent instances where the axon's growth has been slowed or halted by mechanical or other barriers. These swellings might, therefore, represent single-axoned neuromas. In light of the regenerative capacity of dorsal root axons in the frog, an in vivo model is now available for comparing the regeneration of two very different, yet well characterized, populations of axons introduced into the same CNS environment. This model is being used in continuing studies in this laboratory to investigate basic mechanisms of axonal growth and guidance.

204.8 OLFACTORY MUCOSA AND EMBRYONIC BRAIN TISSUE TRANSPLANTATION IN THE ANTERIOR CHAMBER OF THE EYE OF RAT. J. A. Heckroth* and P. P. C. Graziadei. Dept. Biol. Science, Florida State University, Tallahassee, FL 32306

Previous experiments have shown that the olfactory neurogenetic matrix of postnatal rats transplanted into the anterior chamber of the eye of adult rats maintains its capacity to produce mature olfactory neurons (Heckroth et al. Int. J. Devel. Neuroscience, 1983).

In the present experiments we have transplanted olfactory neuroepithelium from neonatal rats into the anterior chamber of the eye together with fragments of different regions of embryonic rat brain to study the possible interaction of the regrowing olfactory axons with a variety of targets. Donor tissue from embryonic rat brains was transplanted into the anterior chamber of the eye of adult rat hosts. Following a 7 day recovery period olfactory mucosa from postnatal rat donors was introduced into the anterior eye chamber and positioned close to the brain tissue. Animals were sacrificed after 60 and 90 days and the tissue processed for light microscopy. A total of 63 transplants involving the olfactory bulb, frontal and occipital cortex, cerebellum, tectum and medulla were performed, 53 of which were successful. Apposition of the brain and the mucosa transplants resulted in the penetration of the regenerating olfactory axons of the reconstituted olfactory neurons into the brain fragments. Bundles of olfactory axons invaded the adjacent brain transplants or the iris without selective differences. The degree of interaction between brain and mucosa transplants varied between preparations but showed no relation to the type of brain tissue available to the regenerating olfactory axons. Large fasicles of axons penetrated adjacent neural tissue without forming the glomeruli characteristic of the olfactory axon terminals. Only exceptionally have we observed the olfactory axon bundles to terminate in circumscribed areas of the brain tissue where structures resembling glomeruli were seen. However, this rare finding deserves further confirmation.

Olfactory neural elements were observed migrating from the epithelium and at instances penetrating into the transplanted brain tissue. Further experiments at ultrastructural level are in progress to determine the detailed nature of interneuronal contacts of the olfactory axons with the brain parenchyma in the in oculo environment, as well as the long term survival and behavior of the olfactory neurons and their matrix.
(Supported by NIH Grant number NS16421)

204.9 IMMUNOCYTOCHEMICAL AND MORPHOLOGICAL OBSERVATIONS OF OLFACTORY NEUROEPITHELIUM TRANSPLANTS IN RAT BRAIN. E. E. Morrison* and G. A. Monti Graziadei* (Spon. L. Beidler). Dept. of Biological Sciences, Florida State University, Tallahassee, FL 32306

The olfactory neurons are replaced normally and when injured, by the olfactory neurogenetic matrix (basal cells) present at the base of the neuroepithelium. The mature olfactory neuron produces a specific low molecular weight acidic protein, olfactory marker protein (OMP), that is found in the perikaryon, axons and axon terminals. In the present study we have transplanted neonatal olfactory mucosa (P4-P10) into the cerebral ventricles of neonatal and adult rats, to examine the neurogenesis and maturation of the olfactory neuron and to determine the presence of OMP within a foreign environment. The OMP was visualized by the peroxidase-antiperoxidase immunohistochemical method. The post operative examination period was 10-100 days. The transplant was promptly vascularized and formed a series of inter-connecting vesicles lined by a respiratory and sensory epithelium. The transplant was in contact with the cerebrum, cerebellum and medulla. At early survival times (10-20 days), few OMP positive neurons are observed. Between 20-35 days survival the number and intensity of OMP positive neurons and sensory axons has increased. All transplants examined up to 100 days survival demonstrate varying amounts of OMP positive sensory neurons and axonal fascicles. Silver preparations and OMP visualization demonstrated mature olfactory neurons characterized by their dendrites and their axons. OMP positive axonal fascicles are observed to originate from the neuroepithelium and penetrate the host brain, however, no characteristic glomerular structures are present. Mature OMP positive olfactory neurons are observed, migrating away from the neuroepithelium, within the lamina propria and in the host brain. We conclude that the transplanted neurogenetic matrix produces mature OMP positive olfactory neurons in the absence of its normal target tissue. Ultrastructure examination of host transplant connectivity is currently being conducted. (Supported by NIH grant NS 16421).

204.10 AUTORADIOGRAPHIC TRACING OF CROSSED CORTICO-THALAMIC PROJECTIONS IN CATS WITH NEONATAL ABLATION OF ONE CEREBRAL HEMISPHERE. B.J. Sonnier, J.R. Villablanca, C. Olmstead, F. Gómez, J.P. MacAllister Ment. Ret. Res. Ctr., Depts. Anat. & Psychiatry, UCLA Sch. Med. Los Angeles, California 90024

In a continuing investigation of brain reorganization and functional recovery, the left half of the telencephalon was removed (hemispherectomy) in 6 kittens ($\bar{X}$ age 12.3 $\pm$ 7.6 days) which were followed into adulthood (companion Abstract) and then used for this study. Six intact adult cats served as controls. All cats received injections of 50:50 mixture of tritiated leucine-proline into the right pericruciate cortex. Starting at about 4 mm from the midline, 5 to 6 injections were made (0.2 to 0.6 μl at 50μ Ci/μl) 1.0 to 1.5 mm in front and behind the cruciate sulcus at a depth of 3 mm. Five days later the cats were perfused intracardially, frozen coronal serial sections were cut at 50 μm and processed for autoradiography. Sections were developed after a 6 week exposure and injections sites and terminal field areas were reconstructed by serial drawings under both light and dark field illumination (atlases of Jasper & Ajmone-Marsan and of Reinoso-Suárez) In intacts cats, there were cortical projections along the entire A-P extent of the thalamus in the following main nuclei: ventralis anterior, reticularis, ventralis lateralis, central lateralis-parafascicularis, centralis medialis (NCM), lateral portions of medialis dorsalis, ventralis medialis, ventralis posteromedialis, and centrum medianum. All projections were strictly ipsilateral except for a few which continued 1 to 3 mm into the left side dorsally at the level of NCM, and more posteroventrally at the level of the nucleus subparafascicularis and dorsal aspects of zona incerta. In all hemispherectomized cats the outstanding, apparently only difference was the presence of fibers crossing the midline and terminating in most of the hemi-thalamus ipsilateral to the ablation. The crossing also was at the two dorso-ventral levels seen in intacts but both portions were more extensive and dense. The dorsal portion terminated along the entire extent of the intralaminary nuclei, while the ventral one filled most of the remaining atrophic left ventral thalamus. These crossed projections were relatively sparse and diffuse, never reaching the high density seen for terminal field areas in the intact side. We are now using computer-assisted densitometry to quantify the extent of this novel crossed terminal distribution. This finding, with additional changes reported elsewhere, indicate that after hemispherectomy there is an extensive anatomical reorganization in subcortical structures which parallels the functional recovery seen.
(Supported by USPHS Grants HD-05958 and HD-04612)

204.11 **IMMUNOCYTOCHEMICAL DEMONSTRATION OF SUBSTANTIA GELATINOSA-LIKE REGIONS AND SEROTONERGIC AXONS IN EMBRYONIC SPINAL CORD TRANSPLANTS IN THE RAT.** Paul J. Reier and Barbara S. Bregman, Dept. Anatomy, Univ. Maryland School of Medicine, Baltimore, MD.

Recent studies in this laboratory have demonstrated survival and differentiation of embryonic rat spinal cord transplants introduced into intracerebral and spinal lesions of adult and neonatal rats. Currently, we are using immunohistochemical techniques to investigate the development and distribution of chemically-defined neuronal elements within these transplants and their innervation by identified neuronal populations of the host CNS. In the study reported here, we have examined whether any topographical differentiation occurs in spinal cord transplants and whether they become innervated by serotonergic fibers from the recipient CNS. Donor tissue was obtained from 14- and 15-day-old fetal rats and placed into either the choroidal fissure or subtotal lesions (i.e., dorsal funiculotomies or hemisections) of the spinal cords of 250 gm. rats. After 1-4 month survival periods, the recipients were sacrificed and tissues were prepared for either indirect immunofluorescence or peroxidase-anti-peroxidase visualization of neuronal processes exhibiting immunoreactivity for antisera (Immunonuclear Corp.) to Met-enkephalin, neurotensin or serotonin. The distribution of myelinated axons was also demonstrated with anti-MBP (myelin basic protein). In some experiments, tissues were prepared for electron microscopy. Sections stained with anti-MBP and plastic 2μm sections revealed numerous unmyelinated regions in these transplants containing small neurons (20-30μm in diameter). Sections stained with Met-enkephalin antiserum showed many immunoreactive fibers in the transplants whether the grafts were integrated with the host CNS or not. In many cases, intense staining was observed in regions corresponding to the myelin-free areas. Neurotensin-positive fibers were also seen with many being concentrated in the unmyelinated zones of the implants. Examination of transplants in lesions of the spinal cord showed that some of the implants which were partially confluent with the host neural parenchyma contained a sparse population of serotonin-positive fibers, whereas transplants which were completely isolated from the host spinal cord, as well as cerebral cortex, failed to exhibit any immunoreactive elements. These initial findings suggest that the distinct, unmyelinated regions which develop in embryonic spinal cord transplants share some morphological and immunohistochemical characteristics of the substantia gelatinosa. In addition, the presence of serotonin-like immunoreactive fibers indicates innervation of these transplants by at least one population of chemically-defined neurons in the host CNS.

(Supported by NIH Grant NS-13836)

204.12 SYNTHESIS AND TRANSPORT OF TWO RELATED PROTEIN MARKERS OF THE REGENERATING GOLDFISH VISUAL SYSTEM. T. S. Ford-Holevinski* and B. W. Agranoff. Neuroscience Laboratory, University of Michigan, Ann Arbor, MI 48109.

The goldfish retina responds to intraorbital axotomy by altered patterns of protein synthesis which can be studied by comparing rates of incorporation of labeled amino acids into protein in in vitro incubations of control (normal, N) and post-crush (PC) retinas. Alternatively, one can inject labeled precursor intraocularly and measure the subsequent arrival of axonally transported labeled proteins in the newly regenerated nerve tract and terminal field in the optic tectum (OT). The in vitro procedure has the advantage that early events following axotomy can be observed, and the drawback that the ganglion cells support only a fraction of total measured retinal protein synthesis. Once new nerve has been formed, the in vitro approach permits us to see only those proteins that are synthesized in the ganglion cells and then transported. The two approaches are complementary, as indicated in the present study. By autoradiography of 2D PAGE gels, we previously detected the presence of a closely-associated pair of axonally transported labeled proteins (M_r 68-70K; IEP=4.8-4.9) in the tectum of the PC, but not the N side. Like tubulin and actin, the two proteins are slowly transported and are found in the 10^5 x g supernatant of OT homogenates. In incubations with ^{3}H-leucine or ^{35}S-methionine, we now find that the 68-70K proteins are not labeled in N retinas, but appear within 4 days of crush, become more intensely labeled for the next few weeks, and disappear by 35 days PC. In mixing experiments, Coomassie Blue-stained spots identified in 2D gels of fish brain soluble proteins comigrated with the labeled retinal 68-70K proteins. By means of a sensitive silver stain, we have visualized the 68-70K proteins which give rise to the autoradiographic spots. However, N and PC retinas contain similar amounts of 68-70K proteins on the basis of their silver stain intensities. In contrast, silver-stained 2D gels of supernatants from regenerating optic nerve indicates prominence of the 68-70K proteins and their absence in the N nerve. Thus in the nerve, the silver stain for the presence of chemical amounts of the 2 proteins parallels autoradiographic findings, while in retina it does not. We are pursuing the identity and possible function of these regeneration-linked proteins. We also find evidence for enhanced synthesis of additional retinal proteins in regeneration, including one at 33K, possibly analogous to the "soluble" 33K growth-associated protein described in the regenerating toad optic nerve by Skene and Willard (J. Cell Biol. 89:86, 1981). (Supported by NIH Grant NS 13743.)

204.13 ADRENAL MEDULLA IMPLANTS IN THE ADULT RAT SPINAL CORD. C. L. Jones*, J. T. Buchanan*, and H. O. Nornes. Department of Anatomy, Col. State Univ., Ft. Collins, CO. 80523.

Neural grafting is an experimental technique for replacing specific missing neural input after lesioning the central nervous system. Since spinal catecholaminergic systems may be involved in the descending control of the stepping generator (Grillner, 1975, Physiol. Rev. 55: 247-304), attempts are being made to reinnervate the transected spinal cord with transplants of catecholamine-containing cells. The purpose of this study was to determine if chromaffin cells from the adrenal medulla (AM) survive implantation in the adult rat spinal cord.

The AM from 10 to 12 day old Sprague-Dawley rats were placed into surgically prepared cavities made in the upper lumbar spinal cord of Sprague-Dawley adult rats. In 12 host animals, the cavity was made subpially in the dorsolateral portion of the spinal cord (dorsolateral cavity) with a special pipette and gentle suction. In 15 animals, the cavity was made in the gray matter, leaving the dorsal white matter largely intact (central cavity). Survival times ranged from 7 to 121 days before the tissue was processed for Falck-Hillarp catecholamine histofluorescence.

Eleven of 12 dorsolateral cavity implants, and 13 of 15 central cavity implants had surviving fluorescent chromaffin cells. The number of cells per implant ranged from 30 to 3,000. Few chromaffin cells formed processes. The few processes that formed were 25 to 30 microns in length. Regeneration of host CA fibers into the implant was rarely observed. There was good tissue continuity between the host spinal cord and the implant, however, the interface contained a layer of collagenous tissue. (Supported by grants from the Paralyzed Veterans Administration, no. OBR-156, and Spinal Cord Society).

204.14 PROPERTIES OF PROTEINS ASSOCIATED WITH OPTIC NERVE REGENERATION IN THE GOLDFISH. Larry I. Benowitz and Ellen R. Lewis. Dept. of Psychiatry, Harvard Medical School, McLean Hopsital, Belmont, MA 02178.

Unlike mammals, the goldfish optic nerve will regenerate within 1-2 months of being damaged, to re-establish central visual connections and restore visually-guided behaviors. During the course of regeneration, marked changes occur in the complement of proteins transported intra-axonally in the optic nerve. In the rapid phase of transport, the group which includes membraneous components destined for the nerve terminals, the most marked increases that occur are for proteins with apparent molecular weights of 24-27K, 44-49K and 210K early in the regenerative process, and 110-140K at later stages (Benowitz, Shashoua and Yoon, J. Neuroscience 1:301, 1981). In the regenerating toad visual pathway, specific increases for membrane-bound, rapidly-transported proteins with apparent MW's of 24K, 43K and 50K have been described (Skene and Willard, J. Cell Biol. 89:86, 1981). Studies using 2-dimensional gel electrophoresis indicate that the greatest change occurring among the rapidly-transported proteins in the regenerating goldfish optic nerve actually involves a group of at least 3 proteins having molecular weights between 44-49,000 and highly acidic isoelectric points. These proteins are abundant in proline relative to methionine, are found in both the soluble and plasma membrane fractions of the optic tectum, and are associated with neuronal processes but not glia (Benowitz and Lewis, J. Neuroscience, in press). The 44-49K acidic proteins become turned on at least 50-100 fold in retinal ganglion cells undergoing axonal regeneration. This increase occurs regardless of whether or not the optic tectum is present (Benowitz, Yoon and Lewis, in prep.; Yoon et al., these Abstracts). The presence of the appropriate target field, the optic tectum, does, however, regulate the abundance of other rapidly-transported components, in particular proteins of 120-160,000 daltons (pI 5.2-5.4) (Yoon et al., these Abstracts). We are currently investigating additional properties of these various proteins associated with the regenerative process, and examining their relationships to identified protein species. Supported by NINCDS Grant R01 NS16943.

205.1 ATTEMPTS TO MAINTAIN ABERRANT RETINO-RETINAL AXONS AFTER OPTIC NERVE REGENERATION IN THE FROG. D.J. Stelzner and J.A. Strauss*. Dept. of Anatomy, Upstate Medical Center, Syracuse, N.Y. 13210

After optic nerve crush (NC) in adult Rana pipiens, optic axons are misrouted out the opposite (opp.) optic nerve and into the ganglion cell fiber layer (GCFL) of that retina. These retino-retinal (ret-ret) axons are in greatest number 4-6 wks. after NC and most have disappeared 3 mo. p.o. (J. Comp. Neurol. 196: 605-643). Two expts. were done attempting to maintain this projection. Autoradiography was used to assess regeneration killing frogs at various p.o. periods 24 hrs. after intravitreal ^{3}H-proline injection (40 µCi/4 µl saline) ipsilateral to NC.

In the 1st expt., kainic acid (KA, 5 ng/4 µl saline) was injected intravitreally to denervate ganglion cells in the eye opp. NC. Pilot work showed that 4d after KA injection, many inner nuclear layer cells had degenerated and heavy degeneration filled the collapsed inner plexiform layer. Most ganglion cells survived. In 18 frogs, NC was performed and KA injected into the opp. eye at the same time (N=8, NC-KA) or 4-6 wks. later when ret-ret axons are maximal (N=10, NC-dKA). Controls showed that axons filled the opp. nerve and ret-ret axons were present 5-6 wks. after NC-KA(N=5). In all frogs studied 3 mo. after NC-KA (N=3), and in most frogs studied 4-6 mo. after NC-dKA(N=7), no evidence was found that KA induced denervation of ganglion cells maintained ret-ret axons. However, in 3 frogs 4(N=1) and 6(N=2) mo. after NC-dKA some labeled axons remained within the nerve and within the GCFL for up to 1 mm around the optic disc. The ganglion cells in this region were disorganized and silver grains distributed among them suggested that actual connections may have formed. Thus, denervating ganglion cells can maintain ret-ret axons but the effect is small and timing may be critical.

In the 2nd. expt. right NC and bilateral optic tectum and posterior thalamic lesions were made to remove the targets of the majority of regenerating optic axons. Right optic axons were studied 6 wks.(N=5), 3 mo.(N=6) and 6 mo.(N=2) later. Ret-ret axons were found at 6 wks. p.o. but the number was less than that seen with NC or NC-KA. Most label in the opp. nerve was along its edge. Three mo. p.o. axons were heavily labeled within the opp. nerve but appeared trapped at the optic disc and did not enter the GCFL. Six mo. p.o. few labeled axons remained in the opp. nerve. Even though bilateral tectal ablation did not maintain ret-ret axons, projections were formed bilaterally in the middle thalamic neuropil and isthmal nuclei, normally targets of tectal efferent axons and, particularly at 3 mo. p.o., within the telencephalon and brainstem.

Thus, most ret-ret axons remain transient even when normal optic targets are missing or when ganglion cells in the opposite retina are denervated. (Supported by NS 14096)

205.2 EFFECTS OF DOUBLE TRANSECTION ON REGENERATION OF LAMPREY SPINAL AXONS. H.S. Yin and M.E. Selzer, Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Following transection, the spinal axons of sea lamprey larvae (Petromyzon marinus) regenerate for short distances past the scar, but then stop. This might be explained if the zone of injury exerted a trophic effect on the growing axon tip. To test this hypothesis, double simultaneous transections, 4-17 mm apart were made and animals allowed to recover 59-88 days. Using intra-axonal injection of HRP, the distance of regeneration was determined for giant reticulospinal axons. When these axons are singly cut high in the cord (i.e. close to their cell bodies), they ordinarily regenerate vigorously (up to 5 mm). Double transections in the rostral cord did not significantly affect the distance of regeneration. However, in the caudal cord where single transection is usually followed by meager regeneration, double transection was followed by an increased distance of regeneration. In single transected animals, none of 11 injected axons regenerated back to the scar after initial die-back. In contrast, of eight neurites in double caudal transected animals, four regenerated past the scar, the longest reaching 1.3 mm below the transection.

We also stained dorsal cells and giant interneurons located between the two transection sites. Both cell types ordinarily project, and also regenerate, rostralward. This pattern was unaffected by the presence of a second lesion caudal to the axotomized cells. In 16 injected dorsal cells, the distal end of the regenerating neurite was oriented rostralward, and only one neurite looped caudalward as it approached the scar. Of eight injected giant interneurons, seven had rostrally projecting regenerating neurites and one had no stained axon. Even when retrograde axonal degeneration was complete and axon-like neurites grew from posteriorly oriented dendrites, these neurites looped and regenerated rostralward.

These results are consistent with the hypothesis that, in some circumstances, the zone of injury may exert a trophic effect on the magnitude of axonal regeneration, but probably does not determine the directional specificity of growth.

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205.3 EFFECT OF MULTIPLE LESIONS ON REGENERATION OF GOLDFISH OPTIC AXONS. Janet R. Sparrow and Bernice Grafstein. Dept. Physiology, Cornell Univ. Med. College, New York, N.Y. 10021

Axonal regeneration may be accelerated in neurons subjected to a previous "conditioning" lesion. This effect is particularly marked in the goldfish optic nerve: the rate of regeneration is doubled if a conditioning lesion precedes the second lesion (testing lesion) by 2 weeks (McQuarrie and Grafstein, Brain Res. 216:253, 1981) and less pronounced increases are observed with shorter intervals (Edwards et al, Exp. Neurol. 72:672, 1981). Since a single conditioning lesion has such a dramatic effect we sought to determine whether the effects of a series of lesions can be cumulative and what the maximum change in the rate of axonal outgrowth might be.

Goldfish were subjected to optic tract transection and/or nerve crushing made at varying intervals. Either single (testing lesion alone), double (conditioning lesion and testing lesion) or triple (2 conditioning lesions and a testing lesion) lesions were made. During regeneration following the testing lesion, we measured 1) the outgrowth distance of the optic axons from the distribution of radioactively labeled axonally transported protein after ^{3}H-proline injection into the eye, 2) the amount of fast transported protein in the nerve, and 3) the accompanying changes in the size of the retinal ganglion cells (RGC).

In nerves receiving one conditioning lesion 14 days before the testing lesion, the rate of outgrowth following the testing lesion was doubled, but not further enhancement of outgrowth was evinced by a third lesion given 7 or 14 days before the conditioning lesion: perhaps with one conditioning lesion given 14 days before a testing lesion the axons elongate at the maximum rate attainable. When the interval between the conditioning and testing lesions was shortened (i.e. to 7 or 14 days) so that the conditioning lesion caused a submaximal increase in the rate of outgrowth, another lesion given either 3 days after or 3 days before the original conditioning lesion produced a further acceleration of outgrowth. In the latter experiment the increase in RGC area was also greater with a triple lesion than with a double lesion, but the amount of fast transported protein was the same in both the triple- and double-lesioned nerve.

It is concluded that regenerating goldfish optic neurons can respond to more than one conditioning lesion when the individual lesions produce a submaximal enhancement of axonal outgrowth.

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205.4 REGULATORY ROLE OF THE SYNAPTIC TARGET TISSUE DURING REGENERATION OF THE GOLDFISH VISUAL SYSTEM. Dana Giulian (SPON: H. Epstein) Department of Neurology, Baylor College of Medicine, Houston, Texas 77030

Transection of goldfish retinal ganglion cell axons initiates a regenerative process that leads to the regrowth of axons, new synapse formation within specific regions of the optic tectum, and the recovery of sight. During this regeneration, ganglion cells of the retina and glia of the optic tract have dramatic increases in new protein biosynthesis. Removal of the optic tectum decreases glial biosynthetic activity during an early phase of regeneration and alters production of a high molecular weight ganglion cell protein during the late phase. Such data suggest that the early phase, or that time prior to axonal regrowth into the target tissue, may be mediated by the release of tectal humoral factors acting upon optic tract glia. In contrast, ganglion cells are influenced by the target at a later regenerative phase, perhaps through cell-to-cell contact mechanisms. In support of this model, further study shows that a trypsin sensitive peptide of less than 20,000 daltons recovered from the tecta of regenerating visual systems stimulates protein biosynthesis of optic tract glia.

205.5 INCREASED FIBROUS COLLAGEN AND REDUCTION OF AXONAL GROWTH INHIBITORS ARE ASSOCIATED WITH ENHANCED REGENERATION, M. Schwartz, V. Lavie* and I. Rachailovich*, Dept. of Neurobiology, the Weizmann Institute of Science, Rehovot 76100, Israel.

We have recently established conditions to study the contribution of non-neuronal cells to the process of goldfish optic nerve regeneration. We adapted the use of X-irradiation to restrict the division of non-neuronal cells associated with the injured nerve. Regenerative capacity was determined subsequent to X-irradiation. At an early period post injury X-irradiation resulted in increased capacity of the corresponding retinae to sprout, in vitro. In this work, we present analysis of events associated with the enhanced regenerative capacity.

Observations at the electron microscopic level of an injured nerve following X-irradiation revealed a striking enlargement of the endoneurial extracellular space which was filled with fibrous collagen.

The observed increase in collagen at the EM level was supported by our finding of a 3-fold increase in ^{3}H-proline incorporation by the non-neuronal cells associated with a segment of X-irradiated injured nerve. The direct association between the appearance of fibrous collagen and the increased ability to regenerate was shown by treating the fish whose optic nerve had been injured with β-aminopropionitrile (BAPN), an inhibitor of the enzyme lysyl oxidase. This leads to disrupted formation of collagenous fibers. Treatment with BAPN was started when X-irradiation was performed. We found that such treatment resulted in a marked decrease in the capacity of the corresponding retinal ganglia cells to sprout, in vitro. The inhibitory effect on X-irradiated regenerating retinae was 3-fold higher than on regenerating retina. It should be noted that the BAPN treatment had no effect on the incorporation of ^{3}H-proline. These results further emphasize the importance of collagenous matrix for regeneration.

The increase in collagen was accompanied by changes in the profile of substances released by the surrounding non-neuronal cells. This included a drastic decrease in the level of axonal growth inhibitors as shown by the bioassay on regenerating retinal ganglia cells, in vitro.

The reduction in the level of axonal growth inhibitors and the increase in collagen at the same time may open a new perspective relating to the capacity of neurons to regenerate. Whether these two events are directly or indirectly related remains to be seen.

205.6 NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF GANGLIOSIDES IN RATS WITH A UNILATERAL LESION OF THE MAGNOCELLULAR FOREBRAIN NUCLEI (MFN). G. Pepeu, L. Bracco*, G. Lo Conte*, L. Bartolini* and F. Casamenti*. Departments of Pharmacol. and Neurol. Florence Univ. 50100 Florence, Italy.

It has been shown (Wojcic, M. et al., Neurosci., 7: 495, 1982) that i.m. administration of gangliosides stimulates the recovery of choline acetyltransferase (CAT) and acetylcholinesterase in the hippocampus of rats after a septal lesion. In order to demonstrate whether ganglioside-stimulated CAT recovery was associated with functional recovery, 30 mg/Kg i.m. of GM_1 ganglioside (Fidia Res. Lab.) were administered daily for 22 days to adult rats with a unilateral electrolytic lesion of the MFN. This lesion was followed by a decrease in the ipsilateral cortical CAT activity and an impairment of passive and active conditioned avoidance responses (Lo Conte, G. et al., Pharmac. Biochem. Behav., 17:933, 1982). The rats received daily sessions in a shuttle box from day 10 to day 22 after the lesion. On day 22 they were also trained in a one-trial passive avoidance task. The rats were then killed by decapitation and brain samples were removed for CAT activity and noradrenaline (NA) level determination. Histological controls for lesion size and placement were also carried out.

The ipsilateral decrease in CAT activity induced by the lesion was significantly reduced by ganglioside treatment. Instead of a 39 and 20% decrease found in the ipsilateral frontal and parietal cortex of saline treated rats, in the ganglioside treated rats a significant decrease was detected only in the ipsilateral frontal cortex. Conversely, a significant increase was found in the ipsilateral parietal (+16%) and controlateral frontal (+36%) and parietal (+35%) cortices. The lesion brought about an increase in controlateral NA level. The ganglioside only induced a further small increase in the controlateral frontal cortex. The impairment of the passive conditioned avoidance response was not modified by GM_1 treatment. Conversely, the lesioned rats treated with GM_1 always performed significantly more active avoidance responses than the controls.

In conclusion, repeated GM_1 administrations appear to stimulate the recovery of lesioned cholinergic pathways associated with a partial functional restoration.

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205.7 EFFECT OF PERIPHERAL NERVE INJURY ON REGENERATION OF AXONS IN THE DORSAL COLUMN OF THE SPINAL CORD. P.M. Richardson. Division of Neurosurgery, Montreal General Hospital and McGill Univ., Montreal, Canada.

To investigate the regeneration of spinal axons ascending from lumbar dorsal root ganglia (DRG), a dorsal incision was made in the rat spinal cord at high cervical level. As a favorable substratum for axonal growth (Soc. Neurosci. Abstr. 8:869, 1982), one end of a peripheral nerve segment was grafted in the midline to the cut spinal cord. Each graft was taken from the right sciatic nerve of the same rat and the left sciatic nerve was not injured. Two months after grafting, horseradish peroxidase (HRP) was injected into the grafts as a retrograde tracer for neurons with regenerating axons. Particular attention was paid to neurons in the L4 and L5 DRG some of which have peripheral axons in the sciatic nerve and central axons reaching the spinal cord. HRP-containing neurons were consistently present in the right L4 and L5 DRG but rarely on the left. These observations suggest that peripheral axotomy of DRG neurons enhances regeneration of their simultaneously injured spinal axons. (Supported by the Medical Research Council of Canada and Multiple Sclerosis Society of Canada.)

205.8 PHYSIOLOGIC PROPERTIES OF RAT BRAINSTEM NEURONS REGENERATING AXONS INTO PERIPHERAL NERVE GRAFTS. M. Munz, M. Rasminsky & A.J. Aguayo. Neurosciences Unit, Montreal General Hospital, Montreal, Quebec.

The regenerative potential of CNS neurons becomes apparent when peripheral nerve grafts are inserted into various areas in the neuraxis (Aguayo et al, Adv. Cell. Neurobiol. 3,215:1982). We have now made physiological recordings from axons regenerating from CNS cells into peripheral nerve grafts.

Autologous grafts of 4 cm lengths of the left peroneal nerve were inserted into the right lower lateral medulla of adult rats, the distal end of the graft ending blindly. After 10 to 24 weeks had elapsed to allow graft reinnervation from brainstem neurons (David & Aguayo, Science 214,931:1981; Munz & Aguayo, Soc. Neurosci. Abstr. 8,869:1982), grafts were reexposed and biphasic extracellular recordings were made from the desheathed grafts.

1) Spontaneous activity: in most preparations little spontaneous efferent activity was recorded from the grafts. The enhancement of spontaneous activity by systemic administration of picrotoxin suggested that brainstem neurons regenerating axons into the grafts are tonically inhibited by GABA mediated input. Some spontaneously active units fired in bursts immediately preceding respiratory muscle activity. Such units presumably reflect activity in respiratory modulated brainstem neurons.

2) Physiologically evoked activity: stimulation of mystacial vibrissae and facial skin elicited responses from putative trigeminal nucleus neurons projecting into the grafts. After adminstration of picrotoxin, responses were elicited by touching the skin in various areas of the body, the stimuli presumably exciting dorsal column nucleus neurons projecting into the grafts.

3) Electrically evoked activity: electrical stimulation of limb nerves evoked unitary responses. Two criteria suggested that such responses were synaptically mediated: i) the latency usually showed appreciable jitter; ii) a collision test in which the responding unit was stimulated in both the periphery and on the graft itself indicated interposition of at least one synapse between the periphery and the graft. Electrical stimulation of limb nerves also caused inhibition of firing of some spontaneously active units for periods of up to 400 msec. Both excitation and inhibition showed somatotopic specificity, being elicited by electrical stimulation of single limbs or nerves in limbs. Presumed reticular units were observed to respond with an increase in spontaneous firing rate to electrical stimulation in the periphery.

We conclude that brainstem neurons regenerating axons into peripheral nerve grafts retain many of the physiological properties of normally functioning neurons in intact animals.

205.9 RAT VISUAL SYSTEM NEURONS GROW AXONS ALONG PNS GRAFTS. U. Bünger* and A.J. Aguayo (SPON: A. Peterson). Neurosciences Unit, The Montreal General Hospital and McGill University, Montreal, Canada.

Axons from neurons in different regions of the adult rat brain have been shown to elongate extensively along PNS grafts (Nature 296: 150, 1982). In this study we examined the regrowth from neurons in the rat visual system.

In adult Sprague-Dawley rats a 2 to 3.5 cm long segment of autologous sciatic nerve was taken and implanted either into the visual cortex, the superior colliculus, or the thalamus in the vicinity of the lateral geniculate nucleus. The other end of the graft was sutured to nearby muscles. From 7 to 15 weeks after grafting, the extracranial portion of the graft was re-exposed, transected and HRP applied to its tip to retrogradely label cells whose axons had grown along the graft.

Labelled nerve cells were found in all three regions of the visual system as well as nearby areas such as other thalamic nuclei and hippocampus. The population of labelled cells in the visual cortex was distributed over layers II to VI with the greatest proportion of cells found in layers IV and V, the diameter of the cells ranging between 12 and 37 μm. In the superior colliculus labelled cells have been found in deep and superficial strata. The yield of labelled cells in the lateral geniculate nucleus is very low, only a few cells have been found. In all cases the labelled neurons were in close proximity to the graft, the position of the graft apparently influencing the location of labelled cells. The total length of the newly grown axons from these labelled neurons is approximately 2 cm.

These results indicate that there are nerve cells in several areas of the adult rat visual system that have the capacity for axonal growth under the conditions provided in these experiments. This potential for growth appears to be expressed when these neurons intrinsic to the brain interact with non-neuronal components of the PNS.

205.10 HISTOPATHOLOGICAL CHANGES IN DEVELOPING NEURAL TRANSPLANTS. J. Brasko* and G.D. Das* (SPON: R.P. Maickel). Dept. of Biological Sciences, Purdue University, West Lafayette, IN 47907.

It is well established that embryonic neural tissues may be successfully transplanted into the brains of neonatal and adult host animals. Following transplantation, both the donor and host tissues undergo a series of histopathological and developmental changes which ultimately result in the achievement of fully grown, integrated transplants. The exact nature, magnitude, and time course of these changes are not known. In this study, we report qualitative and quantitative findings on these histological phenomena of developing neural transplants.

Neocortical tissue, 3.5 mm^3 in volume, from 17-day rat embryos was transplanted into the cerebellum of 30-day and 3-month old hosts (Das, '74, '79). Hosts were sacrificed 1, 3, 5, 7, 9, 12, 15, 20, 25, and 30 days following surgery, and their brains were processed for cresyl-violet, hematoxylin and eosin, and Fink-Heimer histology.

The results demonstrated the presence of areas of viable transplant, necrotic transplant, blood, and CSF filled space in the host cerebellum on day 1 after transplantation. C-V and H & E material showed that the portion of viable transplant underwent sequential phases of regression, recovery, and growth, with an initial decrease in volume on days 1 and 3, followed by a steady increase which plateaued by day 20. The volumes of necrotic transplant and blood increased from days 1-3, but rapidly declined to 0 between days 5-7. The volume of CSF filled space increased to 0.5 mm^3 by day 3, a volume which was maintained through day 9 before falling to 0 by days 12-15. The viable portion of the transplants grew initially by proliferation of the surviving neuroepithelial cells, and subsequently by dispersion and differentiation of neuroblasts.

An additional assessment of the host brain reaction to the developing transplant was obtained from Fink-Heimer processed brains. While there was no degeneration argyrophilia present within the transplants at any time, a significant amount of degeneration persisted in laminae of the cerebellum apposed to the transplant, as well as in the deep cerebellar nuclei, up to day 30. Argyrophilic staining was also observed in folia not directly in contact with the transplant throughout the developmental period studied, though the nature of this staining differed qualitatively between the short and long survival times.

These observations indicate that transplantation of neural tissues causes trauma to both the transplant and the host brain. With the proper techniques of transplantation these histopathological changes can be kept to a minimum.

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205.11 NEURAL REGENERATION AND FUNCTIONAL RECONNECTION FOLLOWING OLFACTORY NERVE TRANSECTION IN HAMSTER. R.M. Costanzo, Department of Physiology & Biophysics, Medical College of Virginia, Richmond, VA 23298

The olfactory sensory neurons in the vertebrate nervous system are unique in that they undergo continuous neurogenesis and replacement. Anatomical studies have shown that transection of the olfactory nerves leads to a degeneration of sensory neurons followed by a neurogenesis and replacement with newly formed cells. Replacement neurons grow axonal processes that are capable of re-establishing morphological connections with cells in the olfactory bulb.

To determine the functional capacity of these anatomical reconnections, single unit responses to odor stimuli were recorded from the olfactory bulb following recovery from unilateral olfactory nerve transection. A total of 56 units were studied, taken from hamsters with recovery times of 4, 35, 60, 90, 120, 180 and 270 days. At day 4, although there was spontaneous activity recorded from bulb units on the experimental side (N=10), they did not respond to stimulation of the olfactory epithelium with odors. Control units (N=9) from the unoperated side of the same animals showed normal odor responses. By day 35 some of the units tested responded to odor stimulation, indicating that connections had been reestablished with sensory neurons. With longer recovery times an increasing percentage of units responded to odor stimuli. In addition, concentration response functions showed that units were capable of signaling differences in stimulus intensity. The response of units to four odors (amyl acetate, 1-butanol, ethyl acetate, and ethyl butyrate) showed differences in odor selectivity, suggesting their ability to discriminate among odors.

Histological verification of the extent of nerve transection was made possible by use of teflon markers. Nerve connections observed above these markers were assumed to come from newly reconnected receptors and presumably provided the pathway for stimulus information to reach bulb units.

These results demonstrate that following nerve transection olfactory neurons are capable of reestablishing connections with the olfactory bulb and, furthermore, that these connections make functional synapses that transmit sensory information to second order cells. These findings in the olfactory system suggest that regeneration and repair of neural pathways within the central nervous system are possible.

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205.12 GM1 GANGLIOSIDE FACILITATES FUNCTIONAL RECOVERY AFTER AN ENTORHINAL LESION; INCREASE IN ACETYLCHOLINESTERASE IN DENTATE GYRUS S.E. Karpiak, F. Vilim & S.P. Mahadik. Division of Neuroscience, NYS Psychiat. Inst. & Depts. of Psychiatry & Biochemistry, Coll. of Physicians & Surgeons, Columbia U., N.Y., N.Y. 10032.

Exogenous gangliosides have been reported to enhance both neurite outgrowth and regeneration (in vitro & in vivo). We had examined the effects of total brain ganglioside on the course of recovery of alternation behavior after an entorhinal cortical lesion in rat (1). It was found that the injection of gangliosides (I.M.:50mg/kg) facilitates recovery of the alternation behavior as seen in: 1) reduction in the initial behavioral loss after lesioning; 2) return to pre-lesion performance levels sooner than controls and 3) long-term improved behavioral performance. Using the same model system, we have now examined the effects of GM1 ganglioside on functional recovery. Male Sprague-Dawley rats (180gr), after 4 days of training on a conditioned alternation behavior, received a unilateral entorhinal cortical lesion. On day 3 of training rats began receiving daily injections (I.M.: 10mg/kg) of GM1 ganglioside. Controls were injected with saline. Results show that GM1 administration significantly limits the magnitude (34% fewer errors than controls) of behavioral deficit seen 24hrs (day 5) after the entorhinal lesion. Return to pre-lesion performance levels was achieved by day 8 for GM1 rats and day 11 for controls. At 2 wks post-lesion the GM1 treated rats made less than 1 error daily, while controls made 2-3 errors daily. Recovery of the alternation behavior has been reported to be dependent upon collateral sprouting of glutaminergic afferents into the denervated dentate. We are presently measuring changes in levels of glutamate (HPLC) in the dentate. However, we have examined the cholinergic input, which also reinnervates the denervated molecular zone of the dentate. To assess whether gangliosides affect the course of this cholinergic reinnervation, AChE was assayed in the dentate gyrus of rats 7 days after a unilateral entorhinal lesion. Rats were injected daily with GM1 (10mg/kg:I.M.) and controls received saline. Controls showed AChE levels of 4.8 0.19±μmoles/hr/mg protein and GM1 rats had levels of 5.9±0.22 μmoles/hr/mg protein, representing a 25% increase above control levels. In summary, we have found that treatment of rats with GM1 ganglioside, like total ganglioside, facilitates functional recovery after an entorhinal lesion and accelerates cholinergic reinnervation of the dentate gyrus. It is hypothesized that gangliosides accelerate functional recovery after an entorhinal lesion by facilitating sprouting.

1. Karpiak, S.E. Ganglioside treatment improves recovery of alternation behavior following unilateral entorhinal coretx lesion. Experimental Neurology, (in press), (1983).

206.1 DISORGANIZATION OF OPTIC NERVE PROJECTIONS MEASURED WITH THE THE VEP: IS IT DUE TO HYPOPIGMENTATION?
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It has been consistently reported that the optic nerve (ON) fibers do not cross-over in a normal manner in mammals with albinotic fundi (hypopigmentation). It has been found that pattern VEPs recorded from human albinos support the view that there exist abnormal routing of ON projection at the optic chiasm. The authors of these reports have postulated that this developmental disorganization of ON fiber projections was associated with the hypopigmentation of the retina.

We have recently tested an individual who, although exhibiting some clinical signs of ocular albinism, such as pendicular nystagmus, low visual acuity and a poorly developed fovea (hypoplasia), did not display any sign of ocular or skin hypopigmentation. We conclude that he should be classified as an example of "isolated foveal hypoplasia".

The fact that this individual, like albinos, demonstrated a marked asymmetry in the amplitude of the monocular pattern VEP recorded from the right and left occipital hemispheres leads us to conclude that abnormal foveal development rather than a mellanin defect, may be the variable responsible for the misrouting of ON projections at the chiasm.

206.2 DENDRITIC AND SYNAPTIC CHANGES IN THE DORSAL LATERAL GENICULATE NUCLEUS AFTER CUTTING ONE OPTIC NERVE IN KITTENS. J.A. Robson.
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In the dorsal lateral geniculate nucleus of cats each lamina is innervated by only one eye and previous studies have shown that removal of an eye in newborn kittens results in severe transneuronal atrophy in the denervated laminae. However, some neurons in the denervated laminae do not atrophy and it has been hypothesized that these "surviving large cells" escape the atrophic effects of deafferentation because they are innervated by axons from the intact eye (Guillery, J. Comp. Neurol., 146:407, 1972).

To test this hypothesis kittens had one eye removed in the first two weeks after birth. Nine to 12 months later neurons in the dorsal lateral geniculate nucleus were filled with horseradish peroxidase (HRP) via injections into the optic radiations. In some animals retinal projections from the intact eye were also demonstrated autoradiographically. To permit combined light and electron microscopic analysis of the HRP-filled neurons, diaminobenzidine was used as a chromogen and every third section was embedded in plastic. The other reacted sections were mounted on glass slides and, in the appropriate animals, processed for autoradiography.

Surviving large cells were densely filled with HRP in several animals. Most of these cells are located near a normally innervated lamina and resemble Guillery's class 1 cells (J. Comp. Neurol., 128:21, 1966). They have large somata and radiate dendrites which give rise to few appendages. In all cases these neurons contribute a large portion of their dendritic tree to regions innervated by the intact eye either by growing dendrites into a normally innervated lamina or by growing them into a region demonstrated autoradiographically to contain translaminar axonal sprouts from the intact eye (Guillery, J. Comp. Neurol. 146:407, 1972; Robson, J. Comp. Neurol., 195:403, 1981).

Electron microscopic studies of the labeled dendrites of surviving large cells provide direct evidence that they are innervated by the intact eye. These dendrites are postsynaptic to axonal profiles that have the cytological characteristics of the terminals of retino-geniculate axons - these profiles have round synaptic vesicles and pale-appearing mitochondria and they are always presynaptic, forming asymmetric contacts. As in normal cats some of these terminals are large and contact the proximal dendrites of labeled cells, often in glomerular complexes, while others are smaller and contact more peripheral dendritic segments. (Supported by EY-03940 and the Alfred P. Sloan Foundation.)

206.3 SEQUENCE OF SYNAPTIC FORMATION IN THE PERIFOVEAL REGION OF THE RHESUS MONKEY RETINA: QUANTITATIVE AND SERIAL SECTION ELECTRON MICROSCOPIC ANALYSES. Y. Nishimura* and P. Rakic (SPON: P. S. Goldman-Rakic), Sec. of Neuroanatomy, Yale University School of Medicine, New Haven CT 06510

The perifoveal region of the retina was analysed by electron microscopy in 19 rhesus monkeys ranging in age between the 45th embryonic day (E45) and 3rd postnatal year. Two EM probes (photomontages) consisting of overlapping electromicrographs were made across the full thickness of the retina for each age. The appearance and density of synaptic contacts were determined in the inner (IPL) and outer (OPL) plexiform layers. In addition, in younger embryos, where the type of synapse was difficult to classify, up to 40 serial ultrathin sections were made to trace the origin and nature of pre- and postsynaptic elements. The IPL becomes recognized at E65 and the OPL appeared one month later (E99) as a thin acellular zone composed of immature neurites. Also, synaptogenesis begins earlier in the IPL than in the OPL. In the IPL, the first conventional synapses (paired membrane specializations accompanied with synaptic vesicles) are recognized in the E78 specimen. Three dimensional reconstructions revealed that at this age all the synapses in the IPL, except for those whose origin could not be identified, occurred between amacrine and ganglion cells. Synapses between pairs of amacrine cells and between amacrine and bipolar cells were observed at E91. The first immature ribbon synapses (complexes between bipolar, amacrine and ganglion cells) were recognized in the IPL of the E105 specimen. Initially, prospective ribbons have the form of dense spherical aggregates and only assume their bar shape by E114. In the OPL, the first synaptic junctions associated with vesicles were observed in the E105 specimen. Although synaptic vesicles were observed in the terminals of photoreceptors cells, they were not as yet associated with the ribbon bar. The typical ribbon synapses consisting of a bar surrounded by synaptic vesicles were first seen at E130. Thus, from this and earlier studies on synaptogenesis in the lateral geniculate nucleus (Hendrickson and Rakic, 1977) and superior colliculus (Cooper and Rakic, 1981) it appears that synaptic connectivity of the primate visual system is initiated in central structures, followed two to three weeks later by formation of contacts between ganglion and bipolar cells in the IPL, and still almost one month later between bipolar and photoreceptor cells. This central to peripheral sequence of synaptogenesis is somewhat unexpected in view of presumed peripheral control of sensory connectivity. Furthermore, local circuits between amacrine and ganglion cells may occur before true line projections from receptors via bipolar cells to ganglion cells. Supported by EY 02593.

206.4 DISTRIBUTION OF CELLS PROJECTING TO THE SUPRAGENICULATE NUCLEUS OF CATS, T. P. Hicks, Wm. Fletcher*, and C.A. Smith* Depts of Medical Physiology and Anatomy, The University of Calgary Faculty of Medicine, Calgary, Alberta, Canada.

Single neurones in the suprageniculate nucleus (SGn) of cats can be activated by visual stimuli. These visually responsive cells have identifiable receptive fields and each responds preferentially to particular light stimuli. Since there is no evidence that the SGn receives direct input from the retina, it was considered important to attempt to determine by neuroanatomical methods the sites of cell bodies sending projections to this nucleus.

Unit activity was recorded with glass micropipettes filled with a 20% buffered solution of HRP in 0.2 M NaCl. When a visually -responsive neurone was located below 15 mm depth from the cortical surface, its receptive field was plotted and the ocularity determined. Ejecting currents of between 0.5 and 2.0 μA were used to expel HRP for about one hour. Animals were perfused and the entire brain removed for cutting in 60 μm-thick sections. All sections were reacted according to the TMB method, were mounted on slides and counterstained with neutral red.

Neurones labelled with HRP reaction product were found in a variety of cortical and subcortical sites. Some of these sites included the intermediate division of the superior colliculus and the medial zone of the pretectum, as well as other pretectal sites less easily identified but associated closely with the medial pretectum. Relatively intense labelling could also be observed in the substantia nigra (reticulata) and in and around the periaqueductal grey (dorsal aspect). Cells were detected in the anterior ectosylvian gyrus and within both banks of the anterior ectosylvian sulcus, with greater labelling being observed in the lateral than in the medial bank. It appears from these data that cortical and tectal inputs are likely to provide at least a major component of the visual information giving rise to the physiological properties of SGn neurones.

(This work was supported by the Alberta Heritage Foundation for Medical Research and the MRC of Canada)

206.5 THE LONG TERM BENEFITS OF REVERSE OCCLUSION IN PROMOTING RECOVERY FROM MONOCULAR DEPRIVATION IN KITTENS. K. Murphy*, D.E. Mitchell and M. Kaye*. Dept. of Psychology, Dalhousie University, Halifax, N.S., Canada.

While the behavioral, anatomical and physiological consequences of monocular deprivation can be extremely severe, considerable recovery can occur if at the time that patterned visual input is restored to the deprived eye, the other eye is occluded (reverse occlusion). Recovery is substantial if this procedure is initiated in kittens at 4 or 5 weeks of age. We have examined the permanence of this recovery in kittens that were monocularly deprived from near birth until either 4, 5, or 6 weeks of age and reverse occluded for periods of from 9 to 84 days. Daily measurements of the grating acuity of each eye made upon restoration of binocular visual input revealed a rapid and reciprocal change in the visual acuity of the two eyes. In the majority of cases the acuity of the initially deprived eye fell dramatically within 2 weeks of termination of reverse occlusion, erasing much of the gains in vision made during this period. At the same time the acuity of the other eye improved to approach normal levels. However, in some cases the acuity of this eye remained substantially below normal. Morphological recovery in the LGNs of these animals as revealed by measurements of cell cross sectional area made on completion of behavioral testing, was significantly less than that observed in other animals on whom measurements were made immediately upon termination of reverse occlusion. Similarly, physiological recordings from the visual cortex (area 17) of selected animals revealed a substantial bias of ocular dominance toward the eye that was open during the initial period of monocular deprivation rather than the deprived eye as would be observed immediately following the period of reverse occlusion. Thus, neither the morphological recovery observed in the LGN or the substantial shifts of ocular dominance towards the initially deprived eye that occur during the period of reverse occlusion appear to be permanent. These various findings suggest that the neural connections formed during the initial period of occlusion are different in a fundamental way from those functional connections established with the deprived eye during the period of reverse occlusion.

206.6 **EXPANDED RETINOGENICULATE PROJECTIONS IN THE CAT FOLLOWING PRENATAL UNILATERAL ENUCLEATION: FUNCTIONAL AND ANATOMICAL ANALYSES OF AN ANOMOLOUS INPUT.** **R. W. Williams and L. M. Chalupa**
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In the adult cat, crossed and uncrossed retinal projections to the lateral geniculate nucleus (DLGn) are segregated within separate cell layers; crossed fibers are limited to laminas A, C and C2, uncrossed fibers to A1 and C1. In contrast to the mature pattern, early in development the retinal input is widespread and fibers from the eyes are intermixed.

The removal of an eye 2-3 weeks before birth (between embryonic days **E-42** and **E-51**) disrupts the formation of the normal lamination pattern: the DLGn is composed of a single thick magnocellular layer (which may correspond to a composite A/A1 layer), and a ventral parvocellular layer. The ipsilateral nucleus is at least 20% smaller than its contralateral mate. Intraocular injections of HRP demonstrate that the retinal influx from the spared eye of cats enucleated on **E-42** and **E-49** arborizes throughout the entire ipsilateral and contralateral DLGn. This diffuse input is thought to result primarily from a failure of fibers to withdraw to their normal domain--the fetal pattern of input is maintained into maturity--and secondarily, from the compensatory sprouting of the remaining optic axons into sparsely innervated regions.

Is this extensive and aberrant retinal projection fully functional? Does the DLGn have a normal visual field organization? In order to answer these questions, extracellular single cell recordings were made in the DLGn of 4 adult animals from which an eye had been removed between **E-45** and **E-51**. For comparison we also studied 8 normal cats and 2 cats from which an eye was removed on the sixth postnatal day. All regions of the DLGn of the prenatally enucleated animals appear to receive functional innervation. In 44 electrode penetrations through the DLGn no visually 'silent' regions corresponding to denervated remnants of layers A or A1 (or to the far lateral monocular segment of the ipsilateral nucleus) were noted. However, in postnatally enucleated animals denervated regions were consistently documented. The topographic order of the expanded projection was, given the limits of the technique, normal. Receptive fields were limited to the contralateral hemifield, and extended 86° peripheral in the contralateral DLGn; 36° in the ipsilateral DLGn. The representation of the zero vertical meridian could not be distinguished from normal. Neurons isolated within the upper magnocellular A/A1 layer had smaller receptive fields than those within the parvocellular C layer.

Unilateral injections of HRP were made into the DLGn of two experimental cats--enucleated **E-45** and **E-51**--to determine whether the decussation of ganglion cell axons conformed to the normal pattern. There appeared to be no significant difference in the origins of crossed and uncrossed retinal projections to the DLGn in the one-eyed cats.

The results demonstrate that the anomalous retinal influx to the DLGn is functional, and that the topography of the aberrant projection, as shown both anatomically and physiologically, is congruent with that of the normal retinal projection.

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206.7 FUNCTIONAL AND MORPHOLOGICAL REORGANIZATION OF AREA 17 AND LATERAL GENICULATE NUCLEUS FOLLOWING MONOCULAR DEPRIVATION IN GM_1 TREATED CATS. G. Carmignoto*, R. Canella*, C. Comelli* A. Gorio, S. Bisti*° (SPON: A. Cangiano). Dept. of Cytopharmacology, Fidia Research Laboratories, Via Ponte della Fabbrica 3/A, 35031 Abano Terme (PD) Italy. ° Institute of Neurophysiology, C.N.R., Via S. Zeno 51, Pisa, Italy.

In the peripheral nervous system gangliosides (GA) have been suggested to be involved in developmental processes, in cellular differentiation, axonal growth and sprouting. The functional reorganization of the cat visual cortex following various forms of visual deprivation could be considered one of the most striking model to investigate neuronal plasticity in the Central Nervous System of the mammalian brain and the possible involvement of GA in this complex process. Monocular deprivation (MD) during the early months of life leads to profound cells atrophy at the level of the Lateral Geniculate Nucleus (LGN) and to large modifications in the development of connections at the level of the striate cortex, where probably inhibitory and excitatory terminals relating to the two eyes input, are redistributed. Sprouting and formation of new synaptic contacts could be involved in the mechanisms underlying the synaptic reorganization of LGN and striate cortex. Experiments were performed on 16 kittens divided in two groups: 9 cats for the untreated group and 7 cats for the GM_1 treated group. All the animals were monocularly deprived at 35th day from birth. Monosialoganglioside (GM_1) treatment at alternate days (30 mg/Kg i.p.), starts at the time of deprivation and continues till the day of recording at about 4 months of age. Ocular dominance distribution was assessed by recording from single unit in area centralis of area 17. Results suggest a modification of cortical reorganization following visual deprivation by exogenous GM_1. In area 17, contralateral to the deprived eye, a higher percentage of cells maintain a binocular response, on the contrary in the hemisphere ipsilateral to the deprived eye, no effect of the treatment seems evident. The reopening of the deprived eye and the lids suturing of the original undeprived eye increase the possibility for the deprived eye to recover. Experiments are in progress trying to verify if GM_1 treatment could enhance the recovery when the deprived eye is reopened and the original undeprived eye is closed, even after the critical period is going to end. At the level of LGN, experiments are in progress trying to correlate the minor loss of cortical binocular cells in treated cats with a hypothetical reduced cells atrophy in the deprived laminae. Moreover, by using Golgi method branching pattern of LGN relay neurons are quantitatively studied to detect possible modifications of the dendritic tree following GM_1 treatment.

206.8 RETINO-THALAMIC PROJECTIONS IN THE RABBIT FOLLOWING NEONATAL MONOCULAR ENUCLEATION. A. M. Grigonis*, E. H. Murphy and H. E. Pearson. (SPON: L. Botticelli). Dept. Anatomy, The Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

The projection of the rabbit optic tract is distributed to 3 divisions of the lateral geniculate complex of the thalamus: the dorsal lateral geniculate nucleus (LGNd), the ventral lateral geniculate nucleus (LGNv), and the intergeniculate leaflet (IGL). All three receive a large crossed and small (10%) uncrossed input from the retinae. Monocular enucleation on the day of birth was performed in order to determine the organization of the remaining visual input to these thalamic targets. Tritiated proline and leucine were injected into the remaining eye after survival $\geq$ 70 days, and planimetric measurements were made to assess the size of the labeled terminal regions in both normal and enucleated animals.

The normal ipsilateral projection to LGNd consists of a band of label at the medial edge of the nucleus. In addition, there is a distinct reduction of label density in this medial region of the LGNd contralateral to the injected eye. In rabbits with one eye removed at birth, the ipsilateral projection from the surviving eye appears normal, despite shrinkage of the nucleus. The contralateral projection from the remaining eye, however, does not appear normal. The area of reduced label density in the ipsilateral projection zone is absent, indicating that the contralateral projection from the intact eye had invaded this area. In contrast to the LGNd in which the contralateral projection was changed but the ipsilateral projection was normal, both LGNv and IGL revealed differences in the ipsilateral projection from the remaining eye. The ipsilateral projection to the LGNd and IGL was increased in size in enucleated rabbits compared with normals, although no changes were observed in the LGNv or IGL contralateral to the intact eye.

The mechanisms responsible for changes in the nucleus either ipsilateral or contralateral to the remaining eye could include an expansion of an existing projection or a failure of retraction of an 'exuberant' projection, or both. The results suggest that following partial deafferentation of a target nucleus which normally receives bilateral innervation, different factors may determine whether the ipsilateral or contralateral projection is altered for different thalamic nuclei.

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206.9 TRANSNEURONAL DEGENERATION OF BETA GANGLION CELLS IN CAT RETINA. B. R. Payne, H. E. Pearson and P. Cornwell.* Dept. of Anatomy, The Med. Coll. of Pennsylvania, Phila., PA 19129; Dept. Psychol. Pennsylvania State Univ., Univ. Park, PA 16802.

Following neonatal visual cortex ablation in the cat, there is rapid retrograde degeneration of cells in the dorsal lateral geniculate nucleus (dLGN). Removal of this principal target nucleus produces transneuronal retrograde degeneration of retinal ganglion cells, in particular cells with medium-sized somata (Pearson et al. '81). In order to determine whether this transneuronal degeneration is restricted to a particular morphological class of retinal ganglion cells, we used the technique of retrograde labeling of ganglion cells following injection of horseradish peroxidase (HRP) into the dLGN. This allowed us to study ganglion cells which we knew projected to the remaining dLGN and also to classify the cells into α, β and γ types on the basis of soma size and dendritic morphology.

Kittens received bilateral ablation of visual cortex at 5 days of age. The ablation involved principally areas 17 and 18, with some involvement of area 19. Following a survival time of 34-48 months, multiple injections of HRP were made in both dLGNs of these cats, and also of a group of normal adult cats. Whole mounts of the retinae were made following reaction with benzidine dihydrochloride and hydrogen peroxide. In all cats, HRP injections into both dLGNs results in labeling throughout the retina of ganglion cell bodies and much of their dendritic arbors. We selected a 1200x1200μm^2 sample area in nasal retina centered at a distance of 9mm from area centralis. Ganglion cells were classified as α, β or γ types on the basis of dendritic morphology. In normal cats, α cells comprised 8-10% of the total population in the sample area, β cells comprised 64-67% and γ cells comprised 23-27%. In the retinae of visual cortex-ablated cats, there were normal numbers of α and γ cells present, but the β cell population was severely depleted by 90% of the normal number.

Therefore, morphological analysis and density determination of cat retinal ganglion cells show that, in peripheral retina, neonatal visual cortex ablation spares α and γ cells but results in almost complete loss of β cells. Survival of some ganglion cell populations and the death of others may be explained in terms of the pattern of projections of the different cell types. We conclude that β cells degenerate following visual cortex ablation because of removal of their sole target cells in the dLGN, whereas α and β cells survive because they have collateral projections to other targets.

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206.10 RELATIONSHIP OF LATERAL GENICULATE NEURON MIGRATION TO STAGES OF OPTIC TRACT GROWTH IN THE HAMSTER. S. Jhaveri*, M.A. Edwards & G.E. Schneider. Research Lab of Electronics and Psychology Department, Mass. Inst. of Technology, Cambridge, MA 02139.

As a prerequisite to understanding the critical cellular events involved in the establishment of CNS connectivity, precise information on the relative timing of arrival of afferent axons and their target cells in a given structure is necessary. We are gathering such information in Syrian hamsters, focussing on the lateral geniculate nucleus and its afferents from the eye. The sequence of optic-tract development has been determined with the aid of HRP transport, and Golgi and Cajal-DeCastro methods (Jhaveri et al., Anat. Rec., 205: 225A,'83): ganglion cell axons emerge from the eyeball and traverse the optic stalk on embryonic day 11 (E11; mating=E0), cross at the chiasm and course over the surface of the dorsal thalamus (E13) and reach the caudal end of the SC (E13.5). During this period, axons grow rapidly (60-80 μm/hr), are fasciculated and unbranched -- characteristics of an elongation mode of growth. It is only after another 2 days at E15.5-P0 (P0=day of birth) that at least some of the axons begin a second mode of growth -- the arborization mode -- in which individual retinofugal axons extend multiple collateral branches among their target cells. These collaterals are non-fasciculated and have a slower apparent growth rate.

Neurons of the hamster's LGd become postmitotic from E10.5 to E12.5 (Crossland & Uchwat, Dev.Brain Res.,5: 99,'82). In order to study the migration of LGd neurons we labelled hamster embryos with ^{3}H-thymidine at ages varying from E10 to E13.5, and processed the brains for autoradiography following survival times of ½ to 4 days. Cells heavily labelled by injections on E10.5 to E11 migrate away from the ventricular zone, and some have reached the dorsal thalamic surface by E12.5, at which time a presumptive LGd nuclear mass can be identified lateral to a primitive external medullary lamina. Thus, the pioneering retinofugal axons grow past the earliest-born LGd neurons in their course over the dorsal thalamic surface on E13. Many neurons labelled on E11.5 (peak of LGd histogenesis) accumulate in the immature LGd from E13.5 to E14, mixing with cells generated earlier. The optic tract increases in thickness during this period but extensive invasion of the LGd by collaterals of retinofugal axons does not begin until E15.5, when most, if not all, LGd neurons are already in their final target positions.

Since the LGd neurons are present prior to the onset of the 2nd axonal growth stage, a hypothesis worth considering is that some aspect of their maturation provides the stimulus for the onset of collateral growth and arborization by optic afferents.

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206.11 **EARLY DEVELOPMENT OF RETINAL SPECIALIZATION: THE DISTRIBUTION AND DECUSSATION PATTERNS OF GANGLION CELLS IN THE PRENATAL CAT DEMONSTRATED BY RETROGRADE PEROXIDASE LABELING.** **B. Lia, R. W. Williams, and L. M. Chalupa** Department of Psychology and The Physiology Graduate Group, University of California, Davis, CA. 95616

In cat, as in most other mammals, the adult retina shows features of regional specialization, including a high cell density area centralis region and a sharp decussation pattern. In the present study we sought to determine whether these characteristics of retinal topography are evident during fetal development or whether they arise subsequent to axon-target interactions and differential cell death.

Horseradish peroxidase (HRP) was injected into the major retino-recipient regions of the fetal brain in order to differentiate unequivocally ganglion cells with centrally projecting axons from other profiles within the immature ganglion cell layer. Bilateral injections were made into the thalamus and midbrain on embryonic days E-34, 37, 41, 44, 49, and 56 (gestation: 65 days). In additional cases unilateral injections of HRP were made into the thalamus. Following a 12-24 hour survival the fetuses were removed and perfused with EM grade fixative. Retinae were dissected and reacted with the chromagen phenylenediamine. To minimize shrinkage the retinae were not counterstained. Labeled and unlabeled cells could be readily distinguished in wholemounts using Nomarski interference contrast optics with a 100x oil immersion objective.

In the youngest retinae, at E-34, a primordial area centralis was already apparent: within this region the density of labeled cells is 38,000/mm^2 while in peripheral regions the packing density falls to 17,500/mm^2. In addition to packing density, central and peripheral regions of the retinae differ strikingly in morphology, even in the E-34 retinae. Peripheral neurons typically had elongated cell bodies and prominent dendrites, whereas those in the central region were round with few labeled dendrites. By E-56 a number of large ganglion cells, presumably the α-cell precursors, could be easily distinguished.

The distribution of labeled ganglion cells following a unilateral injection into an E-44 fetus was similar to that seen after comparable injections in adults. The ipsilateral nasal hemiretina was devoid of labeled cells, and the demarcation between labeled and unlabeled hemiretinae was sharp.

The majority of ganglion cells are generated by about E-32 (Kliot & Shatz, 1982; Walsh et al., 1983) and our findings indicate that shortly thereafter an incipient retinal specialization is already apparent. At this stage ganglion cell axons have just reached the major retino-recipient targets (Williams & Chalupa, 1982; Shatz, 1983). While axon-target interactions may well play a role in sculpting out the mature pattern of retinal topography, our findings indicate that the basic features of retinal specialization are organized very early in development.

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206.12 PHYSIOLOGICAL DEVELOPMENT OF SECOND ORDER NEURONS AND THEIR CONNECTIONS IN THE RETINA OF KITTENS. D.I. Hamasaki*, Gregory W. Maguire, Janie Rudolph and Gail S. Tucker (SPON: S.H. Gruber). Bascom Palmer Eye Institute, Univ. Miami, FL.

Earlier studies have shown that the anatomical and physiological properties of the photoreceptors of kittens are adult-like by 3 weeks of age. For this study, the b-wave and oscillatory potentials of the ERG were used to follow the development of the second order neurons and their connections in the IPL. At 3 weeks of age, the threshold and amplitude of the b-waves were significantly different from that of adult cats. The b-waves were smooth with no signs of oscillatory potentials. There was a rapid increase in the sensitivity and in the amplitude of the b-wave during the next several weeks. The oscillatory potentials were first observed during this period but they were significantly smaller and slower than that of adults. By 7-8 weeks, the amplitude of the b-wave was comparable to that of the adult but the threshold was still elevated. The oscillatory potentials at this age were still smaller and slower than those of the adult. Experiments are continuing to determine when the oscillatory potentials are completely adult-like.

In dark-reared kittens, the threshold and amplitude of the b-waves were not affected but the oscillatory potentials appeared smaller and slower than those of light-reared kittens of comparable age.

These results show that the b-wave and oscillatory potentials of the ERG not only have different origins but also that their course of development is different. In addition, these findings demonstrate that kittens enter and pass through the critical developmental period with a physiologically immature retina.

207.1 NEONATAL CANNABINOID EXPOSURE AFFECTS BIOGENIC AMINES AND PITUITARY FUNCTION IN ADULT MICE. S. Dalterio*, R. Steger* and D. Mayfield* (SPON: N. Hagino). Departs. Pharm. & Ob/Gyn., Univ. Tex. Hlth. Sci. Ctr., San Antonio, TX 78284.

In previous experiments we have demonstrated that maternal exposure to either the major psychoactive component of marihuana, Δ^9-tetrahydrocannabinol (THC), or the relatively non-psychoactive cannabinol (CBN), or cannabidiol (CBD) during late pregnancy resulted in long-term alterations in biogenic amines and endocrine functions. The present experiments examined the effects of postnatal cannabinoid exposure on later neuroendocrine function.

Postcastration, the turnover of norepinephrine (NE) in hypothalamus (H) after α-methylparatyrosine (α-MPT) was significantly less in THC- and CBN-exposed males, 18% and 14%, respectively, compared to 27% in controls. In contrast, dopamine (DA) turnover in brain of THC- and CBN-exposed males was increased 24% and 18%, compared to 6% in controls, while, in CBN-exposed castrates, HDA levels were lower than control [997 ± 96 (8) vs 1652 ± 196 (8) ng/g]. Turnover of HDA in CBD-exposed castrates was increased 45% vs 15% in controls. Postnatal THC exposure significantly increased brain 5-HT [1212 ±101 (7) vs 851 ± 117 (6) ng/g] and 5-HIAA [423 ± 455 (7) vs 251 ± 28 (6) ng/g] post-castration.

Ovariectomized female mice postnatally-exposed to cannabinoids also exhibited alterations in biogenic amines. In CBN-exposed females, HNE levels were reduced [2286 ± 194 vs 3430 ± 366 ng/g]. The % reduction after α-MPT for HNE levels was lower [12% vs 37%] in CBD-exposed, as it was for brain NE [0% vs 32%] in the THC-exposed females. In contrast, THC and CBD-exposed females showed a greater reduction, 18% and 16%, in brain DA levels compared to 2% in controls.

Postnatal cannabinoid exposure reduced plasma LH levels in both intact and castrated animals, while intact FSH levels were reduced only in THC-exposed males [987 ± 33 (16) vs 1221 ± 47 (15) ng/ml]. Administration of α-MPT did not influence plasma hormone levels in male castrates, but ovariectomized females postnatally-exposed to cannabinoids had lower levels of LH and FSH after α-MPT.

Pituitary weights were significantly increased in males postnatally-exposed to THC and CBN, and in vitro pituitary LH production was also significanly increased [3383 ± 298 (9) in THC, and 4030 ± 1036 (6) in CBN] compared to 1956 ± 339 (5) ng/mg/hr in controls. Pituitaries from cannabinoid-exposed males were less responsive to in vitro stimulation by LHRH, with increases in LH production of 195% in THC, 218% in CBN, and 247% in CBD, compared to 425% in controls.

The present findings indicate that postnatal exposure to cannabinoids, whether psychoactive or non-psychoactive, alters biogenic amines and pituitary function in adulthood.

207.2 CHANGES IN ANTERIOR PITUITARY HORMONE SECRETION AND HYPOTHALAMIC CATECHOLAMINE METABOLISM DURING MORPHINE WITHDRAWAL IN THE RAT. S.M. Gabriel*, J.W. Simpkins* and W.J. Millard (Spon: A.J. Dunn) University of Florida, Gainesville, Florida, 32610 and Harvard Medical School, Boston, Massachusetts, 02114.

Studies were undertaken to simultaneously assess changes in anterior pituitary hormone secretion and hypothalamic catecholamine (CA) metabolism during naloxone (NAL) precipitated withdrawal in morphine (MOR) dependent female rats. MOR dependency was induced by multiple s.c. implantation of pellets containing 75 mg MOR freebase. Rats were ovariectomized and immediately received 1 MOR pellet followed by 2 additional pellets 2 days later. After 4 days of morphine exposure, rats received NAL (1 mg/kg, s.c.) and were sacrificed 0, 5, 15, 30 and 60 min later. Trunk blood was collected for serum hormone analysis by RIA and the medial basal hypothalamus (MBH) and preoptic area (POA) were isolated for later determination of CA's by amperiometric methods. Concentrations of norepinephrine (NE), normetanephrine (NME), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were quantified.

Within 5 min after NAL injection to MOR-dependent rats, serum luteinizing hormone levels were increased 8-fold and remained moderately elevated through 60 min. Serum β-endorphin-like-immunoreactivity increased 6- to 8-fold from 5 to 60 min after NAL administration, while serum prolactin levels were increased significantly only at 15 min. In contrast, both growth hormone (GH) and thyroid-stimulating hormone (TSH) secretion decreased during MOR withdrawal. GH levels were significantly decreased by 15 min and were undetectable (<2.5 ng/ml) by 60 min after NAL. Serum TSH levels fell progressively from 5 to 60 min after NAL treatment.

In both the MBH and POA, NME concentrations were increased by 166 to 237% within 15 min of NAL administration and remained elevated through 60 min. This increase in NME levels was followed at 30 and 60 min by a 25 to 40% reduction in NE concentration in both tissues. Thus, a marked increase in NE turnover appears to accompany precipitated MOR withdrawal. In the MBH concentrations of HVA and the ratio of HVA to DA increased significantly by 5 min and remained elevated through 60 min after NAL administration while no alteration in HVA levels were observed in the POA at any of the sampling times. In contrast in both MBH and POA, DOPAC levels were significantly elevated at 30 and 60 min and these increases were coincident with significant increases in DA concentrations. Thus, while DA metabolism is enhanced during MOR withdrawal, this does not appear to represent increased release of DA from nerve terminals. These alterations in CA metabolism and hormone secretion may mediate, in part, the dramatic alterations in physiological processes associated with MOR withdrawal.
(Supported in part by NIH AG02021 to JWS).

207.3 EPINEPHRINE, 5-HYDROXYTRYPTAMINE, 5-HYDROXYINDOLE-3-ACETIC ACID AND DOPAMINE IN HYPOPHYSIAL PORTAL AND PERIPHERAL PLASMA OF INTACT AND ADRENALECTOMIZED RATS. C.A. Johnston, D.M. Gibbs[a] and A. Negro-Vilar. Dept. of Physiology, Univ of Texas Health Science Center, Dallas, Texas 75235; and [a]Department of Reproductive Medicine, School of Medicine (T-002), University of California, San Diego, La Jolla, California 92093.

Hypophysial portal blood was collected from urethane anesthetized male rats by stalk cannulation (Porter method) or by periodic aspiration of portal blood (Worthington and Fink method). Portal and peripheral plasma epinephrine (EPI), 5-hydroxytryptamine (5-HT), dopamine (DA) and 5-hydroxyindole-3-acetic acid (5-HIAA) were concurrently measured using high performance liquid chromatography with electrochemical detection (LCEC). Hypophysial portal plasma samples consistently demonstrated significantly higher concentrations of EPI, 5-HIAA and DA but not of 5-HT independent of the collection method. Adrenalectomy (ADX) abolished peripheral plasma EPI whereas hypophysial portal plasma EPI was only slightly attenuated. The presence of EPI in hypophysial portal plasma following ADX strongly suggests that a significant fraction of the amine is derived from a central (neural) source and that the amine may have a direct physiological role in the regulation of anterior pituitary function. ADX did not alter the concentrations of 5-HT, 5-HIAA or DA in either hypophysial portal or peripheral samples. Although 5-HT concentrations in both portal and peripheral plasma were not different, 5-HIAA levels were 3-fold higher in portal plasma. The high concentration of 5-HIAA in portal plasma was not due to conversion of 5-HT to 5-HIAA by monoamine oxidase in plasma. Although the possible physiological significance of this finding, if any, is yet to be determined, the possibility that a compound thought of as an end product metabolite in the CNS may have a role in the function of another tissue must be considered. As previously reported, DA was 10-15 fold higher in portal plasma. These data demonstrate for the first time a concentration gradient for EPI (derived from a central source) and 5-HIAA in hypophysial portal versus peripheral plasma. Furthermore, they demonstrate the utility of LCEC for the concurrent determination of several monoamines and metabolites in hypophysial portal plasma. Finally, they suggest that the portal aspiration method may be particularly well-suited for measuring potentially labile substances because appropriate preservatives/enzyme inhibitors can be added to the samples within seconds.
Supported by NIH Grant HD-09988-06 to ANV and by a Mellon Foundation Grant to DMG.

207.4 SEROTONERGIC MODULATION OF CORTICOTROPIN RELEASING FACTOR AND VASOPRESSIN SECRETION INTO HYPOPHYSIAL PORTAL BLOOD. D.M. Gibbs and W. Vale. Dept. Repro. Med., UCSD, La Jolla, CA 92093 and The Salk Institute, La Jolla, CA 92138.

Among the several hypothalamic hormones which can stimulate ACTH secretion in vitro, the recently described 41 amino acid corticotropin releasing factor (CRF) and arginine vasopressin (AVP) are both thought to be involved in the physiological regulation of ACTH secretion, but their exact roles are unknown. Since central serotonin pathways appear to be involved in the release of ACTH, we studied the effect of fluoxetine, a serotonin reuptake inhibitor, on the release of CRF and AVP into hypophysial portal blood as well as the release of ACTH and AVP into the peripheral circulation. To define the effects of fluoxetine on pituitary secretion of ACTH and AVP, pentobarbital anesthetized male rats with atrial cannulae were injected ip with fluoxetine 10 mg/kg or saline and blood samples were obtained at 20, 40, 60 and 80 minutes. ACTH levels in fluoxetine treated rats rose from a basal value of 121.7 ± 38.5 pg/ml to a peak value of 20 min of 1192 ± 180 pg/ml. AVP levels continued to rise throughout the 80 min collection period from a baseline of 22.8 ± 1.8 pg/ml to 93.0 ± 15.3 pg/ml at 20 min to 136 ± 33.2 pg/ml at 80 min. Neither ACTH nor AVP concentrations changed significantly in rats injected with saline. In a separate experiment, hypophysial portal blood was collected for 30 min from 7 pentobarbital anesthetized rats injected with fluoxetine 10 mg/kg ip and 7 rats injected with saline. CRF levels in portal plasma from fluoxetine treated rats were 1.37 ± 0.09 ng/ml vs 0.85 ± 0.10 ng/ml in controls ($p < 0.01$). AVP levels in portal plasma from fluoxetine treated rats were 1.54 ± 0.16 ng/ml vs 1.03 ± 0.16 ng/ml in controls ($p < 0.05$). These concentrations of CRF and AVP in portal plasma are in the range which can stimulate ACTH secretion in vitro. The data suggest that modulation of ACTH secretion by serotonin is mediated by changes in both CRF and AVP secretion into hypophysial portal blood.

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207.5 SEROTONIN-CONTAINING CELLS AND FIBERS IN THE PITUITARY OF AN ELASMOBRANCH FISH. R.B. Leonard, T.C. Ritchie, M.G. Hughes* and D.J. McAdoo. Departments of the Marine Biomedical Institute, Physiology and Biophysics and Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas.

Serotonin (5-HT) has been implicated in influencing endocrine functions, including the release of several pituitary hormones. However, it has proved difficult to determine the site(s) of 5-HT's action in the brain-pituitary axis. Despite reports of significant quantities of 5-HT in the pituitaries of several vertebrates, histological demonstration of the cellular localization of pituitary 5-HT has been elusive. Recently, 5-HT fibers and cells have been reported in the pituitaries of frogs and rats with immunocytochemistry although other reports have been negative.

5-HT was localized in the pituitary of the Atlantic stingray, Dasyatis sabina, with immunocytochemistry using the PAP method. Preabsorption controls indicated high specificity for 5-HT. There was no cross-reactivity to bovine serum albumin, or norepinephrine while dopamine, 5-methoxytryptamine and tryptamine caused some reduction in staining intensity.

Cells and fibers exhibiting 5-HT-like immunoreactivity were observed in the stingray pituitary without any seasonal variation. The elasmobranch pituitary is an elongated structure, consisting of a large caudally placed neuro-intermediate lobe (NIL) and a rostrally placed pars distalis with rostral (RPD) and proximal (PPD) subdivisions. The ventral lobe, a subdivision of the PPD peculiar to elasmobranchs, was not included in this study. The 5-HT cells are located ventromedially in the PPD. They were oval (6x12 μm) or round (8 μm) or occasionally polygonal in shape. Short processes were often evident, which occasionally appeared to end on a blood vessel. Thin varicose fibers traversed the median eminence and infundibular stalk to reach and ramify in the NIL. Patches of fibers were variously located surrounding blood vessels or intermedia cell cords. In preliminary HPLC measurements we have detected 5-HT in the PPD and the NIL.

Both the immunocytochemistry and the HPLC results indicate that the stained cells contain 5-HT. However, it is possible that the cells take up 5-HT from blood or CSF, instead of synthesizing it. The demonstration of 5-HT cells and fibers in the stingray pituitary suggests that this situation arose early in phylogeny.
Supported by NIH grants NS07185, NS11255 and NS16093.

207.6 LOCALIZATION OF MONOAMINE OXIDASE-B IMMUNOREACTIVITY IN THE NEONATE AND ADULT RAT PITUITARY GLAND. V. Cooper‡ J. E. Pintar and P. Levitt (SPON: T. J. Cunningham). Div. Endorcinology, Mt. Sinai Sch. of Med., New York, NY 10029 and Dept. Anatomy, The Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Monoamine oxidase (E.C. 1.4.3.4) is the primary enzyme involved in the degradation of biogenic amines such as norepinephrine, dopamine, and serotonin. These and other amines function as primary regulators of specific pituitary cells. One way in which levels of these amines can be regulated is by the action of degradative enzymes such as MAO. Two kinds of MAO activity (MAO-A and MAO-B) have previously been characterized by pharmacological criteria and are mediated by structurally different proteins. We have produced multiple antisera to purified MAO-B that do not cross-react with MAO-A and have previously used these antisera to localize MAO-B immunoreactivity in astrocytes and serotonergic neurons of the adult rat brain (Levitt et al. PNAS 79:6385). We have here used these antisera to localize MAO-B immunoreactivity in the neonate and adult rat pituitary gland and have made the following observations. First, MAO-B immunoreactivity is found in the major cell class of the posterior lobe, the pituicytes. This is consistent with other recent observations that these cells express many astrocyte-like properties and indicates that these cells may inactivate transmitters that are released from hypothalamic nerve endings in the posterior lobe. Second, MAO-B immunoreactivity is found in a boundary layer of cells between the anterior and intermediate lobes; this is the first biochemical characteristic expressed in these previously morphologically defined cells and indicates that they may insulate one lobe from physiologic effectors in the adjacent lobe. Third, about 15% of anterior lobe cells exhibited MAO-B immunoreactivity; we are currently determining whether MAO-B cells correspond to specific peptide-containing cell types. Finally, initial experiments with 14-day neonates have demonstrated a different pattern of MAO-B localization with many anterior lobe cells and fewer pituicytes stained. Together, these results suggest that MAO activity may contribute to regulation of pituitary function.

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207.7 6-HYDROXYDOPAMINE-INDUCED ALTERATIONS IN NEURITES OF THE PITUITARY PARS INTERMEDIA. L.C. Saland and E. Ortiz*. Department of Anatomy, University of New Mexico School of Medicine, Albuquerque, New Mexico 87131.

Pro-opiomelanocortin cells of the rat pituitary pars intermedia receive direct synaptic connections from the hypothalamus. Neurites have been demonstrated to contain catecholamines by fluorescent histochemistry and may be a source of inhibitory neuroregulation for the secretory cells. Previous studies introducing 6-hydroxydopamine (6-OHDA) intravenously have led to variable effects on catecholamine innervation to intermediate lobe. Here, we demonstrate ultrastructural evidence for degenerative changes in neurites after peripheral 6-OHDA injections. Adult male Sprague-Dawley rats received two intravenous injections (150mg/kg) 6-OHDA in ascorbic acid and saline on consecutive days. Controls received vehicle only. At times ranging from 24 hours to one week after injections animals were ether anesthetized and perfused intracardially with buffered aldehydes. Pituitary glands were prepared for transmission EM. Micrographs of glands from 6-OHDA-treated rats revealed swollen neurites containing myelin figures as well as some normal-appearing neurovesicles. Endocrine cells exhibited extensive, granule containing Golgi zones and areas of expanded rough endoplasmic reticulum. Injection of the false transmitter 5-hydroxydopamine to several animals revealed electron-dense "dots" in neurovesicles, confirming uptake ability for the terminals. Results with peripheral 6-OHDA injections suggest that the neurotoxin gains access to pituitary neurites to cause degenerative changes. The drug also appears to induce a cellular activation or "disinhibition" indicative of peptide release and new synthesis. Supported in part by DA-02269 and RR-08139.

207.8 VASOPRESSIN AND NORADRENERGIC NEURONS IN DISPERSED HYPOTHALAMIC AND BRAIN STEM CULTURES. C.D. Sladek, M. Gallagher*, M.F.D. Notter, G. Hoffman, and J.R. Sladek, Jr. Depts. Neurol. and Anatomy, Univ. Rochester Med. School, Rochester, N.Y. 14642.

We have developed a protocol for preparing and maintaining monolayers of dispersed fetal or neonatal brain cells which is compatible for maintenance of the vasopressin (VP) neurons of the supraoptic nucleus (SON) and brainstem catecholamine neurons (A1). To prepare the cultures, the appropriate fragments of brain tissue are dissected and dispersed by trituration following a 10 min incubation with 0.1% trypsin. Whole cells are pelleted by centrifugation and subsequently resuspended in culture medium (Eagles minimum essential medium fortified with 10% fetal calf serum, 0.1% glutamine, 0.6% glucose, 100 uU/ml penicillin, 100 ug/ml streptomycin, and 2.5 ug/ml fungizone). Cells are plated onto poly-D-lysine coated culture slides or cover slips. The hypothalamic region dissociated is the area surrounding the optic chiasm which includes the SON. With approx. 10^6 cells plated/35 mm dish, we routinely detect VP in the culture medium during the 1st 72 hours. When fetal donors are used (19 or 20 days post-coitus, dpc) VP commonly is then undetectable in the culture medium during the next 10-20 days. However, in three different preparations VP reached detectable levels after 1.5 to 2.5 weeks of culture. Dispersed cultures of neonatal SON (10 or 15 days post-natal, dpn) maintained detectable VP release into the culture medium throughout 3 weeks of culture. This correlates with the developmental sequence in the VP neuron in vivo in which VP and neurophysin content of the SON neurons increases progressively from 17 dpc to 21 dpn. Following fixation with acrolein , VP, neurophysin (NP), and oxytocin (OX) positive cells were visualized in the hypothalamic cultures by immunocytochemistry. In one preparation of fetal neurons examined after 3 weeks in culture, there were 90 ± 4 VP positive cells per cm^2. Most of these cells were large bipolar neurons.

In dispersed cultures from the ventrolateral medulla which includes the A1 neurons, neurons were identified by the presence of neuron specific enolase (NSE) immunoreactivity and the noradrenergic phenotype was identified by the presence of glyoxylic acid induced catecholamine fluorescence or the presence of dopamine-β-hydroxylase (DBH) immunoreactivity (antiserum by S. Watson). The A1 neurons were complex multipolar cells with shapes characteristic of reticular formation neurons. In one preparation the DBH positive cells represented 47% of the population of NSE positive neurons.

The abiltiy to maintain VP and NE neurons under the same culture conditions suggests that we will be able to co-culture these neurons. This would provide the opportunity to study the regulation of the VP neuron by the NE neurons which innervate them in vivo.

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207.9 LACK OF EVIDENCE FOR AUTOINHIBITION OF VASOPRESSIN (VP) RELEASE IN VIVO AND IN VITRO. Christine M. Gregg* (Spon: E. Hibbard). Dept. of Biology, Penn. State Univ., Univ. Park, PA 16802.

Many authors have reported recurrent inhibition of vasopressinergic neurons, but the neurotransmitter involved is not known. Nicoll and Barker (Brain Res. 35:501, 1971) proposed VP itself may play some role since application of VP to supraoptic neurons was followed by electrical inhibition. Both in vivo and in vitro studies have been used to test this hypothesis.

For the in vivo study Alzet minipumps were used to infuse either artificial CSF or VP (0.25 to 50 ng/hr) into lateral ventricles of male SD rats (266±24g, n=36). Metabolic data were collected both before (7 days) and during (10 days) central infusion. Water and food intake, urine volume and osmolality, and fractional water, Na, and K excretion were measured. Comparison of pre and post infusion data provided no evidence that central VP infusion inhibited VP release. In fact, at infusion rates of 2.5 ng/hr and above, urine volume decreased, osmolality increased, and there was net water retention. 24 hours of dehydration + CSF VP infusion also failed to blunt the expected rise in plasma VP concentration. At the highest infusion rate (50 ng/hr) plasma VP was elevated 10-fold compared to controls (>130 pg/ml vs. 13±3 pg/ml). These data indicated that VP was probably leaking from CSF into plasma.

To circumvent this problem hypothalamus (HT) and neural lobe (PP) were organ-cultured in separate compartments. The intact infundibular stalk passed through a hole in the fluid-tight barrier which separated the 2 sides (Neurosc. Abs. 8:65, 1982). PP VP release was measured during a control and test hour and data expressed as % of control. During the test HT VP concentration was increased to 40 ng/ml alone or in combination with an osmotic stimulus of +15 mOsm. Since a leak of 1% or less cannot be detected, VP alone was used as the control for VP+ ↑Osm. Δ = (VP+↑Osm)-(VP alone).

Stimulus:	none	VP alone	VP+ ↑Osm	Δ	↑Osm alone
PP VP Release	93±9	177±23[a]	487±114[a]	311±123[a,b]	236±56[a]
% of control	(13)	(9)	(9)	(9)	(10)

[a]p<0.05: control hour vs. test hour, paired t-test;
[b]n.s. compared to ↑Osm alone, ANOVA.

These data provide no support for the hypothesis that VP inhibits its own release. The increase when VP alone was added is most likely due to a small leak in the barrier (<1%/hr).
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207.10 RELEASE OF VASOPRESSIN IN VITRO FROM THE ARTERIALLY PERFUSED HYPOTHALAMO-NEUROHYPOPHYSIAL COMPLEX DURING OSMOTIC, CHOLINERGIC OR ELECTRICAL STIMULATION OF THE SUPRAOPTIC NUCLEUS. R.B. Meeker* , R.S. Greenwood and J.N. Hayward. Dept. Neurology and Neurobiology Program, Univ. North Carolina, Chapel Hill, N.C., 27514.

A hypothalamo-neurohypophysial complex (HNC) containing the intact supraoptic nuclei, the supraoptico-hypophysial tract and neurointermediate lobe was rapidly isolated from the rat brain (Sladek & Knigge, Endocrinol. 101:1977) and placed in a plexiglas chamber support system. The internal carotid was cannulated with a glass micropipette and the HNC was perfused with medium at a rate of 1-2 ml/min (Llinas et.al., Fed. Proc. 40:1981; Bourque & Renaud, J. Neurosci. Meth. 7:1983). Perfusate was collected continuously with temporal discriminations of 1-10 min. Vasopressin, measured by radioimmunoassay (Hayward et.al., Endocrinol. 98: 1976) was released at basal levels (5-15 pg/min) for up to 6-8 hrs Intracarotid infusions of hypertonic medium (20-40 mOsm/kg above controls) over 10-60 min resulted in immediate or delayed elevations in vasopressin output of up to 150 pg/min. Nicotine sulfate (5 n moles/ 50 nL) was directly microinjected into the supraoptic nucleus by means of a glass micropipette (tip diam. 30-40 um) and a hydraulic microdrive system. Microinjections over 10 min resulted in elevations of vasopressin output to 13-20 pg/min (200-400% of baseline). Bipolar electrical stimulation (0.5-1 mA, 0.5 msec, 15 Hz) of the supraoptic nucleus with tungsten microelectrodes resulted in immediate elevations in vasopressin output of up to 150 pg/min. These results can be compared to bipolar electrical stimulation (0.05- 0.2 mA, 0.5 msec, 15 Hz) of the pituitary stalk with platinum-iridium microwires which resulted in maximal elevations of 400-600 pg/min of vasopressin output.

These results demonstrate that the arterially perfused HNC is capable of releasing vasopressin in vitro in response to intracarotid osmotic stimuli, locally microinjected chemicals and electrical stimulation of the supraoptic nucleus. The responsiveness of the supraoptic nuclei to osmotic, cholinergic and electrical stimulation was apparently less than the maximal secretory capacity of the HNC as demonstrated by electrical stimulation of the stalk. We expect that this arterially perfused HNC, used previously for electrophysiological studies of the supraoptic nucleus (Bourque & Renaud, Neuroendocrinology 36:1983), will prove useful for parallel studies of the central neural regulation of vasopressin secretion by supraoptic neurons.

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207.11 CHARACTERIZATION OF NEUROHYPOPHYSEAL HISTAMINE: EFFECT OF SALINE INDUCED DEHYDRATION AND STALK-SECTIONING. K. M. Verburg*, R. R. Bowsher*, R. L. Zerbe* and D. P. Henry*. (SPON: L. Lemberger). Lilly Lab. for Clin. Research, Eli Lilly and Co. and Indiana Univ. Sch. of Med., Indpls., IN. 46202.

High levels of histamine (Hm) and histamine N-methyltransferase (HNMT), the major Hm inactivating enzyme in brain, have been found in the hypothalamus and posterior pituitary. Current evidence indicates that Hm may regulate the secretion of vasopressin (AVP) not only in the hypothalamus, but also in the neurohypophysis since Hm levels in this lobe are selectively decreased in AVP deficient Brattleboro rats (Soc. Neurosci. Abstr. 8:55). To investigate this possibility, we evaluated the effect of 1) chronic oral saline administration or 2) stalk sectioning on Hm, HNMT and AVP levels in the rat posterior pituitary. Both Hm and HNMT were assayed by recently developed radiometric methods that have improved sensitivity and specificity (Fed. Proc. 41, 1709; Fed. Proc. 42, 907). AVP was quantified by radioimmunoassay.

	POSTERIOR PITUITARY		
2% Hypertonic saline (days)	Hm (ng/mg protein)	HNMT (units*/mg protein)	AVP (µg/mg protein)
0	18.8 ± 2.7	0.175 ± .02	14.5 ± 0.9
2	12.9 ± 1.9[a]	0.235 ± .02[b]	4.86 ± 1.3[c]
5	15.7 ± 1.8	0.229 ± .03[b]	0.612 ± 0.22[c]
7	11.5 ± 1.1[a]	0.231 ± .02[b]	0.413 ± 0.15[c]

$\bar{X}$ ± SEM (n=6); [a]p<.05; [b]p<.002; [c]p<.001 compared to control group
*unit = nmoles of T methylhistamine formed/hr

Following hypertonic saline administration, AVP and Hm levels in the posterior pituitary decreased while HNMT activity increased thusly suggesting an increase in Hm turnover. No changes were observed in adenohypophyseal Hm or HNMT.

Hm levels increased 100% in chronic stalk-sectioned rats versus sham operated controls in both the posterior (30.1 ± 5.07 vs. 15.6 ± 2.37 ng/mg protein) and anterior (3.62 ± 0.68 vs. 1.85 ± 0.13 ng/mg protein) lobes. HNMT levels remained unchanged and no differences were observed in hypothalmic Hm or HNMT levels.

The current studies confirm that high levels of Hm and HNMT are present in the rat posterior pituitary. Interruption of descending fibers to the neurohypophysis did not decrease but instead substantially increased Hm levels. This result indicates that Hm is non-neuronal in origin and is possibly located in mast cells that may be regulated by descending input. The changes in the levels of Hm and HNMT in the posterior lobe induced by a physiological stimulus for AVP release support a possible link between the two systems.

207.12 SUPRAOPTIC NUCLEI AND NEURAL LOBES IN RATS WITH CORONAL CUTS CAUDAL TO THE ANTEROVENTRAL THIRD VENTRICLE (AV3V) REGION. J. R. Carithers, S. L. Bealer and A. K. Johnson. Dept. of Veterinary Anatomy, Iowa State Univ., Ames, IA 50011, Dept. of Physiology and Biophysics, Univ. of Tenn. Ctr. for Health Sci., Memphis, TN 38163 and Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242.

Electrolytic lesions of tissue surrounding the preoptic recess (AV3V) region appear to cause loss of stimulatory input to the supraoptic nuclei from angiotensin receptors and osmoreceptors. To investigate the pathways affected by these AV3V lesions, we compared the ultrastructure of supraoptic nuclei and neural lobes in ten rats which had received coronal cuts in a plane just caudal to the AV3V region with that of nine control rats which had undergone a sham procedure.

Seven days after cuts were placed, degenerating axons were present in supraoptic nuclei, and degenerating terminals occurred in axodendritic and axosomatic synapses on neurosecretory cells. However, in contrast to their appearance in rats with AV3V lesions, neuronal somas showed no decrease in size of cells or their nuclei. The percentages of multiple and marginated nucleoli were unchanged, and cytoplasmic organelles evinced no changes indicative of depressed secretory activity. In neural lobes of rats with cuts, terminals tended to be depleted of neurosecretory granulated vesicles.

	Sham Group (%)	Cut Group (%)
empty terminals	2.2 ± 2.1	12.4 ± 4.0*
full terminals	62.6 ± 8.8	36.1 ± 8.1*
volume density of terminals	37.6 ± 6.8	26.5 ± 3.7*
perivascular glial processes	42.8 ± 7.9	31.3 ± 6.9*

The percentage of terminals which contained no neurosecretory vesicles was significantly increased while the percentage of full terminals was decreased after cuts. Depletion of hormone stores from axon terminals also caused a significant decrease in the volume density of terminals in the neuropil. Furthermore, withdrawal of glial processes after cuts were placed permitted axon terminals to occupy a greater proportion of the secretory interface along the basal lamina surrounding fenestrated capillaries. These changes in the neural lobe are associated with enhancement of hormone release and are the opposite of changes seen after electrolytic AV3V lesions.

The presence of degenerating axons and terminals in supraoptic nuclei indicates that afferent fibers were destroyed by the cuts. However, normal morphology of neuronal somas in supraoptic nuclei and evidence of enhanced release of hormone from neural lobes make it clear that stimulatory input remains adequate to induce a robust response. Supported in part by Farm Bill of 1977 (P.L. 14388) Section 1433 and USPHS HL-25877.

207.13 SUBFORNICAL ORGAN CONNECTIONS WITH THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT. A.V. Ferguson & L.P.Renaud. Neurosciences Unit, Montreal General Hospital and McGill University, Montreal, Quebec, Canada H3G 1A4.

The subfornical organ (SFO) has been implicated in the physiological control of water balance and sexual behaviour, the former possibly through an associated release of vasopressin from the posterior pituitary. In view of recent anatomical studies describing a pathway from the SFO to the paraventricular nucleus (PVN), whose projections include brainstem and spinal autonomic centres as well as the neurohypophysis, we have examined the effects of electrical stimulation in SFO on the excitability of neurosecretory and other unidentified PVN neurons. In the course of these experiments we have observed also that discrete electrical stimulation in the SFO (100-400μA, 0.1msec, 5-20Hz) produces a short latency 4-16mm Hg rise in arterial pressure, a response which may be mediated in part by the PVN.

Experiments were conducted on pentobarbital or urethane anaesthetized male Sprague-Dawley rats. Electrical stimulation (0.1 msec,0.2-0.8mA) in the SFO was delivered through 30 gauge concentric bipolar electrodes which were implanted stereotaxically. A ventral exposure permitted access to the hypothalamus for extracellular unit recording, and stimulation of posterior pituitary axon terminals of PVN neurosecretory neurons.

A total of 136 neurons in the region of the PVN were tested. Of these cells 44 were identified by antidromic activation as neurosecretory neurons. In response to single SFO stimuli 32 neurosecretory neurons displayed an enhanced activity(latency 10-50 msec) lasting up to 500msec, 10 cells showed no response, and 2 cells showed an initial depression(15-60msec) followed by a longer latency increase in excitability similar to that observed in the neurons which displayed only an excitation. This enhancement in excitability was observed in both putative vasopressinergic(phasic, blood pressure-sensitive), and oxytocinergic(continuous, non-blood pressure-sensitive) neurons.

Among the remaining 92 non-neurosecretory cells, 58 showed orthodromic activation following stimulation of the SFO, with a latency range of 15-40msec, and a duration of 8-30msec. An initial decrease in activity was observed in 20 neurons, and 14 showed no obvious response.

These observations indicate that SFO stimulation exerts a predominantly facilitatory effect on the excitability of PVN vasopressinergic and oxytocinergic neurons. Our data also suggest that SFO stimulation influences a separate population of PVN neurons that may mediate other functions(e.g the increase in blood pressure) associated with the SFO.

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207.14 EVIDENCE THAT INTRAVENTRICULAR RENIN INCREASES DOPAMINE SYNTHESIS AND TURNOVER IN THE MEDIAN EMINENCE. R.H. Alper and W.F. Ganong, Dept. of Physiology, Univ. of Calif., San Francisco, CA 94143.

We have shown previously that intraventricularly (ivt) infused angiotensin II (AII) caused a small, brief increase in catecholamine (CA) synthesis in the median eminence (Alper et al., Neurosci. Abst. 1982). The present studies were undertaken to provide a quantitative analysis of the effect of ivt renin on the CA innervation of the median eminence.

Male rats were implanted unilaterally with chronic lateral ventricular cannulas. Approximately 1 week later rats were administered either artificial cerebrospinal fluid (ACSF; 10 μl) or hog renin (Sigma) over a 4-5 min period. Renin is dipsogenic so water was removed from the cages after the infusion to eliminate differences in water intake in the 2 groups. CA synthesis was estimated in vivo by measuring the rate of DOPA accumulation 30 min after the injection of m-dihydroxybenzylhydrazine (NSD 1015; 100 mg/kg, ip). Dopamine (DA) and norepinephrine (NE) turnover rates were estimated by measuring the decline of both amines in the median eminence following the inhibition of their synthesis with α-methyl-p-tyrosine (αMT; 250 mg free base/kg, ip).

In 2 separate experiments, renin (0.05 or 0.1 U/rat) had no effect on DOPA accumulation in the median eminence at 30 min and 2 hr, but increased DOPA accumulation at 4 hr; the concentration of DA in the anterior pituitary was unaltered from 30 min-4 hr. In another experiment, renin (0.1 U/rat, ivt) increased DOPA accumulation at 4 and 8 hr, but not at 12 hr as compared to 4 hr ACSF-infused or 12 hr water-deprived controls. The concentration of DA in the anterior pituitary was increased at 8 hr, but was not different from controls at other times. When the αMT-induced decline of DA and NE was examined 6 hr after renin (0.1 U/rat, ivt), the turnover of DA was found to be increased and the turnover of NE was unaltered. Also, the DA concentration of the anterior pituitary was doubled and the serum prolactin concentration was decreased 6 hr following ivt renin. Other rats that received renin (0.1 U/rat, ivt) drank 15±1 ml water in 30 min and 29±2 ml in 8 hr, compared to an intake of 3±1 ml in 8 hr controls. Total daily water intake was increased for 3 days following the single ivt infusion of renin.

These data provide evidence that ivt renin, probably through the generation of AII in the CSF or brain, stimulates the release of DA, but not NE, in the median eminence of male rats. This, in turn, may cause the increased anterior pituitary concentration of DA and the decreased secretion of prolactin. The effects of renin on DA in the median eminence develops more slowly than the effects on water intake. (Supported by USPHS Grants AM06704 and AM07265).

207.15 EFFECT OF INTRAVENTRICULAR (IVT) INFUSION OF ANGIOTENSIN II (AII) ON PULSATILE LH SECRETION IN OVARIECTOMIZED RATS. M.K. Steele, R.V. Gallo and W.F. Ganong, Department of Physiology, University of California, San Francisco, CA 94143.

In ovariectomized rats treated with estrogen, IVT AII increased plasma LH levels; while in untreated ovariectomized rats, LH concentrations were not modified when discrete blood samples were taken 10, 15 and 30 min after IVT AII (Steele et al., Endocrin. 111:722, 1982). However, LH release in ovariectomized animals is pulsatile, with wide fluctuations in blood levels over 15 to 30 minutes. Therefore, we further investigated the effect of IVT AII on LH secretion in ovariectomized rats by employing a blood sampling protocol where the pulsatile nature of LH secretion could be fully characterized.

Rats were implanted with chronic IVT cannulae, ovariectomized, and tested 8 days later. A jugular cannula was inserted 24 hr prior to testing. Rats were bled continuously via a peristaltic pump at 50 μl whole blood/7-8 minutes for 3.5 to 4.0 hrs. Blood samples were analyzed by RIA and values expressed as ngLH/ml whole blood. Following a control bleeding period of 1.5 to 2.0 hrs, artificial CSF or AII (in CSF at a dose of 15, 150, or 600 ng/25 μl/hr) was infused for 1.5 to 2.0 hrs. Mean LH levels during infusion of AII at 15 ng/hr were not significantly different from preinfusion values, nor from levels during infusion of CSF. Infusions of 150 or 600 ng/hr AII, however, produced significant, dose-related decreases in mean LH concentrations compared to preinfusion levels and to those during CSF administration. Pulse frequency and pulse amplitude were also depressed by the two highest doses of AII.

Using an identical protocol, infusions of the AII receptor blocker saralasin at doses of 1.5 μg or 15.0 μg/25 μl CSF/hr produced small increases in mean LH levels, significantly different from values during infusion of CSF, but not different from their respective preinfusion levels. Therefore, under these conditions, saralasin did not exhibit any agonistic AII activity. Co-infusion of saralasin (1.5 μg) plus AII (150 ng) abolished the depressive effects of AII upon mean LH levels, pulse amplitude and pulse frequency.

These results demonstrate that infusion of AII into the cerebral ventricles of ovariectomized rats suppresses, in a dose-related fashion, pulsatile LH secretion. The abolition of these effects by saralasin suggests a specific action of AII at its receptor. Furthermore, when taken together with the data from experiments from estrogen-pretreated rats, these data suggest that the steroid millieu of the animal determine the direction of the LH response to IVT AII. (Supported by USPHS Grants AM06704, HD18020, HD05577 and Kroc Foundation).

207.16 FOLLICULAR DEVELOPMENT AND ENDOCRINE LEVELS: EFFECT OF A NEW MATE ON PREOVULATORY CHANGES E. Sims*, A. Johnson[1] *, & M.-F. Cheng (SPON: J. Cohen). Inst. of Animal Behavior, Rutgers Univ., Newark, N.J. 07102 ; [1] Dept. Animal Science, Cook College, Rutgers Univ., New Brunswick, N.J. 08903

When a male and female ring dove are paired, their behavior patterns change in a complex and predictable sequence. The endocrine changes that culminate in ovulation after 7-10 days of pair interaction presumably also follow a predictable pattern. In the present study we examined the pattern of ovarian development and endocrine levels associated with each day of pairing until egg-laying. We found that follicular development proceeds slowly until two days prior to ovulation at which time it proceeds in an exponential growth pattern and dramatic increases occur in levels of luteinizing hormone and progesterone.

To determine if this sequence of physiological events can be altered by external factors, we introduced new males to previously paired females. In a previous study we found that a two or three day delay in ovulation can occur if a female previously paired for four days is then paired with a new male. Therefore, we compared the physiological changes of females re-paired with new males to those of females remaining with original mates. We found that the phase of rapid follicular growth appears to be a critical period with respect to the new male's effect on the female's ongoing physiological processes. If the new male is introduced prior to the critical period, ovarian development and the corresponding changes in LH and progesterone levels are delayed. If the male is introduced during the critical period, it appears that it is too late for a delay in the physiological processes leading to ovulation. This delay in ovulation may allow time for the male and female to coordinate their respective reproductive changes and activities. Reproductive synchrony between pair members is presumably important for the successful completion of the breeding cycle in monogamous species, such as ring doves. Hence, the delay in the physiological events leading to ovulation may have significance in terms of the reproductive success of the female and the new male.

207.17 ANTISERA GENERATED AGAINST HAPTEN-CONJUGATES CAN YIELD FALSE-POSITIVE STAINING WHEN USED FOR IMMUNOCYTOCHEMISTRY. Gerald P. Kozlowski and W. Les Dees*. (SPON: N.H. McArthur). Department of Physiology, U.T.H.S.C.D., Dallas, TX 75235.

When raising antiserum to peptides, it is a common practice to couple the hapten to a high-molecular weight carrier molecule in order to enhance immunogenicity. Such is the case for several antisera generated against luteinizing hormone releasing hormone-bovine serum albumin (LHRH-BSA) conjugates: Arnel #104, White-Porter #1 (WP-1), Kelch #12 and Nett-Niswender #42. These antisera were used in the unlabeled-enzyme immunocytochemical (ICC) procedure for staining LHRH in the medial basal hypothalamus of the rat. For the first reactant, tissue sections were incubated 24 hrs with a 1:700 dilution of untreated antiserum or antiserum to which was added either: LHRH, BSA or both LHRH and BSA. Following washes between steps, the sections were then incubated in: sheep anti-rabbit IgG, peroxidase-antiperoxidase complex and developed in diaminobenzidine-H_2O_2. When untreated antiserum was used, there was staining of fibers in the zona externa of the median eminence near the intermediolateral sulcus as well as staining of neuronal cell bodies in the median eminence and arcuate nucleus area. When 120 µg of LHRH was added to 1 ml of a 1:100 dilution of the primary antiserum 24 hrs before use for ICC, the staining of fibers in the median eminence disappeared, whereas staining of cell bodies persisted. Conversely, when 500 µg of BSA was added to 1 ml of a 1:100 dilution of the primary antiserum 24 hrs before use, the staining of fibers persisted, whereas the staining of cell bodies disappeared. Adding as much as 1 mg of BSA/ml of antiserum did not affect fiber staining. When both LHRH and BSA was added to the antiserum, all staining was abolished. Therefore, these antisera contain not only anti-LHRH but also anti-BSA and perhaps other antibodies resulting from immunization of the rabbit with an LHRH-BSA conjugate. The anti-BSA antibodies not only stain cell bodies of the median eminence-arcuate nucleus area but also cells and fibers of the magnocellular neurosecretory system. It is likely that neurosecretory cells in contact with fenestrated blood vessels are able to take up serum albumin, or substances associated with albumin, for retrograde transport to their perikarya. For ICC then, the anti-BSA in anti-LHRH antisera untreated by either liquid or solid-phase immunoabsorption can be responsible for false-positive staining for LHRH in cells of the arcuate nucleus area. Similarly, when interferring antibodies are present, this problem can also occur for the specific localization of other peptides and substances. (Supported by AA 06014-01).

REGULATION OF PITUITARY FUNCTION IV

208.1 CHANGES IN DOPAC/DA RATIOS IN THE MEDIAL (MEm) AND LATERAL (MEl) MEDIAN EMINENCE IN RESPONSE TO HYPERPROLACTINEMIA AND SUCKLING. Jeffrey I. Mechanick*, Ilene R. Cohen-Becker*, Karen A. Gregerson* and Michael Selmanoff. Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

We have established a radioenzymatic-thin layer chromatographic assay which simultaneously determines dopamine (DA), norepinephrine (NE) and epinephrine with a sensitivity of 1-10pg and 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylglycol (DOPEG) with a sensitivity of 80-120pg. We have used this assay in three experiments. In each experiment microdissected tissue from 5 rats was pooled for a single assay determination such that experimental groups of 8 comprised tissue from 40 rats. We first completed a localization study in adult male rats in 20 brain structures and the anterior (AP) and posterior (PP) pituitary glands. This is the first such study for DOPEG and the most thorough one to date for DOPAC. DOPAC was detectable in 13 of 22 structures with DOPAC/DA ratios ranging from 8% (caudate nucleus, CN) to 25% (MEl). DOPEG was detectable in 6 of 22 structures with DOPEG/NE ratios ranging from 8% (interstitial nucleus of the stria terminalis, ventral aspect) to 32% (MEm). The second and third experiments attempted to see if the DOPAC/DA ratio in the MEm and MEl would change under circumstances in which we expected to observe the tuberoinfundibular dopamine (TIDA) neurons to increase (in response to elevated prolactin) or decrease (in response to an acute suckling stimulus) their activity. Male rats were made hyperprolactinemic with 7 injections (4mg/kg sc) of ovine prolactin (oPRL) spaced 8 hours apart. In such rats the DOPAC/DA ratio was increased in the MEm (2.2-fold) and the MEl (1.9-fold) but not in the CN nor the PP gland and there was a 2.6-fold increase in the concentration of DA in the AP gland. DOPEG/NE ratios remained unchanged in the MEm and the MEl. In 10 day postpartum mother rats, 10 but not 20 minutes of suckling produced a decrease in the concentration of DOPAC in the MEm compared with 0 time controls. However, there was no change in the DOPAC/DA ratio in the MEm or the MEl nor were any DOPEG/NE changes observed. The results suggest that elevated PRL increases the TIDA neuronal activity which is reflected in increased DOPAC/DA ratios in the MEm and the MEl and the increased DA concentration in the AP gland. The suckling stimulus either produces a small decrease in TIDA neuronal activity which is not detected by this metabolic index or suckling does not affect the activity of these neurons. Supported by NIH grants NS-14611 and HD-15955. KAG is the recipient of NIH National Research Service Award HD-06481. MS is the recipient of NIH Research Career Development Award NS-00731.

208.2 MILK EJECTION AND EEG STATE IN URETHANE-XYLAZINE ANAESTHETIZED RATS. D.W. McKay* and K. Brown-Grant*. (SPON: P. Redfern)., Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland, A1B 3V6.

Isherwood and Cross (J. Endocr., 87, 437, 1980) reported that the frequency of milk ejection (ME) was twice as high in suckled rats anaesthetized with urethane (U) plus xylazine (X) as compared with rats given U alone. Because Lincoln et al. (Exp. Brain Res., 38, 151, 1980) had shown that ME in U treated rats occurs only when the EEG is synchronized, we determined ME frequency while recording the EEG pattern, synchronized (SS) or desynchronized (DS), in rats given U or U + X.

Episodic release of oxytocin was monitored by the stretch reactions of the 10 suckling pups in Long-Evans rats on Day 14 of lactation anaesthetized with either 1.2 g/kg U i.p. or 0.8 g/kg U i.p. plus 3 mg (0.15 ml) X i.m. The EEG was recorded from stainless steel screw electrodes implanted on day 15 of pregnancy and blood samples were taken through an intracardiac catheter implanted on day 8 of lactation.

With U alone the EEG alternated between SS and DS. Three hours after induction of anaesthesia, if pups were applied, the % time in SS increased from about 35 to more than 90 for up to 1 hr, and then gradually decreased again over the next 1.5 hr of suckling to about 60%. In agreement with Lincoln et al., oxytocin release occurred only when the rat was in SS. In rats given U + X the initial EEG pattern was continual SS and when pups were applied at 30 min after the induction of anaesthesia the EEG pattern remained in SS for the subsequent 2.5 hr period of suckling. The mean number of ME per rat was 21.4 ± 3.4 (8) for the U + X rats but only 9.5 ± 2.1 (11) for U rats ($p < 0.02$). However, it does not appear that this difference can be explained wholly by the greater amount of time spent in SS by U + X rats. When the ME number of U rats is multiplied by the ratio of total suckling time to time in SS, the mean ME number is increased only to 13.1 ± 2.5.

A second neuroendocrine response to the suckling stimulus also differs between the two groups. In the U rats the mean plasma prolactin (PRL) concentration was initially low, but began to increase steadily after about 45 min of suckling and reached a mean value of 120 ± 31 ng RP2/ml after 2.5 hr of suckling. In contrast, PRL was initially high in the U + X rats (176 ± 52) but fell rapidly, despite suckling and frequent milk ejections, to a final value of 8 ± 3.

208.3 INFLUENCE OF PROGESTERONE AND ESTRADIOL ON ACUTE, OVARIECTOMY-INDUCED CHANGES IN PULSATILE LH RELEASE IN THE RAT. R.E. Leipheimer*, A. Bona Gallo*, and R.V. Gallo. Physiology Section, Biological Sci. Group, and Department of Biobehavioral Sciences, University of Connecticut, Storrs, CT 06268

The object of this study was to determine whether the rapid increases in LH pulse amplitude and frequency which occurred within 24 h after ovariectomy (ovx) on diestrus 1 (D1; Leipheimer and Gallo, Neurosci. Abst. 8:63, 1982) were due to the removal of progesterone (P) and/or estradiol (E). Initial studies demonstrated that plasma levels of E and P were 18.2 ± 1.2 pg/ml and 34.1 ± 3.2 ng/ml, respectively, between the evening of D1 and the morning of D2 in our colony of intact rats. Immediately following ovx and jugular vein cannulation on the morning of D1, rats were implanted either with empty silastic capsules or capsules capable of restoring physiological levels of E and P comparable to control values reported above. These rats were continuously bled (75ul/6 min) for 3 h one day after ovx for analysis of pulsatile LH release, and then decapitated for determination of plasma E and P levels. Rats with empty capsules had decreased levels of E and P, and increases in mean blood LH levels, LH pulse amplitude and frequency. Animals with E capsules had physiological levels of E, decreased levels of P, but no suppression of pulsatile LH release. In contrast, animals with P capsules had physiological plasma levels of P, decreased levels of E, and a marked reduction in the acute LH response to ovx. This suppression was due entirely to a decrease in LH pulse amplitude, as pulse frequency was not altered. Rats with E and P capsules had physiological levels of these hormones which resulted in an even greater reduction in the acute LH response to ovx. This suppression was due to decreases in both LH pulse amplitude and pulse frequency. The effect of P on LH pulse amplitude was centrally mediated since the in vitro response to LHRH of anterior pituitary (AP) fragments from P-implanted rats was the same as that of AP fragments taken from rats with empty capsules. These studies demonstrate that the acute increase in LH pulse amplitude that occurs within 24 h after ovx on D1 is due to the absence of a central inhibitory effect of ovarian P, while the rapid increase in LH pulse frequency is due to the loss of both ovarian E and P. Thus during the rat estrous cycle (Gallo, Biol. Reprod. 24:771, 1981) the decrease in LH pulse amplitude occurring from D1 to D2 may well be due to the central inhibitory effect of P, while the stable LH pulse frequency between D1 and D2 may be due to the braking effect of E and P combined upon the firing rate of the LHRH pulse generator. Supported by NIH HD05577.

208.4 CHANGES IN LH PULSE AMPLITUDE AND FREQUENCY AS A FUNCTION OF TIME FOLLOWING CASTRATION OF MALE FERRETS. Cheryl L. Sisk and Claude Desjardins*. Dept. Zoology, Univ. Texas, Austin, TX 78712

Plasma LH patterns in intact adult male ferrets are characterized by the occurrence of LH pulses. Peak LH levels range from 3-7 ng/ml, while plasma LH is typically undetectable in the interpulse interval. On average, LH pulse frequency is 0.5 pulse/hr. However, pulses do not occur at regular intervals and may appear in groups of 2-4 spaced within 2 hours' time. In contrast, the frequency of LH pulses in long-term (>60 days) castrated ferrets is a consistent 2 pulses/hr. Post-castration basal LH concentrations are ≥4 ng/ml and peak LH levels are 8-12 ng/ml. In this study we followed the development of this castration response by examining the pattern of pulsatile LH release as a function of time after castration.

Male ferrets (age >6 mos; wt >1.5 kg) were fitted with an indwelling atrial cannula and loosely tethered to a valve mounted outside the cage. Blood was obtained from each ferret during 3 separate sampling periods. Two days after cannulation, blood samples (0.4 ml) were withdrawn every 5 min for 8 hr. Withdrawn blood was replaced by a mixture containing 48% ferret red blood cells and 2.5% human plasma proteins in Krebs-Ringer solution. Five days after this initial sampling period, ferrets were castrated. Twenty-two hr later, a second set of blood samples was withdrawn every 2.5 min for 4 hr. The third set of blood samples was obtained in a similar fashion on day 6 post-castration. Plasma LH concentrations were measured by a heterologous ovine-ovine RIA.

In intact ferrets, LH pulse frequency was 0.3-0.6 pulse/hr. The characteristics of these pulses were similar to those described above for intact ferrets. Within 22 hr after castration, LH pulse frequency increased to 1.5-2 pulses/hr. Basal LH levels rose to 2-4 ng/ml. The amplitude of LH pulses was small, with peak LH levels reaching only 4-6 ng/ml. At 6 days post-castration, LH pulse frequency was 2 pulses/hr. Basal LH levels were ≈5.5 ng/ml and peak LH levels were ≈11 ng/ml.

The frequency of LH episodes increases and becomes maximal within the first 22 hr following castration. In contrast, increases in basal LH levels and in the amplitude of LH pulses continue through at least 6 days following castration. These results suggest that the testes normally inhibit an inherent ≈30 min periodicity underlying pulsatile LH release, and that this inhibition is quickly unmasked following castration. The more gradual post-castration increase in basal LH levels and in pulse amplitude may reflect a change in pituitary responsiveness to LHRH and/or increased LHRH stimulation following castration.

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208.5 LESIONS OF THE PARAVENTRICULAR NUCLEAR REGION SUPPRESS PULSATILE FSH BUT NOT LH RELEASE. M.D. Lumpkin*, W.K. Samson*, J.K. McDonald and S.M. McCann. Department of Physiology, University of Texas Health Science Center, Dallas, TX 75235.

Previously we reported that bilateral lesions of the paraventricular nucleus-dorsal anterior hypothalamic area (PVN-DAHA) would reduce the post-castration blood levels of FSH but not those of LH in male and female rats (Fed Proc 38:1107, 1979) and would also attenuate the progesterone stimulation of FSH but not LH in the estrogen-primed ovariectomized (OVX) rat (Fed Proc 41:984, 1982). However, the pulsatile nature of gonadotropin release was not examined in the above experiments. In addition, we first characterized the ultradian release of FSH in the OVX rat in a previous communication (Endo Soc, Abst. 574, 1981). In the present report, we sought to confirm the presence of pulsatile FSH release in the OVX rat and to examine the role of the PVN-DAHA in selective periodic FSH secretion. This task was accomplished by generating radiofrequency lesions in the PVN-DAHA of female rats ovariectomized for 3 weeks. Control animals consisted of sham-operated rats in which the lesion electrode was lowered to the level of the corpus callosum but was not fired. One week post-lesion, all rats were fitted with jugular cannulae. Blood samples (250 µl) were withdrawn from conscious, undisturbed animals at 10 min intervals for 3 hours and the blood volume replaced. Control animals displayed secretory peaks of FSH in plasma with a frequency of 4.0±0.44 per 3 h (or 1 peak/40-50 min) while LH, as expected, pulsed at a frequency of 5.8±0.49 per 3 h (or 1 peak/20-30 min). The mean peak value was 2401±104 ng FSH/ml (RP-1 ref. prep.) and 43.3±1.32 ng LH/ml (S-1 ref. prep.) in controls. PVN-DAHA destruction reduced FSH peak frequency to 1.2±0.37 per 3 h while the number of LH oscillations remained unchanged at 5.6±0.25 per 3 h. The mean peak value for FSH was reduced to 1477±243 mg/ml but the average for LH peaks did not change significantly (40.6±1.25 ng/ml). Immediately after the 3 h sampling period, synthetic LHRH (200 ng) was infused i.v. into rats and blood samples withdrawn 10 and 40 min later. PVN-DAHA ablation did not alter pituitary gland sensitivity to the LHRH challenge since LH and FSH were released at similar levels in the 2 groups of animals. Since little or no radioimmunoassayable LHRH is present in the PVN-DAHA and because obliteration of the PVN-DAHA does not reduce median eminence levels of LHRH (Fed Proc 38:1107, 1979), we conclude that the PVN-DAHA drives pulsatile FSH release but exerts no control over episodic LH secretion and that the putative FSH-releasing factor and not LHRH is probably responsible for the periodic FSH profile. (Supported by NIH HD-09988 and HD-07062).

208.6 ALTERATIONS OF THE RAT ESTROUS CYCLE WITH KAINIC ACID OR ELECTROLYTIC LESION OF THE ROSTRAL HYPOTHALAMUS. O. K. Rønnekleiv and M. J. Kelly. Oregon Regional Primate Research Center, Beaverton, and Depts. of Anatomy and Physiology, Oregon Health Science Univ., Portland, Oregon 97201.

Previous experiments, using electrolytic lesions and knife cuts, have demonstrated that the suprachiasmatic/medial preoptic (SCN/MPN) region of the hypothalamus is essential for the occurrence of the proestrus surge of prolactin (PRL) and luteinizing hormone (LH) in the female rat. In order to further elucidate the role or the SCN/MPN region in the regulation of proestrus LH and PRL surges two lesion procedures were used: 1. The neurotoxin, kainic acid, at 20-800 µMol doses was pressure-pulse injected bilaterally through a glass pipette (20 µm tip diameter) into the SCN to lesion the cells, leaving fibers of passing intact. 2. Electrolytic lesions were made using Wood's metal electrodes of 20 µm tip diameter and 0.35 mA current for 3 min to destroy cells and fibers.

Young, adult, female rats were treated with kainic acid (n = 25) or received bilateral electrolytic lesions (n = 6). Control animals (n = 5) had the micropipette lowered into the SCN but no lesion was made. Vaginal smears were monitored before lesion and 6-8 weeks afterwards. Serial blood samples (0.4 ml) were obtained every 20 min for 4 hr in the afternoon at various stages post lesion. The plasma was retained for hormone determination, the blood cells were resuspended in a plasma protein solution and reinjected. The majority of animals treated with kainic acid showed some disruption of their estrous cycle. Well placed injections into the SCN caused increased incidence of estrous-type smears, but preliminary data indicate that these animals had normal afternoon surges of LH and PRL. Histological examination of the tissue with nissl and immunocytochemical staining revealed that some cells were still present in the SCN.

Large electrolytic lesions including the MPN and the rostral SCN, but leaving the organum vasculosum (OVLT) intact caused constant estrous animals. These animals had no LH surge, but did show a 1-hr delayed PRL surge. Immunocytochemical staining for luteinizing hormone releasing hormone (LHRH) revealed fiber and cell stain in the OVLT region. LHRH fibers and cells were also present in the mediobasal hypothalamus. These data indicate that lesion of the SCN neurons are effective in altering the estrous cyclicity of female rats. Experiments are in progress using increased doses of kainic acid in the SCN or the MPN to elucidate which neurons control the estrous cycle of the female rat.

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208.7 THE POSTPARTUM PREOVULATORY SURGE OF GONADOTROPIN SECRETION MAY BE INITIATED BY THE LABOR PROCESS. Susan R. Fox* and M. Susan Smith* (SPON: D.M. Koester). Dept. of Physiol., University of Pittsburgh, Pittsburgh, PA 15261.

The rat has a preovulatory surge of gonadotropins postpartum (PPsurge). The signal that initiates this PPsurge could be 1) a time of day signal similar to that occurring on the afternoon of proestrus during the rat estrous cycle, or 2) a time of parturition signal created by either the removal of a placental factor or the addition of a signal from the labor process. To examine what signal initiates the PPsurge blood samples were taken after delivery of the last pup, 2,4,6 and 8 hrs later, and on the following day at 1300,1500,1700 and 1900 hrs. Plasma was assayed for LH and FSH. In 11 rats delivering between 0700 and 2000 hrs the peak gonadotropin concentrations occurred between 1320 and 2325 hrs. Rats delivering in the morning had PPsurges early in the afternoon and rats delivering in the afternoon had PPsurges in the evening. Thus, the PPsurge is apparently a function of both the time of day (as it occurs only in the PM) and the time of parturition. If the time of day signal is the primary signal for the PPsurge then administration of an anesthetic dose of pentobarbital to rats delivering before 1400 hrs should block the PPsurge. Pentobarbital blocked the PPsurge in only 5 of 11 animals on the day of parturition. Suprisingly, these 5 rats had a LH surge the following afternoon despite the presence of 8 suckling pups. The refractoriness of the PPsurge to pentobarbital blockade and to an inhibitory suckling stimulus suggests that although a time of day signal may be necessary for the expression of the PPsurge, it is not the primary signal. Rather the process of parturition may provide the primary signal, perhaps by a mechanism which allows the expression of a time of day signal. This hypothesis is supported by the observation that although estradiol concentrations on the day before parturition are sufficient to initiate an LH surge, a surge occurs only after parturition has been completed. Furthermore, when the labor process was circumvented in 11 animals by cesarian section, the PPsurge in LH was not observed on the day of parturition nor on the following day, whereas the surge of FSH was greatly diminished. Therefore, the signal for the PPsurge provided by the labor process did not seem to be present following cesarian section, suggesting that this signal is not the removal of an inhibitory placental factor. Moreover, when pups were delivered by a hysterectomy procedure involving significant cervical manipulation, a PPsurge was observed that day in 2 of 4 animals. These results suggest that the signal arising during parturition that is responsible for initiating the PPsurge may be created by the cervical stimulation that eventuates during the labor process.

208.8 LONGTERM PULSATILE LHRH INFUSION CAUSES PSEUDO-PRECOCIOUS PUBERTY AND PERSISTENT ESTRUS IN THE PREPUBERTAL FEMALE GUINEA PIG. M.D. Loose*& E. Terasawa, Neurosciences Training Program & Wisconsin Reg. Primate Res. Ctr., University of WI, Madison, WI 53715-1299.

Female guinea pigs were studied to investigate the effects of pulsatile LHRH infusion on the onset of puberty. Prepubertal female guinea pigs (19.6 $\pm$ 1.7 days of age; n=13) received jugular catheters through which a two minute pulse of LHRH (0.05µg or 0.1 µg) was infused once an hour for 14 to 39 days. Animals were placed in a canvas jacket with a flexible tether which lead to a swivel allowing free movement. The swivel was connected to an infusion pump and automatic timer apparatus. The vagina was examined daily and, if open, vaginal smears were taken. Laparotomies were performed shortly before or after the termination of the infusion to determine the presence or absence of corpora lutea and to measure ovarian and uterine dimensions. In control females which received no infusion (n=10), the day of the first vaginal opening (VO) and ovulation were similarly determined. To examine ovarian histology five animals which received the LHRH infusion and 4 prepubertal control animals were ovariectomized at 32-40 days of age. In the control females VO occurred at 47.4 $\pm$ 11.3 days of age and the mean age at the first ovulation was 54.1 $\pm$ 14.4 days. The age at VO of the LHRH infused animals was 24.5 $\pm$ 1.9 days, 5.0 $\pm$ 0.8 days after the infusion was begun and was significantly earlier ($p<0.001$) than the controls. After vaginal opening all 13 animals that received LHRH showed characteristics of persistent estrus; 1) the vagina in 11 of 13 animals remained open until the infusion was stopped, the remaining 2 animals had prolonged vaginal opening; 2) cornified cells were predominant in the vaginal smear; 3) the histological appearance of the ovaries was polyfollicular and 4) the uterus was significantly enlarged, 4.4 $\pm$ 0.3mm in diameter, as compared to the prepubertal controls, 2.6 $\pm$ 0.8mm,($p<0.001$). The effects of LHRH infusion on the day of ovulation were not consistent: Four animals ovulated at 38.8 $\pm$ 3.3 days of age during the infusion period, while the remaining four animals ovulated at 58.9 $\pm$ 9.1 days of age, 5 or more days after the infusion was terminated. None of the histologically examined ovaries had corpora lutea. We interpret these results to indicate that the exogenous LHRH stimulated gonadotropin secretion which in turn stimulated folliculogenesis and steroid synthesis causing premature and prolonged vaginal opening and cornification, as well as increased uterine size, but not precocious ovulation. Therefore, we conclude that the doses and frequency of LHRH infusion employed in this study caused pseudo-precocious puberty followed by persistent estrus in the female guinea pig. Furthermore, the present experiment also suggests that increased release of pulsatile LHRH may be one of the causes of the persistent estrus syndrome. Supported by NIH grants HD11355, HD15433 and RR00167.

208.9 Evidence for serotonergic-catecholaminergic coupling in the central control of LH secretion in normal and androgenized female rats. R.J.Handa*,T.P.Condon and R.A.Gorski (SPON: C.H.Sawyer). Lab. of Neuroendocrinology, BRI and Dept. of Anatomy. UCLA, Los Angeles, CA 90024

We have reported that the timed administration of serotonin (5-HT) induces ovulation in lightly androgenized female rats exhibiting the delayed anovulatory syndrome (DAS; Anat.Rec.202:74a). In this study we examined the role of 5-HT and catecholamines in the estrogen-promoted circadian discharge of LH in both normal and androgenized female rats. To induce the DAS, 10ug of testosterone propionate (TP) was injected on day 5 of life. Controls received oil. Ovariectomy (ovx) was performed 3 weeks after the onset of persistent vaginal estrus in the TP-treated group or in age matched controls. A 5mm silastic capsule filled with crystalline 17B-estradiol (E) was implanted SC 15-18 days following ovx. Blood was sampled by intra-atrial cannulae throughout the following 3 days (day 1-3). This E treatment resulted in a daily afternoon release of LH in control but not in androgenized females. Pretreatment of controls with parachloroamphetamine (PCA,10mg/kg BW; a depletor of 5-HT) 1 day prior to E administration abolished the LH surge. Vehicle injections on day 1 had no effect on LH release in PCA,E-pretreated rats, but 5-hydroxytryptophan (5-HTP; 10,20,50 mg /kg BW IP, given at 1000 h) on days 2 and 3, reinstated the afternoon surge of LH. This effect of 5-HTP was characterized by an acute dose-dependent rise in plasma LH within 15 minutes on injection, followed several hrs later by an afternoon increase in LH similar to that seen in the E-only treated female. Whereas increasing doses of 5-HTP prolonged the acute response of plasma LH, the highest dose resulted in an attenuation of the afternoon rise in LH. In vitro incubation of hemipituitaries from PCA,E-pretreated control females in medium containing 5-HTP (10^{-5}M) failed to increase basal LH levels, suggesting a central site of 5-HTP action. All androgenized females pretreated with PCA and E responded to 5-HTP given at 1000 h on days 2 and 3 with an acute rise in LH. In addition, 60% of these animals also showed afternoon rises on day 2. This afternoon response was much smaller than that of control females but is comparable in amplitude to the proestrous rise in plasma LH seen in lightly androgenized females which are still cycling (Endo.Soc.Abst.#421,1983). Vehicle had no effect. The administration of AMPT (200mg,kg BW; a catecholamine synthesis inhibitor) to PCA,E-pretreated normal and androgenized females on the evening prior to 5-HTP administration, abolished the afternoon LH response while leaving the acute response to 5-HTP intact. These data suggest that a stimulatory afternoon catecholaminergic neural signal which drives LH secretion is temporally coupled to previous serotonergic activity. In the androgenized female this coupling may be lost. HD01182, GM07191

208.10 OVARIAN STEROIDS REVERSE THE INHIBITORY EFFECTS OF NOREPINEPHRINE (NE) ON LH SECRETION IN CASTRATE MALE RATS. T.P. Condon*, R.J. Handa*, C.H. Sawyer and D.I. Whitmoyer. Dept. of Anatomy and Lab. of Neuroendocrinology, BRI, UCLA, Los Angeles, CA 90024.

It has been reported previously that intracerebroventricular (ICV) infusion of NE into ovariectomized (OVX) rats inhibits pulsatile LH secretion whereas similar NE infusion into estrogen-progesterone (EP)-primed OVX rats facilitates secretion of LH. We now report an analogous reversal of the inhibitory effects of NE on LH secretion by EP priming in adult male rats.

Adult long-term (4 wks) castrated male Sprague-Dawley rats bearing chronic 3V-ICV cannulae were maintained under standardized lighting and temperature conditions. Unanesthetized, freely-moving rats were bled via intra-atrial cannulae at 5-min (unprimed) or 15-min (primed) intervals for 4-5 h. Plasma LH and FSH secretory patterns were determined by radioimmunoassay using NIADDK reagents. After each sample (0.25 ml) was drawn, an equal volume of blood substitute was infused. Steroid priming was accomplished by SC injection of 50 µg estradiol benzoate + 25 mg progesterone two days before the bleeding session. After a 2-3 h control bleeding period, animals received an ICV infusion (2 µl over 2 min) of either 0.3 µmoles NE or vehicle (acid saline, pH 5.5-6.0) and were bled for an additional 2 h. Gonadotropin secretory patterns during control periods were similar to those we have reported in OVX rats (Neuroscience 1982 Abst. #18.46). In unprimed rats distinct pulses of LH were observed while FSH excursions were less well defined. The apparent LH pulse periodicity of 15-25 min in castrate male rats was somewhat shorter than that observed in long-term OVX rats (approx. 30 min). NE infusion into unprimed castrate male rats caused a rapid and potent inhibition of pulsatile LH secretion while FSH levels were affected to a much lesser extent. Vehicle infusion had little or no effect on LH or FSH levels. LH secretory patterns in primed animals during control periods remained low and non-pulsatile. However, ICV infusion of NE caused a distinct and immediate increase in LH in 7 of 8 rats, lasting 30-60 min. This facilitation of LH secretion was similar in magnitude and duration to that reported in OVX, EP rats. Again, FSH levels did not appear to be affected. Vehicle infusion was without effect.

From these data we conclude that, in the absence of gonadal hormones, NE has a marked inhibitory effect on LH secretion in male rats as in female rats. However, castrate male rats primed with ovarian steroids can respond to an NE stimulus with a facilitation of LH secretion. We suggest that the central mechanisms by which ovarian steroids modulate noradrenergic effects on LH secretion in adult female rats are also present in the adult male. (Supported by NIH grants NS01162, HD06332-01, and HD01182.)

208.11 ALTERATIONS IN THE PATTERN OF PULSATILE SECRETION OF LH FOLLOWING OVINE PROLACTIN (oPRL)-INDUCED HYPERPROLACTINEMIA IN SHORT-TERM OVARIECTOMIZED RATS. Ilene R. Cohen-Becker*, Michael Selmanoff and Phyllis M. Wise (SPON: N. Pilotte). Department of Physiology, University of Maryland, School of Medicine, Baltimore, MD 21201.

Previous investigators have demonstrated that chronic hyperprolactinemia suppresses the elevated levels of LH found following ovariectomy (Grandison et al., Neuroendo. 23:312, 1977; Smith, Biol. Reprod. 19:177, 1978; Login et al., Neuroendo. 35:327, 1982). In these studies hyperprolactinemia was induced by multiple pituitary grafts under the kidney capsule, lactation or prolactin secreting tumors. Each method used produced high prolactin levels, however, in every case additional hormones were secreted. To obviate this problem, we induced a state of hyperprolactinemia by exogenous treatment with oPRL. The purpose of this experiment was to examine the effect of prolactin on the pattern of pulsatile secretion of LH in the ovariectomized rat, as may be evident by a change in the frequency and/or amplitude of the pulses. Female Sprague-Dawley rats (200-225 gms) were bilaterally ovariectomized under ether anesthesia on Day 0. Animals were given subcutaneous injections of oPRL (4 mg/kg body weight) every eight hours beginning at 0900h two days after ovariectomy (Day 2). Control animals received an equivalent regimen of vehicle. Blood samples (0.1 ml) were withdrawn and replaced with an equivalent volume of saline, from 1400h to 1700h at ten minute intervals. LH concentrations were determined by radioimmunoassay in serum using the RP-2 standard. Control animals exhibited a mean of 1.90 ± 0.16 pulses of LH per hour. Hyperprolactinemic animals showed a decreased frequency of LH pulses with a mean of only 1.29 ± 0.23 pulses per hour. Differences in the peak amplitude were observed between control and hyperprolactinemic animals. The amplitude of the pulses in the control rats averaged 4.58 ± 0.50 ng/ml of LH. In contrast, the amplitude of the pulses in hyperprolactinemic rats was significantly lower, averaging only 2.30 ± 0.15 ng/ml of LH. In conclusion, oPRL-induced hyperprolactinemia in short-term ovariectomized rats resulted in an alteration in the pattern of pulsatile secretion of LH. This change involved both a decrease in the frequency and the amplitude of the pulses of LH. Since LH pulse frequency is thought to be dependent upon a Gn-RH pulse generator, our data may be due to a direct effect of oPRL on the frequency of Gn-RH pulses. Studies are in progress to determine if the decreased amplitude resulted from an alteration in pituitary sensitivity to Gn-RH. Supported by NIH grant HD-15955.

208.12 FURTHER EVIDENCE FOR THE INVOLVEMENT OF SEROTONIN IN STEROID INDUCED LH RELEASE IN OVARIECTOMIZED RATS. S. Iyengar* and J. Rabii. (SPON: A.K. Sinha). Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08854.

We have continued our investigation of the role of serotonin in the hypothalamic regulation of luteinizing hormone (LH) secretion. In our earlier experiments (Neuroscience Abst. 18:48, 1982), using a neuropharmacologic approach, we reached the conclusion that brain serotonin plays a stimulatory role in the control of LH secretion. This abstract presents the results of our recent work in which a direct approach to the brain serotonergic system was used in an attempt to further elucidate the involvement of this amine with LH release. The experimental model is the ovariectomized rat primed with estrogen (5µg) and treated with progesterone (1.5 mg) 48 hours later. This treatment leads to a well defined LH peak about six hours following the administration of progesterone. In the first study, the dorsal raphe nucleus, site of origin of hypothalamic serotonergic input, was destroyed by electrochemical lesioning. A current (anodal-direct) of 2mA was applied via a monopolar stainless steel electrode for 15 seconds. Sham lesioned animals were used as controls. Surgery was performed one week before steroid treatment. Destruction of the dorsal raphe nucleus led to a marked reduction in the steroid induced LH release. In the second study the serotonergic neurons were destroyed by the intraventricular injection of the neurotoxin 5,7-dihydroxytryptamine (150µg), while the catecholaminergic system was protected with desipramine. Sham controls were infused with the ascorbic-saline vehicle. This treatment, a week before steroid administration, also led to a marked reduction in progesterone-induced LH release in the estrogen-primed castrated rats. Finally using a Halasz-type knife (a 1.5 mm blade) the serotonergic pathway to the hypothalamus was transected. A sham group, in which the blade was lowered to the superior aspect of the tract, was used as control. Here again a marked reduction in LH release was observed in transected rats, when treated with estrogen and progesterone a week later. These data are in agreement with those using a pharamacologic approach and strongly suggest a stimulatory role for serotonin in the hypothalamic control of LH secretion. Supported by the Bureau of Biological Research, Rutgers University, and a BRSG grant from the Office of Research and Sponsored Programs (RU).

208.13 LHRH RELEASE IN VITRO IS INCREASED IN ESTROGEN-TREATED CYNOMOLGUS MACAQUES. J.E. Levine and H.G. Spies. Div. of Reproductive Biology and Behavior, Oregon Regional Primate Center, Beaverton, OR 97006.

It has been proposed that gonadotropin secretion in the primate occurs under the permissive influence of tonic hypothalamic input. This view has been challenged by a recent study which demonstrated that a neural signal is required in the generation of a preovulatory luteinizing hormone (LH) surge (Norman et al., Endo 111: 1874). We sought to determine if this hypothalamic signal is composed of an estrogen-dependent change in the hypothalamic secretion of luteinizing hormone-releasing hormone (LHRH).

Diencephalic tissue blocks were obtained at autopsy from individual cynomolgus macaques and placed in ice-cold, oxygenated Krebs-Ringer-Phosphate (KRP) medium. Mediobasal hypothalamic (MBH) and preoptic-anterior hypothalamic (POA/AH) tissue fragments were quickly dissected away from the block and placed in separate superfusion chambers at 37°C. Tissues were superfused at 60µl/min, and effluent LHRH levels were determined by RIA. After superfusion, tissues were homogenized in 0.1N HCL and stored for LHRH RIA. Experimental animals received s.c. silastic implants containing estradiol-17β (E_2) to achieve >250pg/ml serum E_2 levels and were sacrificed 12h, 36h, 42h, or 48h later (n=3 for E_2 groups). Tissues from at least one animal bearing empty capsules (control, n=6) were superfused simultaneously with all experimental groups. LH levels in serum obtained before and after E_2 treatment were determined by Leydig cell bioassay.

LHRH release was typically greater from MBH compared to POA/AH fragments, and both tissues responded to treatment with 60mM K^+-KRP with increased LHRH secretion. Average release rates during 3h of superfusion and subsequent tissue concentrations are summarized below:

	LHRH Release (pg/min)		LHRH content (pg/mg)	
Group	MBH	POA/AH	MBH	POA/AH
CTL	.331±.062	.128±.012	34.1±4.3	6.4±2.1
12h	.731±.204*	.609±.096***	38.0±1.9	15.8±5.1
36h	.239±.088	.141±.022	42.1±7.7	6.4±2.1
42h	.867±.247**	.661±.104***	62.7±11.4**	21.1±2.6***
48h	.118±.018	.106±.003	25.2±20.8	6.2±2.1

*p<.05, **p<.025, ***p<.01; compared to CTL.

LHRH release was maximal at 42h, when serum LH levels were also highest. Paradoxically LHRH release was also higher at 12h post-E_2, during the period of LH inhibition. These results confirm our concurrent in vivo studies (Soc. for the Study of Rep. Abst.#37) which also demonstrate that LHRH release is increased in E_2-treated monkeys. On the basis of both studies we contend that the primate hypothalamus actively participates in the generation of the E_2-induced gonadotropin surge through the increased secretion of LHRH. Supported by NIH grants HD-16631 and HD-07133.

208.14 SUPRESSION OF LH BY ESTRADIOL IS BLOCKED BY LY117018 IN FEMALE C57BL/6J MICE. C. Mobbs*, N. Telford*, M.N. Gordon*, and C.E. Finch (SPON: Z. Wenzel). Dept. of Biology, Andrus Gerontology Center, USC, Los Angeles, CA, 90089-0191.

Selective antagonists to estradiol (E_2) would aid examination of LH secretion and loss of estrous cycles, which are both sensitive to low physiological levels of plasma E_2. However, most known antagonists possess significant agonistic activity, and so are of limited utility in studying such sensitive neuroendocrine phenomena. In contrast, LY117018 is reportedly a selective E_2 antagonist exhibiting little agonistic activity, as shown by effects of the drug and E_2 on uterine weight. The drug's mechanism of action in the uterus involves competition at the estradiol receptor. However, little is known about its effects on neuroendocrine functions. Therefore, we examined whether LY117018 could block the supression of LH by E_2, and if by itself the drug would supress LH secretion. Female, 3-month-old virgin C57BL/6J mice were ovariectomized for one week, then given either E_2 or sham implants; the E_2 implants gave stable plasma E_2 levels of about 10 pg/ml, a low physiological value in intact mice. Three days after implantation, half of the mice in each implant group were given daily subcutaneous injections of LY117018 (50 ug in 100 ul oil) or oil only. After 2 weeks of daily injection, all mice were sacrificed between 1400 and 1600 h by decapitation. LH levels and uterine weights are shown in the table. The drug blocked the effect of the E2 implant on both uterine weight and LH levels (p<.05, ANOVA), and by itself slightly increased uterine weight (p<.05, Newman-Keuls), but did not by itself affect LH levels.

Group	Ut. Wt. (mg)	LH (ng/ml)
Sham+oil	21±3	230±50
E_2+oil	72±3	80±60
Sham+drug	32±3	340±50
E_2+drug	36±3	310±50

These results indicate that LY117018 can antagonize the effect of a physiological level of E2 on LH secretion without, by itself, affecting LH secretion. Since the antagonist showed some agonistic activity on uterine weight, LY117018 may be even more selective as an E2 antagonist at neuroendocrine loci than at peripheral loci.

These studies were supported by NIA grants AG-00117, AG-00443, and 5T32 AG00037-05. LY117018 was generously supplied by Lilly Research Laboratories.

208.15 ELECTROPHYSIOLOGICAL ACTIONS OF 17β-ESTRADIOL ON MEDIAL BASAL HYPOTHALAMIC LUTEINIZING HORMONE-RELEASING HORMONE NEURONS IN THE FEMALE GUINEA PIG. M.J. Kelly, O.K. Ronnekleiv and J.E. Levine*. Depts. of Physiology, Anatomy, and Oregon Regional Primate Center, Oregon Health Sciences University, Portland, OR 97229

A central issue in neuroendocrinology today is the role of medial basal hypothalmic (MBH) luteinizing hormone-releasing hormone (LHRH) neurons in the control of the female reproductive cycle and feedback regulation of gonadal steroids on these neurons. We have utilized the in vitro hypothalamic slice preparation to identify estrogen-responsive, luteinizing hormone-releasing (LHRH) neurons in the arcuate (ARC) and cell-poor zone (CPZ) of cycling female guinea pigs. Sagittal hypothalamic slices were prepared as previously described [Kelly et al., Exp. Brain Res. 40:440-447 (1980)]. Intracellular recordings were made in eighty-four ARC-CPZ neurons using procion yellow and KCl/KCitrate-filled electrodes. Resting membrane potentials ranged from -35 to -75mV. Similar to in vivo findings, ARC-CPZ neurons exhibited slow, irregular spontaneous activity, and fourteen percent of the ARC-CPZ neurons were antidromically identified as projecting to the median eminence (ME). Also, orthodromic activation was possible via stria terminalis and ME stimulation, and spontaneous postsynaptic potentials were observed. Concentrations of 10^{-9} to 10^{-8} M 17β-estradiol (E_2) in the slice medium hyperpolarized and/or inhibited the spontaneous activity of 29 of the 84 neurons. Inhibition of spontaneous EPSPs was observed at similar concentrations in other neurons. Most of these cells were identified through intracellular labeling with procion-yellow. The estrogen-responsive neurons tended to be small (10μm) bipolar, fusiform neurons, and four of these were found to contain immuno-reactive LHRH [for immunocytochemical staining of slices see Kelly et al., Exp. Brain Res. 48:97-106 (1982)]. Furthermore, preliminary evidence indicates that we can measure LHRH release from the slices during the recording and correlate the rate of release of the peptide with the electrophysiological effects of estrogen on LHRH neurons. In conclusion, we have physiological and morphological evidence that E_2 acts directly on MBH LHRH neurons to inhibit spontaneous activity and alter the neurosecretory release of the peptide. We hypothesize that these rapid membrane effects are involved in the negative feedback actions of E_2 on the mammalian hypothalamus.

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208.16 EFFECTS OF FLUROTHYL- AND ELECTRO-CONVULSIVE SHOCK ON LH AND PROLACTIN SECRETION. R. Bhanot and M. Wilkinson. Depts. of Physiol. Biophys. and of Obstet. Gynecol., Dalhousie Univ., Halifax, N.S., Canada, B3H 4H7.

We have previously reported that repeated convulsions exert adverse effects on the hypothalamic-pituitary-gonadal system of the rat (J. Endocr. 95:37; 95:43, 1982). We now report the effects of flurothyl- and electro-convulsive shock (ECS) on the onset of sexual maturation and on LH and prolactin secretion.

Repeated flurothyl convulsions and ECS (one convulsion per day from age 24 days) significantly delay sexual maturation: convulsed rats reached vaginal opening at 37±1 (flurothyl) and 40±1 (ECS) days compared with 34±1 days (control). Moreover, almost all control rats at first estrus showed ova in the oviducts whereas only 20% of the convulsed group ovulated. In an effort to clarify the mechanism of action of convulsions on puberty onset, we examined acute changes in gonadotropin and prolactin secretion and the positive feedback response of LH to EB/P priming. Serum LH levels (ng/ml ± sem) in immature female rats (48 h post-OVX) 15 min following a single flurothyl convulsion were 86±17 (LH, p<0.025) and 6±1 (prolactin, p<0.005) compared with 288±78 (LH) and 19±3 (prolactin) in controls. Thus, flurothyl convulsions potently inhibit prolactin secretion. In contrast, a single ECS stimulates prolactin secretion in immature females (48 h post-OVX). Serum LH and prolactin levels were 175±42 and 55±10 (p<0.005) in convulsed female rats vs. 200±25 and 10±4 ng/ml in the control group. However, both flurothyl convulsions and ECS attenuate EB/P-induced LH surges in OVX immature rats. Repeated flurothyl seizures (one per day x 10 days) reduced LH surge levels to 423±171 from 1040±202 ng/ml (controls). Similarly, peak LH levels were 663±202 following ECS (one per day x 10 days) vs 2310±480 ng/ml (controls). These results show that convulsive seizures do not significantly alter tonic gonadotropin secretion whereas prolactin output is potently and selectively modified. We have also observed a remarkable difference between the effects of flurothyl and ECS on prolactin. This result has important implications toward the understanding of dopamine-mediation of the antidepressant effects of convulsive treatment. Our results show that flurothyl and ECS convulsions selectively block the neural mechanisms underlying preovulatory-type LH secretion and sexual cyclicity. The disruption of the positive feedback mechanism is apparently not mediated by dopamine/prolactin since chronic bromocriptine treatment delays sexual maturation without blocking ovulation at first estrus. Similarly bromocriptine does not disrupt EB/P-induced LH surges in immature OVX rats (Supported by MRC grant to MW).

208.17 MAITOTOXIN, A CALCIUM CHANNEL ACTIVATOR CANDIDATE, STIMULATES THE RELEASE OF PROLACTIN IN VITRO. K. Koike,* G. Schettini,* A.M. Judd,* M.J. Cronin, I.S. Login* and R.M. MacLeod. (SPON: N. Lenn). Depts. of Med., Physiol. and Neurol., University of Virginia School of Medicine, Charlottesville, Virginia 22908.

Maitotoxin (MTX) (kind gift from T. Yasumoto, Tohoku Univ., Sendai, Japan), a dinoflagellate (Gamdierdiscus toxcus) marine toxin, is a potent calcium (Ca^{2+}) channel activator in pheochromocytoma cell lines (Takahashi et al., J. Biol. Chem., 257: 7287, 1982) and stimulates $^{45}Ca^{2+}$ influx and norepinephrine release. We studied the effect of MTX on pituitary prolactin (PRL) release, which is a Ca^{2+}-dependent process. In primary cultures of anterior pituitary cells, MTX stimulated PRL release in a dose-dependent manner (10^{-9} to 10^{-7} g/ml); the stimulation was 10-fold at the highest concentration used (p 0.01). MTX (5 x 10^{-8} g/ml) significantly (p 0.01) enhanced PRL release after 1.5 min of exposure (4-fold increase) and stimulation was maximal after 1 h (10-fold increase, p 0.01). We studied the effect of manganese (Mn^{2+}), a Ca^{2+} channel blocker, on the MTX-mediated increase in PRL release: 300 uM Mn^{2+} partially blocked (67%; p 0.01), whereas 3 mM abolished the stimulation of PRL release by MTX (p 0.01). The effect of MTX was also studied in perifused pituitary cells. A 10-min exposure to 5 x 10^{-9} g/ml MTX acutely stimulated PRL release (5-fold) which remained elevated for 1-2 hr. After PRL levels returned to baseline, the cells responded to 100 nM TRH by increasing PRL release. Preliminary experiments on $^{45}Ca^{2+}$ uptake using dispersed anterior pituitary cells showed that 5 x 10^{-8} g/ml MTX rapidly stimulated the net uptake of $^{45}Ca^{2+}$.

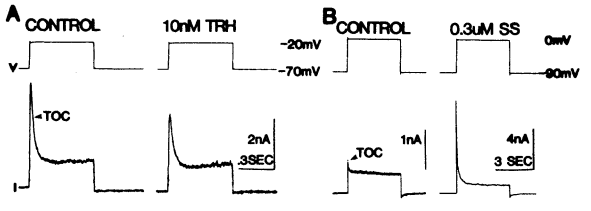
These results indicate that MTX induces a marked release of PRL and $^{45}Ca^{2+}$ uptake, suggesting that at least one component of the stimulatory action of MTX may be ascribed to activation of Ca^{2+} channels. We suggest that MTX may be a useful tool to investigate the involvement of Ca^{2+} in hormone secretory processes.

[1 F05 TW0 3267-01 (GS), 1 F32 CA 07137-01 (AMJ), RCDA 1K04 NS00601, NS 18409 (MJC), TIDA 5R07 NS00454, BRSA 5 S07 RR05431 (ISL), USPHS CA 07535-21 (RMM).]

208.18 TRANSIENT OUTWARD CURRENTS IN CLONAL PITUITARY CELLS ARE REGULATED BY PEPTIDE HORMONES. B. Dufy and J.L. Barker, Laboratory of Neurophysiology, Univ. Bordeaux II, France and Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, MD. 20205

The prolactin-secreting pituitary clone GH3/6 is electrically excitable and individual cells respond to a variety of peptide hormones. Since hormonally-induced changes in excitability coincide temporally with alterations in prolactin secretion, it is evident that changes in one or more ion conductance mechanism may regulate secretory activity. We have applied single- and two-electrode voltage clamp techniques to study the electrically excitable membrane properties resident in GH3/6 cells and the effects of peptide hormones on these properties. Using these techniques we have observed several types of transient and delayed outward currents in addition to inward spike-related currents. Inactivation and activation curves and the rapid kinetics of the transient outward currents (TOCs) indicated that these currents are superficially similar to several types of TOC previously reported in a variety of invertebrate and vertebrate cell types. As in other cells these K^+-dependent TOCs are blocked by either 4-aminopyridine or Co^{2+}, but are relatively insensitive to tetraethylammonium ions. In other cells the TOCs function to control either action potential discharge rate or action potential repolarization rate. The TOCs recorded in GH3/6 cells appear to play similar roles in regulating excitability.

Previous work has shown that thyrotropin releasing hormone (TRH) rapidly stimulates prolactin secretion and increases action potential discharge rate, while somatostatin (SS) has inhibitory actions on both hormone release and cell excitability. We have examined the effects of TRH and SS on TOCs evoked in GH3/6 cells. TRH consistently depresses (Fig. 1A), and SS consistently potentiates TOCs in these cells (Fig. 1B). These effects may help to explain the facilitatory and inhibitory effects of these peptides on action potential discharge rate and prolactin secretion.



208.19 EVIDENCE FOR THE INVOLVEMENT OF ARACHIDONIC ACID METABOLISM IN THE MECHANISM OF PROLACTIN RELEASE IN VITRO. P.L. Canonico* and R.M. MacLeod (SPON: S. Vandenberg). Dept. of Med., Univ. of Va. School of Med., Charlottesville, VA 22908.

Recent evidence suggests the involvement of phospholipid and arachidonic acid metabolism in the secretory mechanisms of several anterior pituitary hormones. We attempted to delineate the role of the fatty acid and its metabolites in the mechanism of prolactin (PRL) release. Arachidonic acid added in vitro to rat pituitary glands significantly stimulated (dose-related) the release of RIA and newly synthesized PRL. Arachidonic acid metabolism occurs primarily through the cyclooxygenase and lipoxygenase pathways. Indomethacin (1-100 uM), a cyclooxygenase inhibitor, did not affect basal PRL release or PRL release stimulated by TRH, phospholipase A_2 or phorbol myristate acetate (PMA). These findings seem to exclude this metabolic pathway as a regulator of PRL secretion. Indomethacin (50 uM) did, however, enhance the stimulatory effect of 100 uM arachidonic acid on PRL release. We have reported that nordihydroguaiaretic acid (NDGA), a rather selective lipoxygenase inhibitor, inhibited basal and TRH-stimulated PRL release in vitro. We now report that 50 uM NDGA significantly inhibited the stimulation of PRL release by phospholipase A_2 or PMA. To further determine lipoxygenase involvement in arachidonic acid metabolism in the mammotroph, we used BW755c, another inhibitor of this pathway. At a concentration of 250 uM, this compound inhibits lipoxygenation of arachidonic acid in pancreatic beta cells and markedly inhibits basal and TRH-stimulated PRL release by hemipituitary glands incubated in vitro and by pituitary cells perifused in columns. In both systems, BW755c also inhibited PRL release induced by 50 mM K^+. Phosphatidylinositol (PI) turnover involvement has been suggested in the mechanism of basal and TRH-stimulated PRL release. PI and other intermediates of the PI cycle (1,2-diacylglycerol, phosphatidic acid) are the cellular sources of arachidonic acid, through the activity of specific lipases, in many cellular systems. We investigated the possible involvement of diglyceride lipase, the enzyme that cleaves the fatty acid from 1,2-diacylglycerol, in the mediation of PRL release by using a selective inhibitor of the enzyme's activity, RHC 80267. At 70 uM RHC 80267 markedly inhibited both basal and TRH- or K^+-stimulated PRL release from hemipituitary glands in vitro. The specificity of this effect is indicated by the finding that neither basal nor stimulated thyrotropin release was affected by the drug. Our findings suggest that pituitary arachidonic acid derived from membranal phospholipids, i.e., 1,2-diacylglycerol, is stimulatory to PRL release presumably through its metabolism via the lipoxygenase pathway.

208.20 CHARACTERIZATION OF ARACHIDONIC ACID INDUCED PROLACTIN RELEASE. L. Grandison and A.M. Camoratto*. Department of Physiology and Biophysics, Rutgers Medical School UMDNJ, Piscataway, N.J. 08854.

We have recently provided indirect evidence that arachidonic acid or its metabolites participate in regulating both basal and secretagogue induced prolactin secretion. Pharmacological agents which inhibit arachidonic acid release from membrane phospholipids block basal, and K^+, TRH or VIP induced prolactin release (Camoratto and Grandison, Fed. Proc. 42:868, 1983) and conversely agents which stimulate arachidonic acid release induce prolactin secretion. To confirm and extend these earlier studies we have characterized the effect of arachidonic acid itself on prolactin release. For this purpose we used GH_3 cells, a clonal cell line producing both prolactin and growth hormone, maintained in vitro. Addition of arachidonic acid to the media was associated with a dose related increase in prolactin release. The lowest effective dose was 10 nM which produced a 50% increase in secretion. Maximal stimulation occurred at 1 to 10μM and was 300% of control. Further increases in the doses of arachidonic acid were associated with inhibition of secretion. The stimulatory effect of arachidonic acid on prolactin release was dependent on extracellular Ca^{++}. Addition of EGTA to a low calcium media blocked the arachidonic acid response. Replenishment of Ca^{++} was associated with a reappearance of arachidonic acid stimulation of prolactin release. These data provide further evidence that arachidonic acid or its metabolites are involved in regulating prolactin secretion.

208.21 THE EFFECT OF GRADED "PHYSICAL" AND "PSYCHOLOGICAL" STRESS ON PROLACTIN RELEASE IN RATS. G. K. Weiss*, D. Yelvington*, A. Ratner*. (Spon: D. Savage). Dept. Physiol., University of New Mexico, School of Medicine, Albuquerque, New Mexico 87131.

Although there have been a number of parametric studies investigating the adrenal response to graded stress it is not clear if the pituitary hormone, prolactin, shows a similar type of correlation. We studied two types of graded stress in rats. One that is considered to be primarily "physical" was produced by various magnitudes of unavoidable footshock and a second, considered to be "psychological", produced by exposing rats to different degrees of novelty. All rats were habituated to handling and blood samples were obtained via right atrial cannulas and analysis for prolactin done using RIA. Footshock was delivered through a metal grid floor of the rat's cage at values between 0.3-10 MA for a duration of 30 sec. Blood was drawn at 5, 10, 15, 25 and 45 min after the shock ended. The magnitude of the prolactin response at 5-10 min was shown to be a function of footshock strength and no habituation of the prolactin response occurred over 15 days. Observation of behavior also showed accentuated "stress related behavior" as the intensity of the footshock increased. Variability of the prolactin response between animals to a given footshock was also correlated to the variability of the behavioral responses.

A second group of rats was exposed either to cages similar to their home cage (low level of stress) were placed on platforms above water (high level of stress). The high levels of stress produced a much greater increase in prolactin than did the low level of stress. Rapid habituation occurred under these conditions. These data indicate that the magnitude of the prolactin response to either a predominantly physical or psychological stress is directly correlated to the intensity of the stress.

208.22 VARIATIONS IN LEVELS OF OXYTOCIN AND VASOPRESSIN IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS DURING STAGES OF THE ESTROUS CYCLE IN RATS. E.R. Greer*, C.A. Pedersen*, M.F. Johnson*, and A.J. Prange, Jr. (SPON: M.C. Diamond). Biol. Sci. Res. Ctr., Univ. North Carolina Sch. Med., Chapel Hill, NC 27514

Oxytocin (OT) and Arginine-8-vasopressin (AVP) were measured by radioimmunoassay in micropunched hypothalamic neurosecretory nuclei of virgin female Sprague Dawley rats during four stages of the estrous cycle.

In the paraventricular nucleus: 1) OT concentration (pg/μg protein) was highest during diestrus, was reduced during proestrus by 45% ($p < 0.001$), during estrus by 48% ($p < 0.001$), and during metestrus by 23% ($p < 0.025$; the OT concentration during metestrus differed from that in proestrus ($p < 0.01$), in estrus ($p < 0.005$), and in diestrus ($p < 0.025$). 2) The concentration of AVP was lowest during estrus, and was 79% higher during proestrus ($p < 0.001$), 38% higher during metestrus ($p < 0.05$), and 66% higher during diestrus ($p < 0.005$). 3) The ratio of concentrations of AVP to OT (AVP/OT) was elevated during proestrus relative to the other three stages: estrus ($p < 0.001$), metestrus ($p < 0.001$), and diestrus ($p < 0.001$).

In the supraoptic nucleus a similar trend occurred: the concentration of OT was elevated during diestrus as compared to estrus (NS); AVP was reduced during estrus relative to proestrus (NS); and AVP/OT was higher in proestrus than in metestrus ($p < 0.05$) and diestrus ($p < 0.005$), while estrus exceeded diestrus ($p < 0.05$).

The variations in concentrations of OT and AVP during the stages of the estrous cycle in the paraventricular nucleus were distinctly cyclic, and may reflect an influence of plasma ovarian steroids on synthesis, axonal transport, and/or release of these peptides.

N: proestrus = 12; estrus = 18; metestrus = 18; diestrus = 10.

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209.1 REGULATION OF ^{3}H-IMIPRAMINE BINDING SITES IN BRAIN BY NORADRENERGIC AXONS. K.J. Kellar and C.S. Cascio*. Dept. of Pharmacology, Georgetown Univ. Sch. of Medicine, Washington, DC 20007

Repeated administration of most tricyclic antidepressant drugs to rats down-regulates beta-adrenergic and 5-HT-2 receptors in brain. In addition, repeated administration of desipramine or imipramine decreases the number of ^{3}H-imipramine (^{3}H-I) binding sites which are associated with presynaptic 5-HT nerve terminals. (Raisman et al., Eur. J. Pharmacol. 61, 1980; Kinnier et al., Eur. J. Pharmacol. 67, 1980). Recently, the possible influence of serotonergic systems on beta-adrenergic receptor regulation following chronic antidepressant drug treatment has been investigated (Brunello et al., Neuropharmacology 21, 1982; Janowsky et al., Science 218, 1982). In these studies, lesions of 5-HT axons were found to prevent the down-regulation of beta-adrenergic receptor binding sites following chronic desipramine treatment. We have investigated the effects of noradrenergic axons on the response of serotonergic receptors (5-HT-2) and imipramine binding sites to repeated administration of amitriptyline or electroconvulsive shock (ECS).

Noradrenergic systems in brain were lesioned with 6-hydroxydopamine (200 ug intraventricular) or received vehicle injections only. Beginning 8 or 19 days later rats received daily injections of saline, amitriptyline (10 mg/kg, 23 days) or ECS (1/day, 12 days). All rats were sacrificed 1 day after last treatment. The lesion resulted in reductions of ^{3}H-NE uptake in cerebral cortex and hypothalamus of 65% and 79%, respectively.

In non-lesioned rats the chronic amitriptyline treatment decreased ^{3}H-spiperone binding to 5-HT-2 receptors in the cortex by 25% and repeated ECS increased binding to 5-HT-2 receptors by more than 40%, as expected. In the 6-OHDA lesioned rats these treatments produced very similar effects on 5-HT-2 receptors: amitriptyline reduced the binding by 24% and ECS increased it by 30%. Thus, noradrenergic systems do not appear to be necessary for 5-HT-2 receptor regulation to occur.

In non-lesioned rats chronic amitriptyline treatment decreased ^{3}H-I binding sites in the cortex (46%), hippocampus (12%) and in the hypothalamus (22%). (ECS did not affect ^{3}H-I sites in cortex or hippocampus.) In contrast to the lack of effect of noradrenergic lesions on 5-HT-2 receptor regulation, the 6-OHDA lesion appeared to completely prevent the down-regulation of ^{3}H-I binding sites in the cortex, hippocampus and hypothalamus following chronic amitriptyline treatment.

These results indicate that noradrenergic and serotonergic systems in the brain can interact in complex ways to regulate either postsynpatic adrenergic receptor regulation or presynaptic sites related to reuptake of 5-HT.

209.2 STIMULATION OF BETA ADRENERGIC RECEPTORS DECREASES BETA RECEPTOR DENSITY AND INCREASES SEROTONIN$_2$ RECEPTOR DENSITY IN RAT CEREBRAL CORTEX SLICES. JAMES A. SCOTT* AND FULTON T. CREWS. Dept. of Pharmacology, University of Florida Medical School, Gainesville, FL 32610.

Recent studies have indicated that administration of beta adrenergic agonists enhanced behavior responses to 5-hydroxytryptamine (Cowen et al., 1982). Since these behavioral responses may be mediated by serotonin$_2$ ($5HT_2$) receptors in the brain (Peroutka et al., 1980), we studied the effects of β-adrenergic stimulation on $5HT_2$ receptor binding sites in rat cerebral cortical slices. Cortical slices were incubated with 100 μM (-)isoproterenol for various periods of time and β-adrenergic and $5HT_2$ receptor binding determined using ^{3}H-dihydroalprenolol (DHA) and ^{3}H-spiperone, respectively. After 90 min of incubation with 100 μM (-)isoproterenol, ^{3}H-DHA specific binding decreased from 95 ± 1 fmol ^{3}H-DHA bound/mg protein to 61 ± 2 fmol ^{3}H-DHA bound/mg protein ($p<0.001$). In contrast, ^{3}H-spiperone specific binding was increased 17 ± 3% ($p<0.001$) from a control value of 241 ± 11 fmoles/mg protein to 282 ± 7 fmoles ^{3}H-spiperone binding/mg protein. Time course studies indicated that these changes require several minutes of incubation, reach a maximum around 30-60 min and are maintained for at least 120 min. Scatchard analysis indicated that there was an increased $5HT_2$ receptor density. Isoproterenol treatment increased ^{3}H-spiperone maximum binding (B_{max}) from a control value of 414 ± 34 fmol/mg protein to 573 ± 46 fmol/mg protein ($p<0.025$). There was no change in apparent K_d. In contrast to the increase in $5HT_2$ B_{max}, isoproterenol treatment decreased the B_{max} for ^{3}H-DHA binding. These post-synaptic changes in serotonergic and noradrenergic receptors could represent an important site of interaction between these two central biogenic amine systems. Possible mechanisms of these receptor interactions will be discussed.

209.3 SEROTONIN INNERVATION OF DOPAMINE NEURONS IN RAT VENTRAL TEGMENTAL AREA. D. Hervé*, T.H. Joh, V.M. Pickel and A. Beaudet, Montreal Neurological Inst., McGill Univ., Montreal, Quebec H3A 2B4 and Lab. of Neurobiology, Cornell Univ. Med. Coll., New York, N.Y. 10021

Biochemical studies have suggested that ascending serotonin (5-HT) neurons might be implicated in the regulation of dopamine metabolism in dopaminergic neurons of the ventral tegmental area (VTA). In an attempt to identify the morphological substrate for such interactions, cellular relationships between 5-HT nerve terminals and tyrosine hydroxylase (TH) immunoreactive neurons were investigated in the VTA by combined radioautographic and immunocytochemical methods. Adult rats were pretreated with a monoamine oxidase inhibitor and submitted to 3h intracerebroventricular infusion of 10^{-4}M tritiated serotonin (^{3}H-5-HT) to which 10^{-3}M non-radioactive noradrenaline was added to prevent uptake of (^{3}H)5-HT by catecholamine elements. Brains were fixed by intraaortic perfusion of 0.5-1% glutaraldehyde and 4% paraformaldehyde. Serial 20 μm-thick vibratome sections were taken across the VTA, immunocytochemically reacted against TH according to the PAP method of Sternberger and processed for light and electron microscope radioautography. In light microscope immuno-radioautographs, (^{3}H)5-HT-labeled varicosities appeared as small and dense clusters of silver grains intermingled with, and often adjacent to, TH-immunoreactive perikarya and proximal dendrites. Labeled varicosities were mainly detected within the ventral third of the VTA (to which the tracer had better access) and predominated in the interfascicular nucleus. Double-labeled structures were never observed, confirming the specificity of the radioautographic reaction. At electron microscopic level, 5-HT varicosities corresponded to small axonal enlargements (0,6 μm in mean diameter) containing a mixed population of small, pleomorphic electrolucent vesicles and a variable number of large granular vesicles. Some characteristically showed an array of minute "microvesicles" associated with microcanaliculi and embedded in a dense cytoplasmic matrix. Twenty percent of 5-HT varicosities detected in the VTA were directly apposed to large TH-immunoreactive dendritic shafts. A number of these exhibited well differentiated, symmetrical junctional complexes in single thin sections. Relationships between 5-HT terminals and immunoreactive perikarya were less frequent by far (<1%). These proportions are likely to be underestimated, however, the method tending to favor detection of either type of labeling over the other. In any event, the results provide solid morphological evidence for direct functional interactions between serotonin and dopamine containing neurons in the VTA. Supported by the Medical Research Council and the Edith and Richard Strauss foundation of Canada.

209.4 RELATIONSHIP OF THE NUCLEUS TRACTUS SOLITARIUS AFFERENTS, MONOAMINES AND PEPTIDES IN THE PARABRACHIAL NUCLEUS OF THE RAT. T.A. Milner, T.H. Joh, R.J. Miller, and V.M. Pickel. Lab of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Anatomical and physiological studies have revealed that a major output of the nucleus tractus solitarius (NTS) is to the parabrachial nucleus (PBN) and that both the NTS and the PBN contain monoaminergic and peptidergic neurons. We sought to determine the distribution and ultrastructural relationship of the NTS afferents to that of the monoamines and peptides within the PBN by combining autoradiography with immunocytochemistry. One to three days following a unilateral injection of 10 to 30 nl of 100μCi/μl mixed ^{3}H-proline and leucine into the NTS of adult rats, the brains were fixed by aldehyde perfusion then the PBN was processed for immunocytochemistry. The antisera included tyrosine hydroxylase (TH), an enzymatic marker for catecholamines, 5-hydroxytryptamine, substance P (SP) and met 5 enkephalin.

Light microscopy. Both the anterograde labeling of the NTS afferents and the immunocytochemical labeling of 5-HT, SP and enkephalin were more extensively distributed in the lateral PBN than in the medial. However, within the lateral PBN, anterograde labeling was predominantly in the ventral region, while immunoreactivity for 5-HT, SP and enkephalin were primarily in the dorsal. Both 5-HT and SP were localized to varicose processes whereas enkephalin was found in a few perikarya as well as varicosities. TH localization differed in that perikarya and processes were heaviest in the medial PBN with fewer processes and scattered neurons found laterally. Within the lateral PBN electron microscopy revealed that enkephalin, SP, 5-HT and TH were localized in terminals having similar morphology. The labeled terminals were .4 to 1.2 μm in diameter, contained large dense core and many clear vesicles and formed primarily axodendritic synapses. In addition to terminals, TH immunoreactivity was detected in perikarya, dendrites and preterminal axons. The perikarya were 12-15 μm in diameter and exhibited vesicle filled evaginations. Axosomatic synapses between TH labeled terminals and perikarya were occasionally detected, however, most of the terminals forming synapses with catecholaminergic soma or dendrites were unlabeled. Terminals showing autoradiographic labeling following injections of ^{3}H-amino acids were found rarely in the immediate vicinity of immunocytochemically labeled profiles. These studies suggest that the NTS probably contributes a minor component, if any, to either the peptidergic or monoaminergic terminals within the PBN.

(Supported by Grants HL 18974 and MH 00078).

209.5 EVIDENCE FOR A GABAERGIC INHIBITORY INFLUENCE ON CENTRAL SEROTONERGIC NEURONS EXERTED IN THE DORSAL AND MEDIAN RAPHE. B. Scatton and T. Nishikawa*. Synthélabo-LERS, 92220 Bagneux, France.

GABA exerts an overall inhibitory influence on serotonergic transmission in the rat brain. Thus, systemic administration of the GABA receptor agonist progabide (50-1200 mg/kg ip) as well as of the indirectly acting GABA mimetics γ-acetylenic-GABA (200 mg/kg ip), dipropylacetamide (150 mg/kg ip) and aminooxyacetic acid (50 mg/kg ip) diminish (by 20-30%) 5-hydroxytryptophan (5-HTP) accumulation (after aromatic L-aminoacid decarboxylase inhibition), an index of serotonin neuronal activity, in a variety of brain areas receiving a serotonergic innervation from either the dorsal (striatum, substantia nigra, olfactory tubercle) or median (hippocampus, frontal cortex) or both (hypothalamus, septum) raphé nuclei. Progabide (400 mg/kg ip), given subacutely, also reduces the release of extracellular 5-hydroxyindoleacetic acid (5-HIAA) (as measured by in vivo electrochemical detection) in the rat striatum.

The GABAergic influence on serotonergic neurons is not exerted at the level of nerve terminals as: 1) the progabide-induced reduction in striatal and hippocampal 5-HTP accumulation is abolished after cerebral hemitransection and 2) intrastriatal infusion of γ-vinyl-GABA (100 µg) or SL 75102, the active and soluble metabolite of progabide (100 µg), fails to alter 5-HTP accumulation in the striatum.

The GABAergic control of central serotonergic transmission is more likely exerted at the level of cell body. Thus, local injection of GABA (10-100 µg), SL 75 102 (10-100 µg) or γ-vinyl-GABA (100 µg) into the dorsal raphe diminishes 5-HTP accumulation in the striatum but not in the hippocampus, whereas intra-median raphe injection of these compounds reduces 5-HTP accumulation in the hippocampus but not in the striatum. In addition, intra-dorsal raphé infusion of these drugs reduces the release of extracellular 5-HIAA from the striatum, the latter effect being antagonized by a local infusion of 20 µg of R 5135, a novel steroid derivative possessing potent GABA receptor antagonist properties.

In conclusion, the present data indicate that GABA exerts an inhibitory influence on central serotonergic transmission which is mediated via stimulation of GABA receptors located in the dorsal and median raphé nuclei. Preliminary studies indicating that progabide (1200 mg/kg ip) reduces 5-HTP accumulation in the spinal cord, the serotonergic innervation of which is originating from the raphe magnus, suggest that GABA might also exert an inhibitory influence on this raphe nucleus.

209.6 ELECTROPHYSIOLOGICAL EVIDENCE SUGGESTING MULTIPLE SEROTONERGIC EFFECTS ON CEREBELLAR PURKINJE CELLS: CORRELATION WITH BASELINE FIRING RATE. Munhyang Lee*, Jean C. Strahlendorf, and Howard K. Strahlendorf (Spon: Richard D. Nathan). Physiology and Medical and Surgical Neurology, Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430.

Purkinje (P) cells represent once class of target neurons receiving serotonergic afferents in the cerebellum. This study was designed to reevaluate the effects of iontophoretically applied serotonin (5-HT) on P cells. 5-HT elicited one of three effects measured during the ejection period: 61% of neurons were suppressed; 27% of the cells responded biphasically (i.e., a brief period of inhibition preceded a dominant period of excitation), and 11% of the P cells displayed an excitation (N=99). 5-HT-mediated inhibition had shorter onsets and durations than 5-HT-mediated excitatory and biphasic responses A correlation between the spontaneous rate of P cells and the action of 5-HT was evident. P cells excited by 5-HT had a significantly lower pre-drug firing rate (37 Hz) than those inhibited by 5-HT (51 Hz). On several occasions, the direction of the P cell response to serotonin reversed after a shift in the baseline firing rate (i.e., a rapidly firing neuron that was depressed initially by serotonin responded subsequently with an increase in rate as the spontaneous rate slowed). When the P-cell firing rate was increased or decreased arbitrarily by glutamate or glycine, respectively, the 5-HT response changed in a rate-dependent fashion. Methysergide appeared to have a differential action on excitation and inhibition: inhibition was augmented, whereas excitation was attenuated. Spiperone attenuated the serotonin-mediated inhibition. These studies suggest that P cells may contain two 5-HT receptor subtypes: inhibitory and excitatory. The response to 5-HT may reflect a functional balance between the two subtypes that is determined in part by the spontaneous rate of the cell. The overall qualitative effect of 5-HT may be to set P cells at a preferred rate. Currently, we are examining the possible presynaptic mechanisms underlying these observations. (Supported by Tarbox Parkinson's Disease Institute, TTU.)

209.7 TRYPTOPHAN HYDROXYLASE INHIBITION BY P-CHLOROPHENYLALANINE (PCPA): EFFECT ON DMI INDUCED CHANGES OF THE NOREPINEPHRINE (NE) RECEPTOR COUPLED ADENYLATE CYCLASE IN RAT CORTEX. D.D. Gillespie*, D.H. Manier*, L.R. Steranka[1] & F. Sulser. Tennessee Neuropsychiatric Institute, Vanderbilt University School of Medicine, Nashville, TN 37232 and Indiana University, Gary, IN 46408.

Previous studies have shown that specific lesions of serotonergic neurons with 5,7-dihydroxytryptamine (5,7-DHT) abolish the desipramine (DMI) induced down-regulation of β-adrenoceptors in brain (Brunello et al. Neuropharmacology 21, 1145, 1982; Janowsky et al. Science 218, 900, 1982) and decrease the agonist affinity for isoproterenol (Manier et al. Europ. J. Pharmacol. 86, 137, 1983). Since the neurotoxin 5,7-DHT reduces not only serotonin (5HT) but also affects the concentration of neuropeptides (substance P and TRH), the lack of down-regulation of β-adrenoceptors by tricyclics following 5,7-DHT could be the consequence of the lack of 5HT or the neuropeptides or both. To determine whether the alteration in NE receptor density and function are the consequence of the loss of 5HT per se, we studied the effect of PCPA on the DMI induced down-regulation of cortical β-adrenoceptors and the concomitant attenuation of the NE sensitive adenylate cyclase. DMI (10 mg/kg b.i.d.) caused within 4 days a significant attenuation of the NE sensitive adenylate cyclase (response to 100 µM NE in pmol/mg protein ± SEM; controls 72.5 ± 5.2; DMI 42.3 ± 3.6; $p < 0.001$) and a reduction in the B_{max} value of [^{3}H] -dihydroalprenolol (DHA) binding from 135.0 ± 8.9 to 95.6 ± 5.4 fmol/mg protein ± SEM ($p < 0.005$). Within 2 days, PCPA (400 mg/kg i.p.) "reversed" the down-regulated β-adrenoceptor population despite continuous DMI administration (B_{max} values: 183 ± 9.6 [DMI + PCPA] vs. 88.5 ± 3.9 [DMI] fmol/mg protein ± SEM). Significantly, the DMI induced attenuation of the NE sensitive adenylate cyclase remained unchanged following PCPA (cyclic AMP response to 100 µM NE: 37.4 ± 4.4 [DMI] vs. 39.9 ± 6.0 (PCPA + DMI) pmol/mg protein ± SEM). The agonist affinity of β-adrenoceptors as determined from the inhibition of [^{3}H]-DHA binding by (-)-isoproterenol, was significantly decreased following PCPA in normal animals and further reduced in DMI treated rats (IC_{50} values in µM ± SEM: controls, 0.38 ± 0.02; DMI, 0.44 ± 0.05; PCPA, 2.4 ± 0.04; DMI + PCPA, 10.1 ± 1.4). Conclusions: 1) 5HT per se is co-required with NE for DMI induced down-regulation of β-adrenoceptors. 2) β-Adrenoceptors following PCPA display characteristics of "uncoupled" receptors. 3) The DMI induced down-regulation of β-adrenoceptors is "reversible" by inhibition of tryptophan hydroxylase with PCPA. (Supported by USPHS Grant MH-29228, the Tennessee Department of Mental Health and Mental Retardation and the Lake County Medical Center Developmental Agency, Lake County, IN.[1]).

209.8 SEROTONERGIC-OPIATE INTERACTIONS IN DISTRESS VOCALISATIONS OF JUVENILE GUINEA-PIGS. Berend Olivier* and L.DiAnne Bradford. Dept. Pharmacology, DUPHAR b.v., P.O.Box 2, 1380 AA Weesp, The Netherlands.

In an animal model of social affiliation behavior, separation-induced distress vocalisation (DV) in juvenile guinea-pigs, the involvement of serotonergic and opiate compounds and their interactions was assessed.

Fluvoxamine, a specific 5-HT reuptake blocker and new antidepressant drug, decreased DV in a dose-dependent way (5-20 mg/kg, i.p.). Methysergide enhanced DV's and antagonized the decrease in DV's induced by fluvoxamine. Methysergide (5 mg/kg, i.p.) antagonized the decrease in DV caused by morphine (2 mg/kg, i.p.), whereas naloxone (10 or 25 mg/kg, i.p.) also antagonized the decrease in DV caused by fluvoxamine (10 mg/kg, i.p.). Fluvoxamine (10 and 20 mg/kg, i.p.) when combined with morphine (0.5 mg/kg, i.p.) potentiated the decrease of the DV's in a dose-dependent way.

These results show that serotonin and opiate systems in the CNS influence social affiliation behavior in guinea-pigs, and that these systems interact in a modulatory manner.

209.9 MEDIATION BY NORADRENALINE OF THE GABAERGIC INFLUENCE OF MUSCIMOL ON RAT STRIATAL CHOLINERGIC ACTIVITY. A. Vezzani*, S. Consolo*, M. Sieklucka*, J-X. Wang* and H. Ladinsky* (SPON. L. Valzelli). Laboratory of Cholinergic Neuropharmacology, Mario Negri Institute for Pharmacological Research, Milan, Italy I-20157.

Muscimol, a rigid analog of GABA and a potent GABA-mimetic drug, increased the content of ACh in rat striatum by about 55% at the optimal dose and time of 10 mg/kg, i.p., 30 min without affecting choline content, the ACh turnover rate, ChAT and AChE activities or NE content. The increase in ACh was mediated by the GABAergic system as pretreatment with picrotoxin completely blocked the effect while atropine, pimozide, metergoline and PCPA pretreatments were ineffective against muscimol. Unilateral degeneration of the corticostriatal input by undercutting of the frontal cortex prevented completely the increase in ipsilateral ACh content induced by muscimol after two postoperative weeks. In addition, the action of muscimol was strongly prevented when α_1-adrenoceptors were blocked by prazosin and when NE synthesis was inhibited by α-MpT. Furthermore, the increase in striatal ACh induced by a supramaximal dose of oxotremorine, an action proposed to be mediated by the noradrenergic system, did not summate with the increase induced by a supramaximal dose of muscimol, suggesting a common neuronal target in the mechanism of the drugs. The drug's cholinergic effect was not influenced by reserpine pretreatment. The results indicate that in addition to suggested glutamatergic mediation, there coexists noradrenergic mediation of the GABA effect of muscimol which relies upon newly-synthesized NE. Conceivably, the activation of α_1-adrenergic receptors by muscimol produces inhibition of glutamate release which in turn leads to suppression of ACh output and to the ensuing buildup of intraneuronal ACh. Consistently, it was found that at least 15% of the specific binding sites for (^{3}H)prazosin were lost in the striatum ipsilateral to the lesioned corticostriatal glutamatergic pathway after two postoperative days. If GABA and NE acted in series, i.e. GABA(-)/NE(-)/GLU(+)/ACh, then muscimol would be expected to decrease the level of ACh. It is proposed instead that GABA and NE act in conjunction at the level of the glutamatergic neuron to regulate glutamate release and then cholinergic activity.

209.10 BIOCHEMICAL EVIDENCE FOR AN INHIBITORY CONTROL OF THE LC BY THE C2 ADRENALINE NEURONS. B. Astier*, B. Renaud. Laboratoire de Neuropharmacologie. Faculté de Pharmacie. Université Claude Bernard. LYON, France.

Since the discovery of the existence of adrenaline (A) in mammalian brain and the localization of both A cell bodies and A nerve terminals in the rat medulla oblongata, several physiological and pharmacological experiments have suggested for these neurons a role in the central regulation of blood pressure which may be different from the possible role of the noradrenaline (NA) neurons. It thus seems important to study the possible interactions between these neurons. An anatomical basis for such interactions, is provided by immunohistochemical observations, showing the existence of A terminals derived from C1 and C2 groups, innervating the NA cell bodies of the locus coeruleus (LC) (Hökfelt et al., Brain Res., 1974, 66, 235-251). Therefore, in this study, we sought to compare the biochemical response of the LC (which innervates almost the entire central nervous system) after the lesion of another catecholaminergic neuronal group of the rat medulla oblongata : either one group of NA neurons : the A2 group (nucleus commissuralis, NCO) or one group of A neurons, the C2 group. The electrolytic lesions were produced on rats previously anesthetized with halothane, by passage of a 3 mA cathodal current for 5 seconds, medially for A2, and bilaterally for C2. The response of NA neurons of the LC was followed after the lesion of the A2 or C2 group, by using as markers the "in vitro" activity of the two catecholamine-synthesizing enzymes tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH). The activity of the adrenaline-synthesizing enzyme phenylethanolamine-N-methyltransferase (PNMT) was used as a specific marker of the A terminals within the LC. In order to measure the changes occuring in an area rich in NA and A terminals, the enzymatic activities were also determined in the tractus intermediolateralis (TIML) of the spinal cord.

No change was observed in the TH and DBH activities within the NA cell bodies of the LC, 5 days after lesion of the A2 group (NCO). The PNMT activity of the LC was not changed either. Within the NA and A terminals of the TIML, these three enzymatic activities were not modified.

On the other hand, the TH activity within the LC was significantly increased (+104%, $p < 0.001$) 5 days after bilateral lesion of the C2 group. Modifications occured neither in the DBH and PNMT activities of the LC, nor in the TH, DBH and PNMT activities within the NA and A terminals of the TIML.

This increase in TH activity occuring in the LC after lesioning of the C2 A neurons favors the hypothesis of an inhibitory control of the LC by the A nerve terminals derived from the C2 A cell bodies.

209.11 NOREPINEPHRINE (NE) POTENTIATES THE VIP-INDUCED ACCUMULATION OF cAMP IN MOUSE CEREBRAL CORTICAL SLICES : EVIDENCE FOR AN ALPHA-ADRENERGIC MEDIATION. P.J. Magistretti and M. Schorderet*. Dept. of Pharmacology, CMU, University of Geneva, 1211 Geneva, Switzerland.

VIP and NE are two cortical neurotransmitters contained in neuronal systems organized in striking contrast : VIP is localized to bipolar, radially oriented, intracortical neurons and NE to tangentially organized cortical afferents which originate in the locus coeruleus. In cerebral cortex, these two neurotransmitters share two sequentially related cellular actions : stimulation of cAMP formation and the subsequent activation of glycogenolysis.

In a series of experiments we have investigated the interactions between VIP and NE on cAMP formation in mouse cerebral cortical slices. We have found that VIP and NE, when tested at supramaximally effective concentrations display overadditive effects on cAMP formation (pmoles cAMP/mg prot.±SEM) : Control (C)=6.65±1.6; VIP 1 μM=210±25; NE 50 μM=31.8±6.6; VIP 1 μM + NE 50 μM= 475±27† (n=6). Furthermore the stimulating effects of VIP on cAMP formation are potentiated by phenylephrine (PE) but not by isoproterenol (ISO) : C=13.6±0.21; VIP 1 μM=192±20.4; VIP 1 μM + PE 50 μM=356±16.7††; PE 50 μM=14.7±0.79; VIP 1 μM + ISO 50 μM=182.4±18.2; ISO 50 μM=19.1±3.5 (n=5). In addition phentolamine but not propranolol antagonizes the NE-induced potentiation of the VIP-mediated cAMP formation.

These results therefore demonstrate that NE potentiates the stimulatory effects of VIP on cAMP formation and that this potentiation is mediated by alpha-adrenergic receptors. They also suggest that VIP and NE neurons may act, at least in part, upon the same target cells in cerebral cortex. Furthermore, these results, demonstrating a functional synergism between VIP and NE, would suggest that the two neurotransmitters could act physiologically in concert in a given region of cortex delineated by the intersection of the tangential trajectory of noradernergic fibers and a radial cortical volume(s) defined by the arborization pattern of activated VIP-containing neurons.

† $p<0.005$ when compared to the effects of VIP and NE.
†† $p<0.01$ when compared to the effects of VIP and PE.

209.12 BETA-ADRENERGIC RECEPTORS IN ASTROCYTES: CHARACTERIZATION AND INTERACTION WITH PEPTIDES. D.L. Niehoff and A.W. Mudge*. MRC Neuroimmunology Res. Prog., Dept. Zoology, University College, Gower St., London, WC1E 6BT, England.

Rat cortical astrocytes in culture have been demonstrated to respond to the addition of norepinephrine by an increase in the production of cAMP (Rougon et al, Soc. Neurosci. Abst. **8**: 239, 1982). This response is potentiated by substance P, somatostatin, and VIP, and inhibited by met-enkephalin, all of which have little or no effect on basal cAMP levels. These results suggested the presence of interacting β-adrenergic and peptide receptors on astrocytes in culture. We have now demonstrated and characterized β-adrenergic receptors on these cells in situ, using the antagonist [^{125}I]-iodocyanopindolol (ICYP) and examined the effects of neuropeptides on this binding.

Astrocytes were prepared by a modification of the method of McCarthy and de Vellis (J. Cell Biol. **85**: 890, 1980) and plated at a density of 2×10^5 cells/well in multiwell plates. Only cultures which were 95-98% positive for glial fibrillary acidic protein were used in binding assays. 3-8 days after plating cells were washed in a Hepes-buffered balanced salt solution and incubated with ICYP and various drugs or peptides at 37° for 1 hr. Non specific binding was determined in the presence of 2.5×10^{-7} M (±) propranolol.

The binding of 10-200 pM ICYP was saturable; Scatchard analysis revealed a KD of 55 pM and Bmax of 1.4 fmol/10^6 cells. Displacement of ICYP by the β_1-selective antagonist, atenolol (IC_{50}=120nM) or the β_2-selective antagonist ICI 118,551 (IC_{50}= 300nM) yielded shallow plots indicative of receptor multiplicity, with about 65% of the total receptor population composed of the β_1 subtype. Displacement by the agonist isoproterenol was also shallow (IC_{50}=0.9 μM), and the high affinity component was selectively shifted to the right by 10^{-5} M GTP. VIP, met-enkephalin, and somatostatin (10^{-8}-10^{-6} M) had no effect on ICYP binding. Preliminary experiments indicate that somatostatin may reverse the GTP-induced alteration in agonist displacement of ICYP.

These results indicate that astrocytes possess β-adrenergic receptors which are similar to those previously described in rat cortex and demonstrate the efficacy of the in situ assay as a technique for the study of adrenergic and peptidergic receptors on astrocytes. In addition, these data suggest that peptidergic modulation of catecholamine action does not proceed via a direct effect on β-receptors, but may instead occur by altering receptor interaction with another component of the receptor-cyclase complex, e.g. the nucleotide regulatory subunit.

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209.13 EFFECTS OF NEUROTENSIN ON DOPAMINERGIC ACTIVITY FOLLOWING INTRANIGRAL OR INTRASTRIATAL INJECTIONS. H.D. Everist, C.C. Chieuh and A. Pert. Biological Psychiatry Branch and Laboratory of Clinical Science, NIMH, Bethesda, MD 20205

Neurotensin (0, 1.0 or 10.0 nmoles in 1 ul saline) was administered unilaterally into the substantia nigra or caudate nucleus of male Sprague-Dawley rats. Rotational behavior was automatically recorded for 30-45 min. following neurotensin administration. The rats were then immediately decapitated and the ipsilateral and contralateral substantia nigra and caudate nuclei were microdissected. The fresh frozen brain samples were assayed within 24 hr. by an HPLC procedure for the simultaneous determination of L-DOPA, dopamine, serotonin and their metabolites. While the administration of neurotensin failed to elicit consistent rotational behavior, significant dose-dependent increases in the acidic metabolites of dopamine ipsilateral to the side of injection were found. Nigral administration of neurotensin (10.0 nmoles) produced 30 to 80% increases in the nigral contents of DOPAC and HVA with concomitant 100 to 190% increases in these metabolites in the ipsilateral caudate nucleus. The striatal content of dopamine was increased about 60% ipsilaterally after injections of the high dose of neurotensin into either the substantia nigra or caudate nucleus. Neurotensin increased the NSD-1015-induced accumulation of L-DOPA in the caudate nucleus from 1.8 $\pm$ 0.2 to 2.9 $\pm$ 0.3 ng/mg. Similar effects on dopamine and dopamine metabolism were observed in the nucleus accumbens following intranigral injections. Serotonin and 5-HIAA contents in the nigrostriatal system were not altered significantly by treatment with neurotensin. These behavioral and neurochemical observations indicate that neurotensin increases the synthesis of dopamine and the intraneuronal metabolism by MOA, whereas it decreases the neuronal activity or shuts off the release of dopamine. This notion is consistent with the results of the following experiment with d-amphetamine. Unilateral intranigral administration of neurotensin resulted in no significant rotational behavior. However, when d-amphetamine (2.5 mg/kg, i.p.) was administered 15 min. later, significant turning contralateral to the injection side was observed in 10 of 14 rats. AMPT pretreatment significantly attenuated this amphetamine-induced rotational behavior. In conclusion, neurotensin could turn off the dopaminergic activity in the nigrostriatal system in a manner similar to axonal transsection or treatment with gamma-butyrolactone.

209.14 ELECTROPHYSIOLOGICAL EVIDENCE FOR MODULATION OF DOPAMINE NEURONS BY 8-HYDROXY-2(DI-N-PROPYLAMINO)TETRALIN. S.L. Fallon*, H.S. Kim* and J.J. Welch. Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

Serotonin (5-HT) neurons originating in the dorsal raphe nucleus are known to have a modulating influence on the impulse activity of dopamine (DA) neurons in the substantia nigra. This connection is thought to be responsible for the enhanced DA transmission caused by the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Christoph et al., Life Sci., 21:1585, 1977). By interacting with 5-HT autoreceptors to reduce raphe activity, 5-MeODMT presumably disinhibits postsynaptic DA neurons. Hjorth et al. (J. Neural Trans., 55:169, 1982) have described a new potent 5-HT agonist, 8-hydroxy-2(di-n-propylamino)tetralin (8-OH-DPAT) which also is believed to activate 5-HT autoreceptors. Their biochemical and behavioral investigations have indicated that 8-OH-DPAT lacks appreciable effects on DA neurons. Using electrophysiological techniques we have investigated the 5-HT and DA properties of 8-OH-DPAT. For comparison, 5-MeODMT and quipazine were also tested.

Extracellular activity was recorded from single neurons in either the dorsal raphe nucleus or the substantia nigra zona compacta of chloral hydrate anesthetized rats. Small intravenous doses of 8-OH-DPAT, from 2 to 10 µg/kg, inhibited completely the firing rate of 5-HT neurons located in the dorsal raphe. In contrast, the predominant effects of 8-OH-DPAT on DA neurons in the substantia nigra were clearly excitatory. In 70% of the DA neurons tested, 8-OH-DPAT at doses up to 1 mg/kg caused a marked and long-lasting firing rate increase. The average increase was approximately 30% of baseline regardless of the dose tested. The activity of the remaining neurons was either depressed or not affected. At doses above 1 mg/kg, 8-OH-DPAT caused a dose-dependent firing rate inhibition of DA neurons. Haloperidol treatment (0.1 mg/kg) antagonized the inhibitory effects of the higher doses of 8-OH-DPAT on DA activity but enhanced its low dose excitatory effects. 5-MeODMT (30-80 µg/kg) and quipazine (0.5-1 mg/kg) inhibited 5-HT neurons completely and caused excitation of DA neurons equal in magnitude to the 8-OH-DPAT-induced increase.

These results are consistent with other studies showing that 8-OH-DPAT is a potent 5-HT agonist. Additionally, our electrophysiological findings suggest that low doses of 8-OH-DPAT may, by virtue of its ability to enhance DA neuronal transmission, cause an indirect activation of DA receptors. However, at high doses, 8-OH-DPAT appears to be a direct acting agonist at DA receptors, sharing a property common to most 2-aminotetralin derivatives.

209.15 CHOLECYSTOKININ MODULATES THE IN VIVO RELEASE OF DOPAMINE IN THE NUCLEUS ACCUMBENS OF THE RAT. M. Voigt* and R. Y. Wang. Dept. of Pharmacology, St. Louis Univ. Sch. Med., St. Louis, MO 63104.

Cholecystokinin (CCK) and dopamine (DA) have been shown to coexist within a subset of A10 neurons located in the ventral tegmental area. These CCK/DA neurons project primarily to the medial nucleus accumbens (NAc). Previous studies in this lab (White and Wang, in preparation) have demonstrated that iontophoretically applied CCK increases, whereas DA suppresses, the firing rate of neurons in the medial NAc. This suggests that CCK and DA are functional antagonists in this structure with regards to postsynaptic effects. In the present study, we have attempted to examine the effects of CCK on a presynaptic event, the release of endogenous DA, within the NAc. This was done through the use of push-pull cannula perfusion.

Concentric cannulae were implanted stereotaxically into the medial NAc of chloral-hydrate anesthetized rats. Perfusion of a previously described artificial cerebrospinal fluid was begun and maintained at a constant rate of 15 µL/min. Perfusate was collected in ten minute fractions and endogenous DA was measured using HPLC coupled with electrochemical detection. Cannula placement was verified for each experiment using standard histological procedures, and only those experiments where the lesion was confined to the tip of the cannula were used for data analysis.

During perfusion with normal buffer, basal DA levels were below the limits of detection. d-Amphetamine at 10 µM elevated DA levels to 40 pg/min. Buffer containing 55 mM K^+ (hi-K), when perfused for 10 min, increased DA levels to 61.8$\pm$8 pg/min ($\bar{X}\pm$SE, n=5). This stimulated release was demonstrated to be Ca^{++} dependent. In experiments (n=3) where the cannula was placed lateral to the NAc proper, no DA was detected during stimulation by hi-K. The effects of various concentrations of CCK on hi-K induced DA release were then tested. At 20 nM, CCK-8SO_4 had no effect (62.3 $\pm$5 pg/min, n=4). At 200 nM, CCK-8SO_4 decreased hi-K stimulated release by 63% (23.0$\pm$6 pg/min, n=5, $p<0.0005$ vs. control). Interestingly, CCK-8SO_4 at 2 µM only decreased release by 47% (33.2$\pm$5, n=4, $p<0.025$ vs. control). In marked contrast to CCK-8SO_4, unsulfated CCK at 200 nM had no effect on K^+-induced DA release (68.0$\pm$3, n=3).

In conclusion, our results showed that: 1) K^+ increases DA release in the NAc in vivo in a Ca^{++} dependent manner, and 2) only the sulfated form of CCK-8 suppressed K^+-induced DA release. This latter finding supports the concept that CCK may functionally antagonize the action of DA in the NAc. (Supported by USPHS Grants MH34424 and MH00378.)

209.16 EFFECTS OF THYROTROPIN-RELEASING HORMONE ON GLUTAMATE EXCITATION OF RAT SPINAL MOTONEURONS. S.R. White, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6.

Thyrotropin-releasing hormone (TRH) and serotonin (5HT) appear to coexist in some bulbospinal neurons which send terminals to the lumbar ventral horn of the rat spinal cord (Johansson, et al, Neurosci. 6, 1981, 1957). 5HT facilitates glutamate excitation of rat spinal motoneurons (White & Neuman, Brain Res. 188, 1980, 119), but TRH has been found to selectively depress glutamate excitation of rat cerebral cortical neurons (Renaud, et al, Science 205, 1979, 1275). The present study was undertaken to determine the relationship between the effects of TRH and 5HT on glutamate excitation of rat spinal motoneurons.

The center, NaCl-containing barrel of a multibarrel micropipette was used to record extracellular action potentials from physiologically identified motoneurons in the lumbar spinal cord of urethane-anesthetized rats. Other barrels contained 4 M NaCl for automatic current balance or control current ejection, and 1 M monosodium-L-glutamate (GLU), 0.16 M serotonin bimaleate (5HT) and 0.01 M TRH for iontophoresis. Stable baseline responses to GLU were established by manipulating GLU ejection currents and cycle durations. The effects of TRH or 5HT on the baseline activity evoked by GLU were then examined.

TRH (20-30 nA, 60-90 sec) had a prolonged facilitatory effect on GLU excitation without affecting spike amplitude in 16 out of 19 motoneurons tested from five different rats. A brief decrease in GLU-evoked activity often accompanied the onset of current to the TRH barrel, but facilitation of firing usually began well before current offset and lasted two to four minutes after offset. This effect of TRH was very similar to that produced by 5HT (15-20 nA, 60 sec). However, unlike for 5HT, a marked tachyphylaxis developed for the facilitatory effects of TRH. This tachyphylaxis hampers investigation of interactions between effects of TRH and 5HT, but preliminary results suggest that the facilitation of spinal motoneuron excitability produced by 5HT may be enhanced by prior application of TRH.

The finding that TRH ehanced glutamate excitation of rat spinal motoneurons corresponds to previous reports that systemically administered TRH increased EMG activity in hindlimb muscles, depolarized ventral roots and increased MSR amplitude in spinal rats (Barbeau & Bedard, Neuropharm. 20, 1981, 477; Ono & Fukuda, Neuropharm. 21, 1982, 739) and that bath application of TRH depolarized motoneurons in frog isolated spinal cords (Nicoll, J. Pharm. Exp. Ther. 207, 1978, 817).

209.17 CHANGES IN OVARIECTOMIZED RAT BRAIN MONOAMINE AND PEPTIDE NEUROTRANSMITTER CONCENTRATIONS FOLLOWING ESTRADIOL ADMINISTRATION. N. Barden, V. Picard* and T. Di Paolo, Dept. of Molecular Endocrinology, Le Centre Hospitalier de l'Université Laval, Ste-Foy, Quebec G1V 4G2.

We have previously reported effects of chronic estradiol (E_2) administration on the nigro-striatal dopaminergic system. In order to extend these studies to possible interactions of E_2 with neurotransmitter systems other than dopamine, we have investigated the effects of E_2 on rat brain serotonin (5-HT) and certain neuropeptides including TRH and substance P (SP). Ovariectomized rats were treated with 17β-estradiol (1 µg b.i.d.) for 3 weeks or with one dose (50 µg) 12 h or 24 h before sacrifice. Individual brain nuclei were removed from frozen brain sections by a micropunch technique and the 5-HT content measured using HPLC with electrochemical detection or peptide concentrations determined by specific radioimmunoassay. Whilst the appearance of anti-dopaminergic actions necessitate chronic E_2 administration, effects on brain 5-HT were produced by acute, but not chronic, treatment and were restricted to increased concentrations in the substantia nigra and dorsal raphe nucleus. Similarly, acute, but not chronic E_2, reduced the SP content of substantia nigra. This reduction in SP may be secondary to effects on 5-HT in view of reciprocal changes in SP and 5-HT concentrations of several brain nuclei following inhibition or stimulation of 5-HT synthesis. Concentrations of TRH in anterior hypothalamus and arcuate nucleus were significantly increased by acute E_2 administration, whilst chronic treatment was without effect. In contrast, chronic E_2 administration prevented the d-amphetamine-induced decrease in sriatal SP concentrations, suggesting mediation via an anti-dopaminergic mechanism. These results indicate that E_2 effects on the CNS are not restricted to dopamine-containing neurons but are also exerted on at least one other monoaminergic system and either directly or as a secondary effect can influence peptidergic neurotransmission. These actions of E_2 on monoamines and peptides in both the nigro-striatal system and hypothalamus may be of significance in relation to movement disorders and control of pituitary hormone secretion.

209.18 CHOLECYSTOKININ OCTAPEPTIDE (CCK-8) DOES NOT INTERACT IN VITRO WITH RAT HYPOTHALAMIC ADRENERGIC RECEPTORS. R.L. Kochman, T. Grey* and J.D. Hirsch. Dept. of Biological Research, G.D. Searle & Co., Skokie, IL 60077.

Adrenergic receptors are involved in appetite regulation in the rat: α-agonists such as clonidine can elicit feeding, while β-agonists such as isoproterenol suppress feeding. Cholecystokinin (CCK), a peptide found in both brain and gut, may be an important endogenous modulator of appetite. Both CCK and the adrenergic agonists may produce their appetitive effects via the hypothalamus, which has been proposed as the central locus for appetite regulation. In order to determine a possible direct action of CCK on adrenergic receptors, we studied the effects of sulfated CCK octapeptide (CCK-8) on agonist and antagonist binding to the α- and β-adrenergic receptors in the rat hypothalamus.

Crude P_2 hypothalamic homogenates were incubated at 37°C for 30 minutes with 0.3-30nM ^{3}H-p-aminoclonidine (^{3}H-PAC)± 10µM phentolamine to assess α-agonist binding; 1nM ^{3}H-PAC ± 1-30nM phentolamine to assess α-antagonist binding; 0.3-30nM ^{3}H-l-dihydroalprenolol (^{3}H-DHA)± 30µM propranolol for β-antagonist binding; 1nM ^{3}H-DHA ± 1-30µM d,l-isoproterenol for β-agonist binding. In some cases, the membranes were pre-depolarized with 50mM KCl + 2.5mM $CaCl_2$ to release endogenous CCK. Since guanine nucleotides are known to modulate both CCK and adrenergic binding, the effects of 10nM CCK-8 on 0.1mM 5'-guanylylimidodiphosphate (Gpp(NH)p)-modulated adrenergic binding were determined.

α- and β-Agonist and antagonist binding were unaffected by 1, 10,or 100nM CCK-8. In depolarized membranes, these concentrations of CCK-8 had no effect on ^{3}H-PAC or phentolamine binding. Similarly, the effects of 0.1mM Gpp(NH)p on binding were not affected by 10nM CCK-8. The results of these studies do not support the hypothesis that CCK-8 produces satiety via a direct action on hypothalamic adrenergic receptors.

	K_D(nM)	B_{max}(fmol/mg protein)
^{3}H-PAC	2.5 (2.4)	1296 (1564)
+ 1nM CCK	2.3 (1.9)	1319 (1387)
+ 10nM CCK	2.2 (2.9)	1203 (1764)
+100nM CCK	2.4 (1.5)	1335 (1081)
+0.1mM Gpp(NH)p	7.0 (5.8)	967 (1537)
+0.1mM Gpp(NH)p/ 10nM CCK	9.9 (5.5)	1142 (1530)

*values in parentheses were calculated using depolarized membranes.

209.19 TRANSMITTER INTERACTIONS IN THE HYPOTHALAMUS OF THE RAT. R.S. Flint and W.J. McBride. Depts. Psychiatry and Biochemistry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46223.

The interactions of amino acid, monoamine and cholinergic transmitter systems were studied in minislices of rat hypothalamus by measuring release of endogenous neurotransmitters. The hypothalami from three adult male Wistar rats were sliced into 300 x 300 µm sections oriented perpendicular to the horizontal plane. The slices were combined and aliquots of the tissue suspension were transferred to polypropylene columns containing G-50 Sephadex. The tissue slices were dispersed in the Sephadex and superfused with physiological media in equilibrium with 95% O_2/5% CO_2 at 37°C and pH 7.4. A submaximal Ca^{+2}-dependent release of norepinephrine (NE), dopamine (DA), serotonin (5-HT), GABA, glutamate (Glu) and aspartate (Asp) was obtained with 35 mM K^+. The effects of 100 µM (a) carbachol on the release of NE, 5-HT, Glu and Asp; (b) DA, NE and 5-HT (individually added) on the release of Glu and Asp; and (c) GABA and Glu (individually added) on the release of endogenous NE, DA, 5-HT, Glu and Asp were determined in the presence of 35 mM K^+ first in the absence of Ca^{+2} and then in the presence of 2.5 mM Ca^{+2}. The cholinergic agonist, carbachol, had little effect on the 35 mM K^+-stimulated, Ca^{+2}-dependent release of NE, 5-HT, Asp and Glu (N=3). NE potentiated the release of Glu in 2 of 3 cases and had little effect on the release of Asp. In addition, both 5-HT and DA significantly enhanced the release of Glu by 40 and 70%, respectively. Neither amine appeared to alter the stimulated release of Asp. GABA had little effect on the release of NE, DA and 5-HT but did produce a 5 and 2-fold increase in the release of Asp and Glu, respectively. Glu had little affect on the release of NE and DA and appeared to reduce the K^+-stimulated, Ca^{+2}-dependent release of 5-HT by 35% (N=3). On the other hand, Glu potentiated its own release as well as the release of Asp. All the observed changes upon release appeared to be dependent on the presence of Ca^{+2}. Overall, the data indicate that (a) the monoamine systems in the rat hypothalamus are not regulated by cholinergic or amino acid transmitter systems; (b) the monoamines, but not the cholinergic system, are capable of acting upon glutamatergic neurons; and (c) the amino acid transmitter systems are capable of interacting with one another. Whether any of the interactions are direct pre- or post-synaptic actions or are indirectly mediated cannot be determined with the present data. (Supported in part by NS19286, MH00203 and AA03243).

209.20 EFFECT OF β-PHENYLETHYLAMINE ON REGIONAL BRAIN CATECHOL-O-METHYLTRANSFERASE ACTIVITY. Charles J. Hannan, Jr. and William F. Shivers, Jr.*. Clinical Investigation and Psychiatry Departments, DD Eisenhower Army Medical Center, Ft Gordon, GA 30905.

This preliminary study examined the change in regional brain catechol-O-methyltransferase (COMT) activity in gerbils after 10 daily i.p. injections of β-phenylethylamine (PEA, 50 mg/kg). Brains were divided into the 6 parts indicated in the Table below. COMT was estimated by incubating tissue supernatants with an excess of dopamine (2.3 mM) and S-adenosylmethionine (0.5mM). Phosphate buffer (pH 7.8), magnesium chloride (0.5mM) and adenosine deaminase (3U) were also added to a final volume of 0.55 ml. The reaction was stopped after 100 min incubation with 4M HCl containing methoxyhydroxybenzylamine internal standard and the pH adjusted to 6.5 with borate buffer. This was passed through CG-50 cation exchange resin and then injected onto a reversed phase HPLC column equipped with an electrochemical detector. The amount of 3-methoxytyramine (3-MT) and 4-O-methyldopamine (4-MT) produced was calculated and results are given as ng product/min/mg wet weight of tissue.

COMT Activity as 3-MT Production in Gerbil Brain Parts:

	COMT ACTIVITY (Mean ± SD)		
Brain Part	Breeders*	Young Males*	Young Male Cont.
1 Frontal Cortex†	7.72 ± 1.60	24.47 ± 19.71	10.32 ± 2.34
2 Midbrain	6.92 ± 2.87	12.49 ± 6.73	10.58 ± 3.56
3 Hippocampus	8.80 ± 4.74	19.25 ± 13.53	15.02 ± 7.44
4 Brain Stem	7.16 ± 3.44	28.21 ± 32.80	23.07 ± 21.93
5 Cerebellum §	6.56 ± 3.64	19.66 ± 5.22	12.23 ± 4.14
6 Remaining Cortex	5.94 ± 2.87	20.12 ± 17.95	17.00 ± 17.77

* B-PEA daily 50 mg/kg for 10 days
† Significant as P = 0.02367, one-way ANOVA
§ Significant as P = 0.00002, one-way ANOVA

Retired breeder male gerbils treated with PEA were found to have generally lower COMT activity than PEA treated or saline control young males. There was also a general increase in mean COMT activity of young animals treated with PEA over those not treated. PEA has been proposed to exert its behavioral effects by release of catecholamines or by direct stimulation of neurons. Using COMT activity to reflect the prolonged activity of catecholamine systems, it appears that PEA stimulates catecholamines sufficiently to induce an increase in COMT.

210.1 WHOLE BLOOD SEROTONIN AND PLATELET IMIPRAMINE BINDING IN AUTISTIC AND NORMAL SUBJECTS. G.M. Anderson,[1*] E.L. Hoder,[2*] P.P.G. van Benthem,[3*] F.R. Volkmar,[4*] C.R. Hansen, Jr.,[5*] R.B. Minderaa[6*] B.A. Shaywitz,[7] D.J. Cohen[8*]. Departments of Laboratory Medicine[1] Pediatrics,[2,8] and Neurology[7] and the Child Study Center,[1-8] Yale University School of Medicine, P.O.B. 3333, New Haven, CT 06510.

A number of researchers have reported that group means of plasma or whole blood serotonin (5-HT) are elevated in autistic children compared to normal subjects. The cause of this hyperserotonemia and its relationship to central serotonergic functioning remain unclear. We have examined 5-HT levels in large groups of normal, autistic, and mentally retarded subjects in order to better characterize this basic finding. We have also measured platelet imipramine (IMI) binding in a group of autistics and normals because of the known association of the IMI binding site with brain and platelet 5-HT uptake systems. Whole blood serotonin (5-HT) and tryptophan (TRP) were measured using an HPLC method in a group (N=87) of normal children and young adults and in an autistic population (n=40) having a similar age distribution. Whole blood 5-HT was significantly elevated in the drug-free (N=21 autistic group (205±16 ng/ml, 776±87 ng/10^9 platelets) compared to normal subjects (136±54 ng/ml, 552±26 ng/10^9 platelets). Platelet counts were similar in drug-free autistic and normal subjects. Autistic subjects on anticonvulsants and neuroleptics had signifilower 5-HT levels (117±12 ng/ml) than drug-free autistic subjects. This drug induced lowering of 5-HT levels makes it imperative that autistic and retarded subjects be taken off medication when tested for the presence of hyperserotonemia. A sex difference was observed in the normal group with young males (1-6 years old) having significantly higher levels than young females (181±15 vs 113±15 ng/ml). The significant negative correlation (-.48, p.001) observed in the normal group between 5-HT concentration when expressed as ng/10^9 platelets and the platelet count suggests that the concentration is best expressed as ng/ml. It is also an indication that the increase in 5-HT is due to increased production rather than a alteration in platelet function. Additional correlations of 5-HT (ng/ml and ng/10^9 platelets), TRP, platelet count, and the ontogeny of the measures in the normal, autistic, and retarded groups will be presented along with group means and correlations observed for platelet IMI binding.

210.2 CHRONIC ELECTROCONVULSIVE SHOCK DIFFERENTIALLY AFFECTS BRAIN AND PLATELET ^{3}H-IMIPRAMINE RECOGNITION SITES, M.S.Abel*, L.R.Meyerson, D.E.Clody* and L.P.Wennogle*. (SPON:B.Beer). Dept.CNS Res., Med. Res.Div. of American Cyanamid Co., Lederle Labs, Pearl River, NY 10965.

The density of ^{3}H-imipramine (IMI) receptors on the human blood platelet has been used as a marker for certain psychopathological states, including depression. For example, in a number of studies a decreased density of IMI sites is found in platelets derived from depressed individuals as compared to normal controls. It is tempting to speculate that these altered peripheral IMI sites may reflect a similar state in the central nervous system. The present study was designed to assess the effects of various chronic antidepressive treatments on the regulation of IMI binding in cortical and platelet membrane preparations from the same animal. The well-established effects of these treatments on the cortical β-adrenergic system were also monitored as verification of the efficacy of the chronic regimens. Rats were treated chronically (14 days) with either distilled water, desipramine (DMI, 10mg/Kg i.p., b.i.d.), electroconvulsive shock (ECS, 75 mA 0.5 sec duration, once per day, corneal electrodes),ECS plus DMI, or diazepam (5 mg/Kg i.p., once per day). After a 24 hour washout period, Scatchard analyses were performed from saturation isotherms using ^{3}H-IMI to identify the serotonergic transporter/allosteric regulator complex. ^{3}H-Dihydroalprenolol (DHA) was used as a marker for β-adrenergic recognition sites. Previously reported alterations in DHA binding were replicated in our laboratory, thereby verifying the proper administration of the chronic treatments; ECS, DMI and the combination of ECS plus DMI all produced a down-regulation in the cortical β-adrenergic receptor with no changes in affinity(K_D). However, regarding ^{3}H-IMI binding, after ECS or ECS plus DMI a markedly elevated Bmax was observed in platelets whereas no significant change was seen in the brain. We found no significant change in ^{3}H-IMI binding parameters in platelet or cortex after DMI administration. Therefore, the supersensitivity observed in the ECS treatment groups can be attributed to the effects of ECS per se. No statistically significant changes in ^{3}H-IMI affinity were observed after any experimental regimen. The mechanism underlying elevations in IMI binding to platelets after ECS is unclear. It is, however, interesting that a treatment localized to the CNS (i.e. ECS) produced a large change in the periphery. These results are indicative of independent regulation of recognition sites in platelets and CNS tissue by antidepressive therapy and suggest that the platelet may not be an entirely adequate model for the CNS component of the biochemistry of psychopathologies.

210.3 PLATELET ALPHA-2 ADRENERGIC RECEPTOR SENSITIVITY IN MAJOR DEPRESSIVE DISORDER. S.M. Stahl, P.M. Lemoine,* R.D. Ciaranello, and P.A. Berger*. Stanford University, Stanford, CA 94305.

Recent investigations suggest that antidepressant medications can decrease (i.e. "down regulate") the number of receptors for a variety of neurotransmitters. Specifically, central nervous system beta adrenergic, α-2 adrenergic and serotonin-2 receptors may all be down regulated by antidepressants over a two to three week time course comparable to the onset of clinical antidepressant effects. Accordingly, numerous investigators have hypothesized that depression may be linked to supersensitive neurotransmitter receptors, and that antidepressant medications may exert their clinical effects by down-regulating these receptors. Platelets contain α-2 adrenergic receptors which have the same kinetic and pharmacolocic properties as central nervous system α-2 adrenergic receptors, and, thus, may provide an acceptable receptor functioning in depressed patients. We have investigated the functioning of α-2 adrenergic receptors in patients with major depressive disorder by measuring the specific binding of ^{3}H-yohimbine, an α-2 adrenergic receptor antagonist, to platelet membranes. Patients with the diagnosis of major depressive disorder (unipolar or bipolar, Research Diagnostic Criteria) hospitalized on a clinical research ward, and who had been medication free for at least four weeks, consented to venepuncture for collection of platelets. Bmax and K_D values for platelet ^{3}H-yohimbine binding were normal in unmedicated patients with major depressive disorder, and did not correlate with scores on the Hamilton rating scale for depression. Platelet α-2 adrenergic antagonist sites were also unchanged in number or affinity in depressed patients after long term treatment with a variety of antidepressant medications. Thus, our results with ^{3}H-yohimbine fail to demonstrate supersensitive platelet α-2 adrenergic antagonist sites in unmedicated patients with major depressive disorder. Also, platelet α-2 adrenergic antagonist sites are apparently not down regulated by chronic antidepressant treatment.

210.4 DOWN REGULATION OF BRAIN β-ADRENERGIC RECEPTOR BY IPRINDOLE. O. Gandolfi*, M.L. Barbaccia*, D.M. Chuang* and E. Costa (SPON: B.T. Ho) Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032.

In rats receiving two daily doses of iprindole (5 or 10 mg/kg i.p.) repeated for 21 days, the number but not the affinity of β-adrenergic receptor recognition sites in the frontal cortex is decreased. In rats receiving such a dose regimen of iprindole the Vmax of NE (100 μM) stimulated adenylate cyclase activity in minces of frontal cortex is attenuated. In addition this long term administration of iprindole decreases the Bmax of [^{3}H]-mianserin and [^{3}H]-ketanserin binding to crude synaptic plasma membranes prepared from rat frontal cortex and hippocampus, whereas the characteristic of [^{3}H]-5HT binding displaced by LSD (1 M) is unchanged.

The decrease in [^{3}H]-mianserin and [^{3}H]-ketanserin binding is due to a reduction in their densities but not their affinities with binding sites. Protracted iprindole injections modify neither the uptake of [^{3}H]-5HT by minces of frontal cortex nor the number of imipramine binding sites. An acute injection of iprindole does not modify the binding of [^{3}H]-dihydroalprenolol, [^{3}H]-mianserin, [^{3}H]-5HT to crude synaptic membranes of rat brain. While the lesion of 5HT axon terminals with 5,7-dihydroxytryptamine prevents the down regulation of β-adrenergic receptors (decrease in recognition sites and attenuation of the NE stimulation of receptor-coupled adenylate cyclase) elicited by long term imipramine or desipramine treatment, this specific lesion with the neurotoxin fails to modify the down regulation of β-adrenergic receptor and of cAMP accumulation induced by repeated iprindole administration.

Our results suggest that iprindole may act on a specific recognition site(s) located on neuronal membranes postsynaptic to 5HT terminals, resulting in a decrease of β-adrenergic receptor function and perhaps an appearance of its therapeutic effect upon long term treatment. In conclusion iprindole acts on a site that bears some similiarities to that of mianserin, though it is not identical because mianserin does not down regulate its non binding while iprindole down regulates the Bmax of mianserin binding.

210.5 INCREASED β ADRENERGIC RECEPTOR BINDING IN HUMAN FRONTAL CORTEX. M. T. Zanko* and Anat Biegon, Department of Pharmacology, Hoffmann-La Roche Inc., Nutley, NJ 07110 (Sponsored by R. A. O'Brien)

β Adrenergic receptors in rat brain are decreased in number following chronic treatment with tricyclics, MAO inhibitors and electroconvulsive therapy, suggesting involvement of these receptors in the therapeutic action of these treatments. Therefore, we have undertaken a study of β adrenergic receptors in postmortem brain tissue from drug-free suicide victims and matched controls. Since changes in receptors may be highly localized, we have chosen a quantitative autoradiographic approach to screen for differences in discrete brain regions. Coronal sections (50 μ) were cut through the frontal cortical level of the right hemisphere of 6 suicide victims and 6 age and sex matched controls (5 men and 1 woman per group). The sections were incubated with 150 pM iodopindolol, 2200 Ci/mmole (nonspecific binding was assessed by co-incubation with 0.5 μM propranolol) and apposed against tritium sensitive sheet film (LKB ^{3}H-ultrofilm) alongside tissue-iodine standards. After 4.5 or 24 hours exposure the autoradiograms were subjected to computerized densitometry and converted to fm/mg protein using the tissue standards. We find significant elevations in β adrenergic binding in the frontal inferior, cingulate and orbital gyri of the cortex. Samples of these regions from the same subjects were homogneized and saturation binding studies were carried out to determine whether the change is due to increase affinity or Bmax. Using ^{3}H-DHA (NEN 90 Ci/mmole) we find and increase in Bmax in the suicides and identical Kd's. Thus using two different techniques we were able to show an elevation in β adrenergic binding in suicides, which may be relevant to the biochemical changes underlying depression and suicide.

210.6 MUSCARINIC CHOLINERGIC BINDING SITES ON FIBROBLASTS: A TOOL FOR INVESTIGATING AFFECTIVE DISORDERS. E.S. Gershon and N.S. NADI*, Sect. on Psychogenetics, NIMH, Bethesda, MD 20205

Using ^{3}[H]-quinuclidinyl benzilate (QNB) as ligand, we have demonstrated saturable and specific binding with a single binding site (Kd = 10^{-10}M, B_{max} = 200 fmol/mg protein). A series of 8 pharmacologic agents at 10^{-8}M showed similar displacement of QNB binding from rat brain homogenate and fibroblasts in vitro (r = 0.97). A physiologic role of this binding site as a receptor is suggested by demonstration that 10^{-5}M arecoline, or oxotremorine, or 10^{-6}M acetylcholine inhibits norepinephrine sensitive adenylate cyclase in the fibroblasts by an average of 51%.

The number of binding sites increases by 25% after exposure to 10uM atropine for 24 hours, with return to previous values one passage after removal. 10uM lithium carbonate produces a 16% decrease in number of binding sites.

These cells also have some choline acetyltransferase activity (150 fmols/mg protein/15 minutes). High affinity choline uptake similar to brain has been demonstrated in human fibroblasts by others (Riker et al., J. Neurochem. 36, 746, 1981) and confirmed by us.

Differences in B_{max} among cell lines from different individuals are stable over several cell passages, suggesting genetic variation controlling B_{max}. The percentage increase in B_{max} in the presence of atropine is also a stable individual characteristic.

Twelve patients with major affective disorder have higher B_{max} (329.0 ± 63.9 fmol/mg protein) than 12 normal controls (277.8 ± 47.3 fmol/mg protein). Nine relatives of these patients who also have had a major affective diagnosis also have significant B_{max} elevation (294.8 ± 47.3 fmol/mg protein). No significant differences in Kd are observed. This suggests that increased muscarinic receptor density in fibroblasts may be associated with genetic vulnerability to major affective disorder.

In addition, cells from 7 of these patients had greater decrease in B_{max} after Li^{+} incubation (46.6 fmol/mg protein) than did cells from 6 controls (9.5 fmol/mg protein). This observation may offer several interesting investigational leads for the study of the mechanism of lithium action in relation to the pathophysiology of affective disorders. These findings suggest that fibroblasts may be useful in the studies of affective disorders.

210.7 **EFFECTS OF CHRONIC L-DOPA AND BROMOCRIPTINE TREATMENT ON DOPAMINE RECEPTORS IN AN ANIMAL MODEL OF PARKINSON'S DISEASE.** **M.B. Schneider*, L.C. Murrin and R.F. Pfeiffer*, Depts. of Pharmacology and Neurology, Univ. of Nebraska Medical Center, Omaha, NE 68105. (Spon: F.M. Skultety)**

Parkinson's Disease is characterized by degeneration of the nigrostriatal pathway resulting in a loss of striatal dopamine (DA) and development of denervation supersensitivity in striatal dopaminergic receptors. The 6-hydroxydopamine (6-OHDA) lesion of DA neurons in the striatum has provided an animal model for this disease. The objective of the present study was to evaluate dopaminergic markers in this model following chronic treatment with either l-dopa or bromocriptine. DA receptors were measured using standard membrane binding techniques. ^{3}H-Spiroperidol (spiperone, ^{3}H-SP) was used as the ligand in concentrations from 0.1 nM to 10 nM in saturation studies. Specific binding represented the difference between ^{3}H-SP binding in the presence and absence of 1 μM d-butaclamol. Data were anlyzed by Scatchard plot and by computer-assisted fitting using LIGAND. DA and dihydroxyphenylacetic acid (DOPAC) levels were measured using HPLC and electrochemical detection. Male albino rats under chloral hydrate anesthesia received lesions of the right striatum by stereotaxic injection of 8 μg of 6-OHDA in 4 μl of 0.02% ascorbate. Two weeks postoperatively animals were tested for contralateral rotational behavior with apomorphine (0.5 mg/kg i.p.) and only those animals rotating at least 5 revolutions per minute were used. Animals were treated with l-dopa (55 mg/kg i.p.), bromocriptine (2 mg/kg i.p.) or polyethylene glycol (vehicle) every 12 hrs for 30 days. Twenty-four hours after the last drug administration the animals were sacrificed and striata were dissected. Lesion with 6-OHDA produced a supersensitivity in dopamine receptors as indicated by an increase in Bmax of binding. This supersensitivity was reversed by either l-dopa or bromocriptine treatment. Neither lesion nor drug treatment produced a significant change in affinity (K_D) for ^{3}H-SP. Drug treatment alone had no effect on either K_D or Bmax. DA and DOPAC levels were reduced >90% in the 6-OHDA lesioned striatum compared to the unlesioned striatum. Neither drug treatment affected DA or DOPAC levels compared to controls. These results suggest chronic administration of bromocriptine or l-dopa reverses the denervation supersensitivity seen following 6-OHDA lesioning but does not affect either DA or DOPAC levels in the striatum. This may play a role in the clinical changes seen with chronic use of these drugs. Supported by grants from the Frances and Edgar Reynolds Foundation and Sandoz, Inc. and BNS-7921105.

210.8 Neurochemical effects of L-dihydroxyphenylalanine (L-DOPA) on N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonian monkey. C.C. Chiueh, R.S. Burns*, S.P. Markey* and I.J. Kopin. Lab. of Clinical Science, NIMH, Bethesda, MD 20205.

N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine(NMPTP) produces a chronic and persistent extrapyramidal motor dysfunction in Rhesus monkeys (Burns et al., Proc. Nat. Acad. Sci. in press, 1983) which resembles the parkinsonian syndrome in drug abusers after i.v. administration of illicit synthetic batches of N-methyl-4-phenyl-4-propionoxypiperidine and the contaminant, NMPTP (Davis et al., Psych. Res. 1: 249-254, 1979; Drug Enforcement Agency Microgram 15: 165, 1982; Langston et. al., Science, 219: 979-980, 1983). However, NMPTP does not produce severe Parkinson's syndrome in animals other than primates (Chiueh et al., Proc. 5th Int. Catecholamine Symp. in press, 1983). The antiparkinsonian drug, L-DOPA, reversed the NMPTP induced extrapyramidal motor syndrome (flexed posture, rigidity, tremor, immobility, akinesea, freezing syndrome) in man and monkey.

In the present experiment, we investigated the effects of L-DOPA on the metabolites of monoamines in the CSF of the monkey model of Parkinson's disease. The ventricular CSF was collected through a chronic implanted catheter in the lateral ventricle of monkey treated chronically with NMPTP. After the monkey developed extrapyramidal syndrome, L-DOPA(100-200 mg) and MK-486 (10-20 mg) were suspended in 50 ml of Tang orange drink and administered orally. The CSF was collected during 30 min intervals and assayed for L-DOPA, dopamine, serotonin and their metabolites by high performance liquid chromatograph. The basal levels of HVA, 5HIAA, and MHPG in CSF were decreased. As had been shown previously, L-DOPA effectively reversed the NMPTP-induced extrapyramidal syndrome. The behavioral effects of L-DOPA peaked at one hr and lasted for 3 hrs. This coincided with increased levels of dopamine and L-DOPA in the ventricular CSF. The increase in HVA occurred much later and peaked at 3-4 hrs after administration of L-DOPA. The CSF levels of O-methyl-DOPA, 5HIAA and MHPG also increased, but like HVA, did not correlate with the time-course of behavioral improvement.

Despite dramatic decrease in striatal dopamine (5-10% of control) in the chronically treated monkey surviving dopaminergic terminals still appear to synthesize dopamine from L-DOPA and meet the demand of supply of dopamine in the nigrostriatal system. It is also possible that dopamine could be synthesized in the intact serotonergic neurons and reached the postsynaptic dopaminergic receptors in the striatum as a "false transmitter" (Ng et al., Science 170:76-77, 1970). Thus L-DOPA can be converted to dopamine even in dopamine deficient parkinsonian brain and reverses the extrapyramidal motor dysfunction following the chronic administration of NMPTP in primates.

210.9 CLINICAL RESPONSE TO L-DOPA DEPENDS UPON ITS ENTRY INTO BRAIN BY LARGE NEUTRAL AMINO ACID TRANSPORT SYSTEM. J.G. Nutt, W.R. Woodward, and J.P. Hammerstad. Department of Neurology, Oregon Health Sciences University, Portland, Oregon 97201.

L-DOPA crosses the blood-brain barrier by a saturable large neutral amino acid (LNAA) transport system. This potentially rate limiting step in the clinical response to L-DOPA has been investigated in 6 parkinsonian patients with a rapid fluctuating response to L-DOPA ("on-off" phenomenon). Patients received continuous intravenous infusions of L-DOPA producing constant plasma L-DOPA concentrations and therapeutic effects for 12 to 36 hours. During the infusion, the patients received various amino acids (100 mg/kg) administered orally in solution. The large neutral amino acids, L-phenylalanine, L-leucine, and L-isoleucine produced a rapid and marked deterioration in the clinical response of the patients beginning 45 minutes after the administration of the amino acids and persisting for 2 to 6 hours with a gradual return to the optimal clinical state. The plasma L-DOPA concentrations were not altered by the amino acid administration. Glycine and lysine, both transported by other amino acid transport systems, had no effect on the therapeutic response or plasma L-DOPA level. High protein meals also abolished the clinical response to intravenously administered L-DOPA. The rise in plasma concentration of total LNAA's was similar after the high protein meal or phenylalanine administration.

These results suggest that transport of L-DOPA across the blood-brain barrier by the LNAA transport system can be a rate limiting step in the clinical response to L-DOPA and that the ratio of plasma L-DOPA concentration to total plasma LNAA concentration may correlate better with therapeutic effects than the plasma L-DOPA concentration alone.

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210.10 THE EFFECT OF CHRONIC DOPAMINE AGONIST TREATMENT ON TYROSINE HYDROXYLASE ACTIVITY AND DOPAMINE-ß-HYDROXYLASE ACTIVITY AND ON D-1 AND D-2 POST-SYNAPTIC DOPAMINE RECEPTORS. K.D. Wilner, I.J. Butler and Y.C. Clement-Cormier. Depts. of Pharmacology, Neurobiology and Neurology, University of Texas Medical School, Houston, Texas 77025.

The effect of chronic levodopa or bromocriptine treatment was tested on D-1 and D-2 post-synaptic receptors in the striatum; and on tyrosine hydroxylase and dopamine-ß-hydroxylase activities as enzymatic markers of pre-synaptic neuronal activity in selected rat brain regions. Levodopa (1000 mg/kg) in combination with carbidopa (100 mg/kg) was administered by the oral route whereas bromocriptine (10 mg/kg) was administered by the intraperitoneal route. Each study lasted for 28 days. At intervals, rats were sacrificed and (1) dopamine-sensitive adenylate cyclase (D-1 receptor activity), (2) (^{3}H)spiroperidol binding (D-2 binding sites), (3) tyrosine hydroxylase (TH) and (4) dopamine-ß-hydroxylase (DBH) activities were evaluated after drug treatment. The data from these studies demonstrated contrasting results for levodopa, an indirect dopamine agonist and bromocriptine, a direct dopamine agonist. Levodopa altered D-1 receptor function after 1 day treatment as shown by a significant reduction in the Vmax for adenylate cyclase. However, after 12 days of levodopa, there was no longer a change in maximal enzyme activity but there was a two-fold increase in the EC_{50} for dopamine. In contrast, bromocriptine treatment had no apparent effect on dopamine-sensitive adenylate cyclase activity. Binding studies using (^{3}H)spiroperidol revealed a significant increase in D-2 binding sites after 5, 12 and 21 days of levodopa treatment. Yet bromocriptine produced no change in D-2 binding sites but did produce a three-fold increase in the dissociation constant compared to control after 12 days of treatment. Finally, both drug regimens differentially affected the enzyme markers of pre-synaptic neuronal activity. Levodopa, following 5 to 21 days, significantly increased the Vmax for the phosphorylated and non-phosphorylated forms of tyrosine hydroxylase in striatum and cortex whereas bromocriptine decreased the activity of both forms of the enzyme but only in the striatum. Levodopa dramatically reduced DBH activity in serum and brain regions after 12 to 21 days of treatment. In contrast, bromocriptine increased DBH activity. Possible causes for the alterations in enzyme activity after levodopa and bromocriptine treatment will be discussed. Still these results suggest that levodopa induces excess dopaminergic activity whereas bromocriptine decreases this activity and thus, these two apparently opposing effects on the dopamine system may explain a possible biochemical mechanism for the success of combination levodopa and bromocriptine therapy in the treatment of Parkinson's disease.

210.11 DOPAMINERGIC ACTIVITY IS REDUCED IN DIABETIC RATS. C. F. Saller* (Spon: M. E. Goldberg). Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, MD 20205.

Acute injections of D-glucose suppress the firing of nigrostriatal dopamine (DA)-containing neurons in chloral hydrate anesthetized rats [Saller and Chiodo, Science 210, 1269 (1980)] and reduce striatal DA release in conscious rats [McCaleb and Myers, Brain Res. Bull. 4, 651 (1979)]. Moreover, the number of striatal DA receptors, as evidenced by increased [^{3}H]spiperone binding to striatal membranes, appear to be increased at six and ten weeks after rats are made chronically hyperglycemic using either alloxan or streptozotocin to destroy insulin-secreting cells [Lozovsky, Saller, and Kopin, Science 214, 1031 (1981)]. These findings suggest that the increased number of DA receptors in diabetic rats might be a compensatory response to a reduction in the activity of striatal DA-containing neurons. To evaluate this hypothesis, rats were given either alloxan (185 mg/kg, s.c.) or saline (1 ml/kg, s.c.), and six weeks later dopaminergic activity was assessed by measuring the striatal levels of DA and its two major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Neither DA nor DOPAC concentrations in the diabetic rats were significantly different from control values. However, striatal HVA levels were 30% lower ($p<0.05$) in the diabetic rats (592 $\pm$ 66 ng/g, mean $\pm$ S.E.M.) than in controls (848 $\pm$ 34 ng/g), suggesting that DA release may be reduced in diabetic rats. In addition, the biosynthetic capacity of DA-containing nerve terminals in the striatum of diabetic rats appears to be reduced. This was evidenced by a 27% decrease ($p<0.05$) in the V_{max} of synaptosomal [^{3}H]DA synthesis from [^{3}H]tyrosine in the diabetic rats (18.12 $\pm$ 1.61 and 24.70 $\pm$ 1.40 pmole/mg protein/min in diabetic and control animals, respectively). The K_m for tyrosine of the diabetic rats (0.61 $\pm$ 0.05 µM) was also reduced ($p<0.05$) by 33% (controls, 0.91 $\pm$ 0.07 µM). Thus, it appears that DA synthesis and release may be reduced in diabetic rats. Whether or not these effects are due to chronic hyperglycemia or hypoinsulinemia remains to be determined. Nonetheless, it is clear that dopaminergic transmission in the striatum is markedly affected by changes in carbohydrate metabolism, and it seems likely that the apparent increase in the number of striatal DA receptors in diabetic rats may be a consequence of a reduction in the activity of nigrostriatal DA-containing neurons. (Present address: Stuart Pharmaceuticals, Div. of ICI Americas Inc., Wilmington, DE 19897)

210.12 CHRONIC FLUPHENAZINE AND CLOZAPINE ELICIT OPPOSITE CHANGES IN BRAIN MUSCARINIC RECEPTOR BINDING. IMPLICATIONS FOR UNDERSTANDING TARDIVE DYSKINESIA. E. Friedman,* G. Gianutsos, J. Kuster*. Depts. of Psychiatry & Pharmacology, N.Y.U. School of Medicine, New York, NY 10016 and Section Pharmacology & Toxicology, UConn, Storrs, CT 06268.

The effects of long-term treatment with the antipsychotic drugs haloperidol (HL), fluphenazine (F) and clozapine on brain dopaminergic and muscarinic receptor binding sites was examined. Mice were treated with HL and F in their drinking water (0.005 and 0.001%^{w}/v) or with clozapine-containing diet (0.075% ^{w}/w) for up to 12 weeks. The 3 neuroleptic drugs elicited increases in striatal ^{3}H-spiroperidol binding. Muscarinic binding of ^{3}H-QNB was decreased only in striatum of animals treated with HL or fluphenazine for 12 but not for 3 weeks. This reduction in binding density was not accompanied by alteration in K_d nor was the relative distribution of high to low affinity agonist sites affected. Tolerance to fluphenazine-induced catalepsy was obtained after 14 days, while catalepsy to a challenge dose of pilocarpine was observed after 42 days of fluphenazine treatment. Twelve weeks of clozapine administration elicited increases in ^{3}H-QNB binding densities in the striatum, cortex and hippocampus. No alteration in striatal muscarinic agonist sites was obtained in clozapine treated mice. Furthermore, no alteration in fluphenazine- or pilocarpine-elicited catalepsy was detected in clozapine-treated animals. Choline acetyltransferase activity was not affected by long-term fluphenazine treatment; however, clozapine administration induced an increase in striatal enzyme activity. These data indicate that while HL, fluphenazine and clozapine elicit increases in striatal dopamine receptor sensitivities, they differ with regard to their effects on brain muscarinic cholinergic receptors. This differential response may be attributed to the high antidopaminergic/low anticholinergic potency of HL and fluphenazine and the potent direct antimuscarinic efficacy of clozapine. These data suggest that the incidence of tardive dyskinesia during long-term treatment with antipsychotic drugs may be related to the ratio of dopamine supersensitivity/muscarinic subsensitivity induced by the drugs.

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210.13 MOLINDONE COMPARED TO HALOPERIDOL IN ANIMAL MODELS OF TARDIVE DYSKINESIA. W.C. Koller[1], J.C. Curtin[1] and J.Z. Fields[2]. Neurology Research Lab., Loyola Univ. Coll. Med., Chicago[1] and Dept. Pharmacology, Chicago Med. Sch., North Chicago, IL[2].

Tardive dyskinesia (TD) is a hyperkinetic movement disorder resulting from long term neuroleptic usage. Since TD may be irreversible and unresponsive to treatment, prevention of TD has become a major concern. The ability of various neuroleptic drugs to induce TD may differ. It is claimed that the newer neuroleptic, molindone, is less likely to induce TD. We have therefore compared molindone to the standard neuroleptic haloperidol in an animal model of TD. Both behavioral supersensitivity to dopamine agonists and alteration of striatal dopamine receptor binding were measured. Groups of 10 guinea pigs were initially challenged with apomorphine (0.4 mg/kg) and the behavioral response of each group recorded (pre-test). Animals were then treated for 12 days with saline or molindone (1,3, or 6 mg/kg) or haloperidol (0.1, 0.5 or 5.0 mg/kg). Six days after cessation of treatment, animals were re-challenged with apomorphine and stereotypy was measured (post-test). Two days after that, animals were sacrificed, the striatum removed, and binding of (3H) spiroperidol to striatal dopamine receptors was determined (Control Kd=29 pM, Bmax=13.9 fmol/mg tissue). Molindone at 1 mg/kg produced no change in stereotypy as compared to chronic saline. Molindone at 3 mg/kg produced a slight (+21%) but statistically significant increase in stereotypy. At 6 mg/kg, molindone produced an even greater behavioral increase (+55%). Behavioral supersensitivity was also seen with all haloperidol groups (+37% to 50%). In the haloperidol treated group, the maximum number of D-2 dopamine receptors labelled by high affinity (3H) spiroperidol binding was increased (+63 to +77%). At the same time, the affinity also was higher (+28 to +156%). These changes are consistent with the haloperidol induced increases in stereotypy. In the molindone group the Bmax was increased only where there was also behavioral supersensitivity (3 and 6 mg/kg; (+35%, +36%). The affinity for these two groups simultaneously increased (+37%, +59%). These changes are consistent with the molindone-induced increases in stereotypy. Haloperidol is thought to be clinically about 6 times more potent than molindone. Comparison of equipotent doses in the animal model (0.5 mg/kg vs. 3.0 mg/kg) suggest that molindone causes less behavioral supersensitivity and smaller changes in dopamine receptor binding. This study, therefore, supports the possibility that molindone may have less potential for eliciting tardive dyskinesia than haloperidol. (Supported by BRSG grant RR-386 to JZF and VA Medical Research)

210.14 CHANGES IN MUSCARINIC CHOLINERGIC BINDING SITES FOLLOWING SPINAL CORD TRANSECTION. S.M. Lasley, J.K. Wamsley†, J.E. Smith‡ and J. D. Lane. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107; † Department of Psychiatry, University of Utah Medical Center, Salt Lake City, UT 84132; and ‡ Department of Psychiatry, LSU Medical Center, Shreveport, LA 71130.

Irreversible spinal cord injury results in a flaccid paralysis (spinal shock) followed by the progressive evolution of hyperreflexia (spasticity). The neural mechanisms responsible for these events are hypothesized but only tentatively understood. To investigate potential effectors, adult cats were transected at the T5-7 level and maintained for periods of up to three months. Neurological signs were monitored. At selected time points, the animals were sacrificed and the lumbar cords removed. Frozen sections were processed for in vitro autoradiography. After mounting, tissue was incubated with [^{3}H]-N-methylscopolamine (1nM) in the absence or presence of carbachol or atropine, then affixed to LKB Ultrofilm. Compared to sham-operated controls, total binding was increased 10-11% during spinal shock, had returned to normal by 7 days, afterwhich it decreased slightly but not significantly over the remaining time course. High affinity sites (sensitive to carbachol displacement) and low affinity sites were monitored, and differences were observed beginning circa 14 days which persisted up to 3 months. In the dorsal horn, there was a 38% increase in high affinity and concomitant decrease in low affinity binding. In the ventral horn (Rexed VII, lateral aspect) there was a 35% decrease in high affinity and concomitant increase in low affinity binding. Based on the concept that high affinity sites are transported from the cell body to the terminals, and low affinity sites are those inserted into the membrane, i.e., "functional", the changes in the ventral horn could be due to loss of suprasegmental cholinergic input onto motorneurons, resulting in a denervation-like supersensitivity, i.e., increase in "functional" receptors. The change in the dorsal horn could be a down regulation of sites resulting from excess agonist assault due to hyper-reflexic arcs at the segmental level. It appears that two elements of plasticity are active in the dorsal and ventral horns. These changes could be a consequence of and/or result in spasticity. GABA, glycine and opioid binding in the same tissue will also be compared to the cholinergic system. [These studies were supported in part by TCOM OR 34180 and NIH NS19622 (to JDL) and MH36563 (to JKW).]

210.15 ELECTROPHYSIOLOGICAL AND BIOCHEMICAL ANALYSIS OF CEREBELLAR SEROTONERGIC MECHANISMS IN THIAMINE DEFICIENCY. Howard K. Strahlendorf, Jean C. Strahlendorf, Rong-Sheng Lee* and Kim Light+. Medical and Surgical Neurology and Physiology, Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430, †Univ. of Arkansas for Med. Sci. Little Rock, AR.

Wernicke's encephalopathy is a neurologic disorder in which patients display gross motor abnormalities as a result of thiamine (Vitamin B_1) deficiency (TD). A selective impairment of the high affinity neuronal uptake system for serotonin (5-HT) in rat cerebellar synaptosomes occurs as a result of thiamine deficiency. Recent ultrastructural and autoradiographic studies in rats have demonstrated a selective loss of labeling of cerebellar serotonergic nerve terminals in hypovitaminosis B_1. A functional impairment of cerebellar 5-HT neurons should alter the responsiveness of target cells to 5-HT. These changes in cerebellar serotonin systems probably account for some of the neurologic signs of thiamine deficiency. The electrophysiological changes in the cerebellum induced by the lack of thiamine have not been studied: neither have the serotonin receptor binding properties been examined. Rats were fed a thiamine free diet for 5 weeks. Sensitivity of Purkinje cells (PC) to iontophoretic 5-HT was determined in urethane anesthetized animals. In controls 5-HT elicited three effects: 61% of the neurons were depressed; 27% of the cells responded biphasically, inhibition followed by excitation; and 11% of the neurons were excited. PC from TD rats responded solely with inhibition to 5-HT. Comparisons of threshold currents to elicit a just noticeable slowing of firing rate revealed a significant difference between control (8NA) and TD rats (2NA). The entire dose response curve from TD rats was shifted parallelly and significantly to the left. These data suggest that the response of cerebellar PC to 5-HT in TD rats is qualitatively and quantitatively different from controls; reflecting a shift to more pronounced inhibitory actions of 5-HT. Receptor binding assays are currently in progress utilizing ^{3}H-LSD as the ligand for 5-HT_1 and 5-HT_2 receptors. Preliminary results reveal no difference in binding properties of LSD between TD rats and controls (Kd and Bmax). However, analysis of the slopes from Hill plots reveal a Hill number greater than one in both TD rats and controls which suggests the existence of more than one serotonin receptor (binding site) in the cerebellum. This is in contrast to the cortex in which no such evidence was found. Additional experiments using more selective ligands for the two receptor subtypes are underway. (Supported by the Institute for Nutritional Sciences, TTU)

210.16 **EVIDENCE SUGGESTING ALTERATIONS IN CEREBELLAR HISTAMINERGIC MECHANISMS IN THIAMINE DEFICIENCY.** Jean C. Strahlendorf, Howard K. Strahlendorf, Kim Light+ and Munhyang Lee. Departments of Physiology and Medical and Surgical Neurology, Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430, +Univ. of Arkansas for Med. Scis., Little Rock, AR 72201.

Thiamine deficiency is believed to cause a relatively selective impairment of cerebellar serotonergic mechanisms. Other putative neurotransmitters in the cerebellum are, for the most, unaffected or only slightly compromised by thiamine deficiency, e.g. norepinephrine, GABA, glutamate and aspartate. Histamine however, has not been studied. The cerebellum has both histamine-1 (H_1) and histamine-2 (H_2) receptors. It has been shown by others that Purkinje cells are primarily inhibited by iontophoretically applied histamine and this response is mediated by both H_1 and H_2 receptor subtypes. As part of our investigation of serotonergic mechanisms in thiamine deficiency (TD) we have uncovered large changes in cerebellar histamine receptor binding and apparent concomitant electrophysiologic changes. Utilizing ^{3}H-pyrilamine as a H_1 receptor ligand, a significant decrease in receptor affinity occurred in cerebellar preparations from TD animals (Kd - 44.8 mM) when compared to controls (Kd - 19.6 mM). This affinity decrease was accompanied by a large increase in receptor number (Bmax: Control - 0.1639; TD - 0.4043). These effects are intriguing in light of the apparent specificity of thiamine depletion for cerebellar neurotransmitter systems. Parallel assays done on cortical tissue from the same groups of animals showed no difference between controls and TD rats. Electrophysiological experiments examining the effect of histamine applied directly to Purkinje cells are underway. Preliminary results indicate Purkinje neurons may be more profoundly inhibited by histamine in TD rats. These data present a new finding that thiamine deficiency affects cerebellar histaminergic systems as well as serotonergic systems. Additional experiments are underway to confirm and expand these results. (Supported by the Institute for Nutritional Sciences, TTU).

210.17 GABAERGIC SYSTEM IN SCRAPIE INFECTED HAMSTERS. C. Masullo*, M. Pocchiari*, W.D. Lust†, C.J. Gibbs, Jr.* and D.C. Gajdusek. Lab. of CNS Studies and †Lab. of Neurochemistry, NINCDS, N.I.H., Bethesda, Md 20205.

Golden Syrian hamsters inoculated with the 263K strain of scrapie virus show an abnormal aggressive behaviour suggesting an alteration in the GABAergic system. Isonicotinic hydrazide, a drug that decreases the level of GABA, injected subcutaneously in control and scrapie infected hamsters induces tonic-clonic seizures in scrapie hamsters significantly earlier ($P(t)<0.0000$) than in control animals. These data indicate a derangement in the GABAergic system. In an attempt to determine how the virus alters the GABAergic system we studied the concentration of GABA, glutamate, cyclic GMP and cyclic AMP in the brains of control and scrapie infected hamsters.

To minimize fixation artefact, 8 control and 8 scrapie infected hamsters were sacrified 56 days after inoculation during clinical disease by freezing in liquid nitrogen. Brains were removed and dissected at -20°C into three areas: cerebral cortex, cerebellum and brain stem. GABA and glutamate were determined by fluorometric methods and the cyclic nucleotides by radioimmunoassay.

No significant alteration was detected in the concentration of GABA, glutamate, cGMP and cAMP in the brain regions tested in scrapie infected hamsters. This suggests that the GABAergic neurons might be spared by the scrapie virus and that the defect might be in the post-synaptic GABA receptor or dependent on other neurotransmitter systems which are closely related.

Further studies are in progress to evaluate the activity of the glutamic acid decarboxylase and GABA transaminase and to test the functionality of the GABA-benzodiazepine-chloride receptor complex.

210.18 DIABETES IN RATS: ALTERED CENTRAL AND PERIPHERAL CATECHOLAMINE FUNCTION, AND EFFECTS OF MANIPULATING DIETARY FAT AND PROTEIN INTAKE. N.Rowland, J.N.Joyce, L.L.Bellush and M.J.Fregly*. Depts Psychology and Physiology, Univ. Florida, Gainesville FL 32611.

Mildly diabetic (D) rats showed a preference for dietary fat compared with nondiabetics (ND), but severely D rats self-selected a high protein regimen (Bartness & Rowland, Soc.Neurosci. 82). We now examine whether different diet selections have functional consequences. Rats were made D with streptozotocin (35 or 70 mg/kg) and received chow for 1 mo. They and ND groups then received either high protein (50%P,40%C,10%F), high fat-protein (50%P,15%C 35%F), or normal (25%P,65%C,10%F) purified diets for 4-6 wk. Food intake, body weight, and metabolic data are presented at this meeting by Bellush & Rowland. The rats were then tested for amphetamine (AM, 3 mg/kg)-induced stereotypy in their home cage. All D rats showed decreased maximum intensity of stereotyped sniffing relative to ND, but the duration of activation was the same in both groups. No effect of diet type was evident. We subsequently found no difference in the amount of 3H-AM in the striatum of D and ND rats 1 hr after injection. These data agree with previous findings of decreased AM stereotypy (Marshall, 78), but do not confirm a partial restoration with high fat/protein. The decreased intensity does not simply reflect a behavioral ceiling effect in either group: 5 mg/kg AM produced stronger stereotypy in both D and ND, and the behavioral ratings of the D rats were now comparable to those observed in the ND after 3 mg/kg AM. We also examined the behavioral response to the dopamine (DA) receptor agonist apomorphine (APO, 1 mg/kg). The maximum stereotypy rating was not different between the D and ND rats; the duration of the behavioral activation was much reduced in D. Again, no dietary effects were noted. A presynaptic dose of APO (.05 mg/kg) produced comparable decreases in open field activity of D (64%) and ND (51%) rats. These findings argue strongly against any functional role for reported (Lozovsky et al 81; Trulson & Himmel 83) increases in 3H-spiroperidol binding to D striatum. In several experiments we have been unable to observe consistent increases in spiroperidol binding, or relation to severity of diabetes.

Peripheral β-adrenergic function was assessed using isoproterenol (ISO). ISO (5 μg/kg) produced larger maximal increases in heart rate in D (125 bpm) than ND (70 bpm). However, tail skin temperature rise with ISO (25 μg/kg) was less in D (basal 32.5°C, max 33.2°) than in ND (basal 28.4°, max 33.8°). There were no differences in colonic temperature. We are currently assessing whether the difference in basal tail skin temperature reflects altered BMR. We shall present data on β-receptor binding to heart and cerebral cortex; parallel data from saturated vs. unsaturated high fat diets for ISO and AM responses are being collected.

210.19 EFFECTS OF SOMAN ON ENERGY STATE AND SELECTED METABOLITES IN MOUSE BRAIN. G.B. Viana* and F.C. Kauffman Department of Pharmacology and Experimental Therapeutics, University of Maryland School of Medicine, Baltimore, Md., 21201.

The effects of soman (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester) on various metabolites in mouse brain were examined 30 min after a single administration of either 175 μg or 330 μg/kg body weight of soman. The drug was administered subcutaneously and animals were killed by immersion in liquid nitrogen. ATP, phosphocreatine (PCr), alanine, glutamate, GABA and citrate were measured by coupled enzymatic procedures. A dose-dependent increase in PCr was noted in the cerebral cortex and basal frontal part of the brain, however, concentrations of this intermediate remained constant in the brain stem and cerebellum. Increases in PCr occurred in the absence of any change in ATP. Administration of soman resulted in significant increases in glutamate and decreases in alanine in several brain regions. Patterns of change in the alanine:glutamate ratio were opposite to that noted after ischemic injury in which alanine:glutamate ratios increase (Conger et al., J. Histochem. Cytochem. 26: 423, 1978). These results suggest that the metabolic changes produced by soman cannot be ascribed to alterations secondary to ischemia. The possibility that soman stimulated oxidative metabolism in mouse brain is suggested by the finding that steady state concentrations of citrate were elevated in the cerebral cortex and brain stem of mice exposed to soman.

Increases in both PCr and ATP were observed when animals were treated with 70-90 μg/kg of soman 3 times over a 24 h period. The increase in high energy phosphate content does not appear to be sex related since ATP and PCr were elevated in various brain regions of both male and female mice. Animals that had received the low doses of soman over a 24 h period did not show alteration in steady state concentrations of glutamate, alanine or GABA.

The results indicate that exposure to sublethal amounts of soman produce a significant increase in high energy phosphate content in mouse brain. Changes in steady state concentrations of alanine, citrate and glutamate support the hypothesis that increases in high energy phosphate content after soman treatment may result from stimulation of metabolism via the citric acid cycle. It is not known whether these metabolic changes represent a primary action or occur secondary to stimulation of cholinergic systems. (Supported by U.S. Army Medical Research and Development Command Contract No. DAMD17-81-C-1279 and CNPq, Brazil).

210.20 PHARMACOLOGICAL AND NEUROCHEMICAL CHANGES FOLLOWING CHRONIC γ-BUTYROLACTONE: A POSSIBLE GABAERGIC ROLE IN A MODEL OF DEPRESSION. P.D. Suzdak and G. Gianutsos. Section of Pharmacology and Toxicology, University of Connecticut, Storrs, CT 06268

Numerous investigations into the mechanism of action of antidepressant drugs have focused on the change in amine receptor function induced by long-term drug administration, with a decrease in β-adrenergic receptors gaining favor as a possible model (Sulser, Pharmakopsychiat. Neuropsychopharm. 11:43, 1978) We have been interested in the pharmacological effects of γ-butyrolactone (GBL), an analog of GABA which produces a down-regulation of GABA receptors following long-term treatment (Suzdak & Gianutsos, Fed. Proc. 42:878, 1983). Long-term oral administration of GBL (0.75% v/v in fruit punch) to mice also resulted in a 23% decrease in maximal specific binding of ^{3}H-DHA, a ligand which labels β-adrenergic receptors, in the "frontal" cortex without affecting binding in the hippocampus. These results suggest that a) GBL may be an effective antidepressant drug; b) GABA may be involved in depression; and/or c) GABA neurons participate in the regulatin of β-adrenergic function in the cortex. In order to test the first hypothesis, GBL (5-100 mg/kg) was tested for activity in the Porsolt behavioral despair test (Arch. Int. Pharmacodyn. 229:327, 1977). GBL was at best only weakly active after acute administration. However, after chronic GBL administration, the dose-response curve for the tricyclic antidepressant, imipramine, was shifted to the left. Furthermore, small doses (10-20 mg/kg) of the GABA-T inhibitor aminooxyacetic acid (AOAA) were found to be active in this test for potential antidepressant activity. These results may suggest a possible role for GABA in depression.

210.21 PROPHYLACTIC AND REVERSAL EFFECTS OF PROLYL-LEUCYL-GLYCINAMIDE (PLG) ON DOPAMINE RECEPTOR SUPERSENSITIVITY. P. Chiu*, G. Rajakumar* and R.K. Mishra* (SPON: M. Steiner). Dept. of Psychiatry and Neurosciences, McMaster Univ., Hamilton, Ontario Canada.

Tardive dyskinesia, a major adverse effect associated with chronic neuroleptic therapy reflects the presence of hypersensitive postsynaptic dopamine receptors in the basal ganglia. PLG has been found to preferentially enhance the affinity of ^{3}H apomorphine binding to dopamine receptors in human and rat brains, and reverse the morphine- and haloperidol-induced catalepsy in rats. This suggests that PLG binding sites are capable of differentially modulating dopaminergic neurotransmission. In the present study, the possible desensitizing effect of PLG on dopamine receptor supersensitivity was examined. Four groups of six rats per group received various drug treatment: Groups 1 and 3 A,B,C were given isotonic saline (1 ml/kg, ip) daily for 28 days; Groups 2, 4 A,B,C received haloperidol treatment (3 mg/kg, ip) daily for 28 days. Groups 3 A,B,C and 4 A,B,C were treated with PLG (20 mg/kg sc) daily, for 5 days prior to, and 5, 10 days after saline or HAL treatment respectively. Animals were sacrificed 5 days after the last drug session and dopamine receptor binding eas carried out on striata of each rat with (^{3}H) spiroperidol as radioligand and 1 um butaclamal as displacing agent. Binding data were analyzed by Scatchard plot.

Our data indicate that chronic haloperidol treatment induced supersensitivity of dopamine receptors as evidenced by an enhancement of (^{3}H) spiroperidol binding sites in the striatum of the treated group. Administration of PLG prior to and after chronic HAL administration inhibited the supersensitivity of dopamine receptors as shown by a statistically significant decrease in B max (the number of maximal binding sites). The longer treatment group exhibited a more dramatic reversal effect of PLG. In all drug treatment groups no statistically significant difference was observed in the dissociation constant (K_D). Administration of PLG alone to saline treated rats did not modify specific (^{3}H) spiroperidol binding.

Our findings suggest that PLG can effectively prevent or reverse the fully developed dopamine receptor supersensitivity, and that this PLG mediated biochemical antagonism of altered receptor sensitivity in the striatum is reflected primarily in the relative density of the ligand binding sites. Clinically, PLG should be considered as a potential prophylactic and therapeutic antidyskinetic agent in the management of tardive dyskinesia.

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REGIONAL LOCALIZATION OF RECEPTORS AND TRANSMITTERS II

211.1 IDENTIFICATION OF PICROTOXIN/BARBITURATE BINDING SITES IN RAT BRAIN BY LIGHT MICROSCOPIC AUTORADIOGRAPHY WITH [^{35}S]-t-BUTYL-BICYCLOPHOSPHOTHIONATE. K.W.Gee, J.K.Wamsley+ and H.I.Yamamura. Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724 and +Department of Psychiatry, University of Utah Medical Center, Salt Lake City, Utah 84132.

t-Butyl-bicyclophosphothionate(TBT) is a potent convulsant agent and has been shown to inhibit GABA neurotransmission in electrophysiological studies. Its ability to displace ^{3}H-dihydropicrotoxin binding suggests that TBT produces its convulsant effect through the picrotoxin/barbiturate(P/B) site. The specific binding of ^{35}S-TBT in rat brain has been recently demonstrated by a filtration binding assay(Squires et al.,1983). ^{35}S-TBT binds to P/B sensitive sites with high affinity(K_d=17 nM) and with relatively low non-specific binding. In the present study we describe the distribution of ^{35}S-TBT binding sites in the rat brain by light microscopic autoradiography.

Freshly removed whole rat brains from male Sprague-Dawley rats were prepared for microtome sectioning by coating in plastic embedding medium and freezing onto microtome chucks+liquid nitrogen. Coronal sections of brain(10 μm) were cut and thaw mounted onto microscope slides. Slide mounted sections were preincubated in 5 mM Tris buffer, pH 7.4(+200 mM KCl & 1 mM EDTA) at 25°C for 90 min. Incubations were terminated by rinsing in Tris buffer and distilled H_2O. Sections were air dryed and autoradiographs prepared by exposing the sections to tritium-sensitive film for 10 days at 4°C.

Under the conditions used, specific binding accounted for approximately 60% of the total ^{35}S-TBT bound. Most brain regions were uniformly labelled with a significant amount of specific binding. A high degree of specific labelling was observed in the granule cell layer of the cerebellum; lamina I & IV of the cerebral cortex; the zona incerta; subthalamic nucleus and the parafascicular nucleus. Moderate binding was observed in the caudate/putamen; molecular layer of the cerebellum; stratum oriens(CA1) of the hippocampus and the inferior olivary nucleus. Binding in white matter areas was slightly above background. The regional distribution is somewhat similar to the observations made in corresponding brain regions using a filtration binding assay. In conclusion, ^{35}S-TBT appears to be a suitable radioligand for light microscopic visualization of the P/B binding site in the rat brain. (Supported by PHS grants MH-27257 and MH-30626, KWG is a recipient of a NRSA(NS 06923) and HIY is a recipient of a RSDA type II(MH-00095))

211.2 NON-CORRESPONDENCE OF ^{3}H-GABA UPTAKE AND GAD LOCALIZATION: TWO POTENTIAL MARKERS OF GABAergic AMACRINE CELLS IN GOLDFISH RETINA. C.L. Zucker & S. Yazulla, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, NY, and J-Y. Wu, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX.

In retina, as well as many other regions of the CNS, GABA is a strong candidate as an inhibitory transmitter. Uptake of ^{3}H-GABA or the presence of the GABA biosynthetic enzyme glutamic acid decarboxylase (GAD) have both been used as markers for GABAergic function. The assumption has been made that either one is a good probe to identify a cell population which uses GABA as its transmitter. In the inner plexiform layer (IPL) of the goldfish retina, GAD immunoreactivity (GAD-IR) and ^{3}H-GABA uptake have been localized to amacrine cell processes in a similar but not identical labeling pattern. Unistatified pyriform amacrine cells which ramify in the proximal 20% of the IPL and make many synaptic contacts with Mbl bipolar cell terminals take up ^{3}H-GABA. Uptake by this cell type accounts for the very dense autoradiographic labeling seen in the proximal IPL relative to more distal laminae. In contrast to this, GAD-IR is found throughout the IPL with strong bands at the 20%, 50% and 80% levels. Although coexistense of ^{3}H-GABA uptake and GAD has generally been assumed, it has not been demonstrated. We have used a double labeling technique (EM immunocytochemistry/autoradiography) to study the correlation between these two markers in goldfish amacrine cells. GAD-IR is found in amacrine cell processes which make synapses onto the processes of other amacrine cells (which may or may not contain GAD-IR), bipolar cells, and possibly ganglion cells. In most cases, GAD containing processes do not take up ^{3}H-GABA. Processes that accumulate ^{3}H-GABA are found adjacent to and occasionally postsynaptic but never presynaptic to GAD-IR processes. Many processes taking up ^{3}H-GABA are not associated with GAD containing processes. The only examples of coexistence of ^{3}H-GABA uptake and GAD-IR are when two or more GAD-IR processes are adjacent to or in a synaptic relationship with each other. The speciousness of ^{3}H-GABA uptake is seen further in synapses involving bipolar cell terminals where neither presynaptic GAD-IR processes nor the postsynaptic terminals take up ^{3}H-GABA, unless two or more presynaptic GAD-IR terminals are adjacent to each other. These relationships are consistent throughout the IPL. This study suggests that in goldfish IPL, GAD-IR is a marker for GABA releasing neurons, whereas ^{3}H-GABA uptake is not. ^{3}H-GABA uptake may be involved in the inactivation of GABA after release, however, its localization provides equivocal results regarding the actual location of GABAergic synapses. This work was supported by NIH grant EY01682 to S.Y. and NS13224, EY03909 to J-Y.W.

211.3 AUTORADIOGRAPHIC DISTRIBUTION OF GLUTAMATE RECEPTOR SUBCLASSES IN RAT BRAIN. D.T. Monaghan*, V.R. Holets, D.W. Toy* and C.W. Cotman. Psychobiology Dept., Univ. of Cal., Irvine, CA 92717.

The action of glutamate in the CNS is mediated by multiple receptors. Four acidic amino acid receptors have been characterized physiologically by their selective sensitivity to N-methyl-D-aspartate (NMDA), kainic acid (KA), quisqualate, and 2-amino-4-phosphonobutyric acid (APB). We now report that receptor subtypes can be localized autoradiographically using ^{3}H-L-glutamate. Rat brain tissue sections were incubated at 30°C for 30 min. with 100 nM ^{3}H-L-glutamate. After rinsing and drying, the sections were placed against LKB ^{3}H-Ultrofilm for 10-30 days, then analyzed by quantitative densitometry.

Our results indicate that there are at least 4 distinct ^{3}H-L-glutamate binding sites. The differing sites exhibit a similar affinity for L-glutamate (k_D = 0.7 uM), yet have distinctly different distributions and pharmacologies. The major population appears to represent the NMDA receptor. The affinity of both NMDA and D-2-amino-5-phosphonovaleric acid (D-APV) is approximately 2 uM whereas L-APV, APB, KA, and quisqualate are considerably less potent. The distribution of these sites is quite anatomically specific. In the hippocampus the greatest density of these sites is found in the CAl stratum radiatum. In general, the distribution is appropriate for a corticofugal and allocortical neurotransmitter. Differing subdivisions within the cerebral cortex, basal ganglia, thalamus, hypothalamus, amygdala, septum, brain stem and other regions are readily differentiated.

A second site is NMDA-insensitive (k_i > 100 uM) but easily displaced by quisqualate and KA (KA k_i = 70 nM). The most dense localization of these sites is within the stratum lucidum of the hippocampus. The distribution of these sites and their affinity for both glutamate and kainate suggest that these sites are the previously described ^{3}H-KA binding sites.

A third population of glutamate binding sites are those which are not displaced by either NMDA or KA. In the hippocampal formation these sites are found enriched in the stratum oriens and subiculum. A fourth population is found when Ca^{++} and Cl^- ions are added. These sites are also NMDA and KA insensitive but within the hippocampus these sites are found within the stratum lacunosum-moleculare and in the molecular layer of the dentate gyrus. None of the glutamate binding sites observed in this study appears to correspond to major class of glutamate binding sites that have been extensively characterized in membrane preparations.

These data demonstrate that there are multiple populations of glutamate binding sites and that at least two of these may represent acidic amino acid receptors which have been shown to be involved in neurotransmission.

211.4 AUTORADIOGRAPHIC LOCALIZATION OF BENZODIAZEPINE RECEPTOR SUBTYPES IN SPASTIC AND CONTROL MICE USING A COMPUTERIZED SINGLE BIT PLANE IMAGE ANALYSIS SYSTEM. L.J. Regan* AND W.F. White (SPON: T.O. Fox). Department of Neuroscience, Children's Hospital, and Neuropathology, Harvard Medical School, Boston, MA, 02115.

Behavioral and electromyographic abnormalities observed in the mutant mouse spastic suggest a decrease in inhibitory neurotransmitter function. Pharmacolgic and receptor binding studies suggest that there is a decrease in inhibitory neurotransmission mediated by glycine; there is an 80-90% decrease in binding to the postsynaptic glycine receptor in the spinal cord, brainstem, and midbrain of spastic compared with littermate control mice. In addition there is an increase in the binding of ligands for both the GABA and benzodiazepine receptors and this increase appears dependent on the relative density of glycine to GABA or benzodiazepine receptors. These data suggest the possibility of interregulation between inhibitory neurotransmitters in the affected regions. In order to further localize and characterize the nature of the increase in benzodiazepine receptor binding observed in the mutant mouse spastic autoradiographic analyses of benzodiazepine binding were performed on ^{3}H-flunitrazepam binding to cryostat sections of spastic and control lumbar, thoracic, and cervical spinal cord, brainstem, and midbrain using diffusable substance autoradiographic techniques. Benzodiazepine receptor subtypes were investigated using 1 uM clonazepam, 1 uM Ro5-4864, 200 nM CL 218,872, 10 uM GABA, and 10 uM diazepam to define nonneuronal, neuronal, type 1 and 2, GABA potentiated, and nonspecific binding. The procedures described in "Quantification of microautoradiograms by single bit plane image analysis (this meeting) were used to analyse the autoradiograms and quantitate the binding in fmoles bound per mg dry weight. Two dimensional maps of receptor density were used to localize benzodiazepine receptor subtype density and to compare densities between homologous regions in the spastic and control animals.

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211.5 TO WHAT EXTENT DOES [H-3]SPIPERONE LABEL SEROTONIN RECEPTORS IN VIVO? D.C. Chugani, J.R. Barrio and M.E. Phelps. Departments of Pharmacology and Radiological Sciences, Division of Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

To define the requirements for imaging human neuroreceptors in vivo with [F-18]spiperone and positron computed tomography, it is important to fully characterize spiperone's(SP) in vivo binding properties. In particular, the resolution of its serotonergic and dopaminergic components is critical. In order to correlate the in vivo localization of SP with the large body of in vitro SP binding data, we compared the anatomical localization of [H-3]SP using contact autoradiography (LKB tritium sensitive film) with rat brain sections labeled by intravenous administration (1000 µCi/kg, 17 Ci/mmol, sacrifice at 2 hr) (in vivo) to sections labeled by incubation in buffer containing [H-3]SP (1 nM, 17 Ci/mmol, 30 min incubation, two 5 min washes) (in vitro). Visual examination of the autoradiographs revealed several striking differences between the in vivo and in vitro sections: a. the choroid plexus was heavily labeled in vivo, but not in vitro; b. the hippocampus was heavily labeled in vitro, but not in vivo; and c. the cortex, especially lamina IV, was more heavily in vitro than in vivo. Microdensitometry was used to quantify this cortical difference in sections matched for similar optical density in striatum after 4 weeks of film exposure. The mean ratio of optical density in striatum to lamina IV was 4.8 in vivo and 1.3 in vitro. Labeling in the choroid plexus in vivo might be attributed to a high rate of delivery of SP to this tissue coupled with nonspecific binding. The significance of the hippocampal binding in vitro is not clear because it has been reported that it is not displaceable by either dopaminergic or serotonergic drugs. The fact that SP binding in cortical lamina IV was considerably less in vivo than in vitro, however, is very important as these sites have been reported to be solely serotonergic in nature (Palacios, J.M., et al, Brain Research, 213:277, 1981). This finding suggests that SP may be more selective for dopamine receptors in vivo than in vitro. The selective retention of SP at dopaminergic sites 2 hours after intravenous injection may be a reflection of the 3-4 fold higher in vitro dissociation rate constant for SP at serotonergic sites as compared to dopaminergic sites.

211.6 ^{3}H-SPIPERONE BINDING TO DOPAMINE-2 RECEPTORS IN AREA POSTREMA AND OTHER BRAINSTEM AREAS REGULATING EMESIS. N. W. Pedigo and K. R. Brizzee. Dept. Pharmacology, Louisiana State Univ. Med. Ctr., New Orleans, LA. 70112 and Neurobiology Lab, Delta Regional Primate Ctr., Covington, LA. 70433.

Dopamine receptor antagonists may exert their antiemetic effects via peripheral dopamine receptors, dopamine type-2(D2) receptors in the area postrema (AP) or D2 receptors in other brainstem areas regulating emesis. The purpose of these studies was to characterize D2 receptors in bovine AP, vagal nuclear complex (VNC), including nucleus tractus solatarius and dorsal motor nucleus of the vagus, reticular formation (RF), and vestibular complex (VC). Specific binding of ^{3}H-spiperone (^{3}H-S) to D2 receptors in brainstem tissue was defined at equilibrium (30 min at 37°C) using 10µM (+)butaclamol or chlorpromazine. The AP is rich in ^{3}H-S binding sites, measured at a saturating concentration of ligand (2.7nM), as is bovine VNC (572 ± 84 and 335 ± 37 fmol/mg protein, respectively). In comparison, bovine caudate nucleus has only 50% more binding (about 800 fmol/mg protein), while RF and VC have considerably fewer ^{3}H-S binding sites (167 ± 17 and 150 ± 26 fmol/mg protein, respectively).

In further experiments, the antiemetic drugs domperidone (DOM), chlorpromazine (CPZ)and thiethylperazine (THI) potently inhibit ^{3}H-S binding in AP with Ki values of 0.40, 0.51 and 0.98µM, respectively. Interestingly, CPZ and THI are each very effective in preventing motion-induced emesis in squirrel monkeys, whereas DOM is only partially effective. This indicates that CPZ and THI may exert additional antiemetic activity at D2 receptors in other brainstem areas, such as VNC, or through other neurotransmitter systems. For example, CPZ has significant anticholinergic activity, but THI and DOM are much weaker muscarinic blockers. Muscarinic cholinergic receptors are heterogeneously distributed in bovine brainstem (VNC>RF>VC), and are also found in AP. Hence, it is likely that the antiemetic activity of these drugs is due to a combination of antidopaminergic, antimuscarinic and, possibly, antihistaminergic effects mediated via specific brainstem nuclei and the area postrema. (Supported in part by the E. G. Schleider Foundation, NASA-Ames NAC-2-101 and NIH RR00164).

211.8 AUTORADIOGRAPHIC LOCALIZATION OF MUSCARINIC RECEPTOR AGONIST BINDING SITES USING [^{3}H]-CIS METHYLDIOXOLANE. J. K. Wamsley, W. R. Roeske* and H. I. Yamamura. Dept. of Psychiatry, University of Utah, Salt Lake City, UT 84132 and Dept. of Pharmacology, University of Arizona, Tucson, AZ 85724.

Displacement of radiolabeled muscarinic antagonists using muscarinic agonists, has been used to indirectly demonstrate muscarinic receptor heterogeneity (Birdsall et al., Mol. Pharmacol. 14:723-736, 1978). Microscopic localization of the high affinity vs. low affinity agonist sites has been accomplished using carbachol displacement of [^{3}H]-N methyl scopolamine binding. This study indicated the high affinity sites exist independently in many distinct brain regions (Wamsley et al., Brain Res. 200:1-12, 1980). Direct labeling of muscarinic receptors with a tritiated form of the agonist cis methyldioxolane ([^{3}H]-CD) had been attained in membrane homogenate preparations and demonstrate the specificity of this ligand for the muscarinic receptor (Ehlert et al., Life Sci. 26:961-967, 1980). We have labeled slide mounted tissue sections with [^{3}H]-CD in order to microscopically localize the binding sites for this compound using receptor autoradiographic techniques.

Slide mounted sections of rat brain were pre-incubated 20 minutes in ice cold 10mM sodium potassium phosphate buffer containing 200mM sucrose. This was followed by a 120 minute incubation period in the same buffer containing 5nM [^{3}H]-CD with a subsequent momentary rinse in fresh buffer. Nonspecific binding was represented on adjacent sections labeled using the same parameters with the added presence of 1 micromolar atropine. Labeled sections were rapidly dried, apposed to a sheet of LKB Ultrofilm in an X-ray cassette and allowed to expose the emulsion for several weeks. After development of the film, the receptor autoradiograms were analyzed by computer assisted microdensitometry. Muscarinic agonists characteristically show only small amounts of specific binding and this study was no exception. However, examination of the receptor autoradiograms showed the presence of highly specific labeling concentrated in many discrete regions of the brain. These include several lamina of the cerebral cortex (predominantly lamina IV), the diagonal band of Broca, anterior thalamic nuclei, olfactory tubercle, lateral posterior nucleus of the thalamus, facial nerve nucleus, nucleus tractus solitarius and the nucleus of the hypoglossal nerve. Thus, these regions represent the subpopulation of muscarinic receptors preferentially labeled by agonist (high affinity sites) as evidenced by direct labeling with [^{3}H]-CD.

211.9 IN VIVO MUSCARINIC RECEPTOR BINDING. K. A. Frey, R. L. Ehrenkaufer* and B. W. Agranoff. Neuroscience Lab and Division of Nuclear Medicine, University of Michigan, Ann Arbor, MI 48109.

The recent advent of in vitro autoradiographic methods for regional visualization of neurotransmitter receptors in animals has encouraged clinical neuroscientists to seek analogous receptor images in humans using emission computed tomography. We report here progress towards quantitative in vivo imaging of muscarinic receptors using [^{3}H]scopolamine in rats. [Methyl-^{3}H]scopolamine was synthesized by heating (-)norscopolamine with [^{3}H]methyl iodide. The product was purified by thin layer chromatography using tetrahydrofuran-diisopropylethylamine (95:5) as solvent. [^{3}H]Scopolamine was administered intravenously to rats using an infusion protocol calculated to yield constant arterial plasma concentrations. At various times following initiation of the infusion, animals were killed and samples of discrete brain regions were extracted with ethanol. Scopolamine concentration was quantitated following chromatography. Regional scopolamine uptake was greatest in the striatum, with lower values in cerebral cortex and hippocampus. Cerebellar uptake was still lower (cortex/cerebellum = 7:1 after 240 min of infusion). Binding was displaced by carrier scopolamine and by atropine, but not by methyl scopolamine. A model is presented which describes regional tracer uptake in terms of muscarinic receptor density, cerebral blood flow and capillary permeability, and endogenous neurotransmitter levels. Potential clinical applications of the method will be discussed. (Supported by NIH Grant NS 15655.)

211.10 LOCALIZATION OF MUSCARINIC ACETYLCHOLINE RECEPTORS IN CINGULATE CORTEX. Brent A. Vogt. Dept. of Anatomy, Boston University School of Medicine, Boston, MA 02118.

Cingulate cortex is considered to be a major component in the cholinergic limbic system because of its extensive acetylcholinesterase (AChE) activity. However, very little is known of the distribution of muscarinic acetylcholine receptors (AChR) in this cortex and nothing is known about their association with specific afferents and AChE-positive axons.

The distribution of AChR was assessed by determining the specific binding of ^{3}H-propylbenzilylcholine mustard (PrBCM; 2.4 nM) to cryostat sections of rat brains using standard autoradiographic techniques. Specific binding was defined as the total number of autoradiographic grains/2500 μm^2 minus the number of grains in control sections incubated in atropine (10^{-6} M) and PrBCM.

Binding of PrBCM throughout all layers of anterior area 24 was relatively even (67.7±8.7 grains/2500 μm^2). In contrast, area 29c of posterior cingulate cortex had particularly high levels of binding in layers Ia and IV (116±18.1 and 75±7.8,respectively), while the mean for all other layers was 54.9±9.4. Since these two layers of the rat area 29c receive most afferents from the anterior thalamic nuclei (ATN), the hypothesis was explored that these receptors are localized to the axons of this afferent.

Ablations of the ATN were made and PrBCM binding and AChE (Koelle method) assayed 2 days to 2 weeks postoperatively in serial sections. PrBCM binding was reduced 41% in layer Ia and 27% in layer IV, while no changes were apparent in other layers. Microdensitometric measurements indicated a loss of AChE activity also in layers Ia and IV. Since these losses appeared very soon after lesion placement (2-3 days) at a time when axons have begun to undergo morphologically distinct degeneration but are not yet pinched from the postsynaptic element, it is presumed that these changes are associated with axonal changes and possibly reduced transport of receptors from the ATN to area 29c.

Since undercut lesions also only reduce binding in layers Ia and IV, it is concluded that the heterogeneity of AChR binding in area 29c is due almost entirely to the ATN axonal receptors. All other binding, therefore, is intrinsic to cingulate cortex, i.e. nonsynaptic dendritic, postsynaptic and/or presynaptic to intrinsic neurons. Finally, the same axons that have AChR are also AChE-positive. Since lesions of the ATN do not alter activity of the synthetic enzyme choline acetyltransferase, it is proposed that these afferents may not be cholinergic. Rather, the release of an unknown transmitter from ATN afferents may be presynaptically modified by acetylcholine.Supported by grants NS 18745 and 16841.

211.11 EVIDENCE FOR A CHOLINERGIC INNERVATION TO FACIAL MOTONEURONS OF THE RAT. D. B. Hoover and J. C. Hancock. Dept. of Pharmacology, Quillen-Dishner College of Med., East Tenn. State Univ., Johnson City, TN 37614.

The facial nucleus (FN) is one of several cranial nerve nuclei reported to contain a high density of muscarinic receptors (Brain Res. Rev. 1: 167-183, 1979; J. Neurosci. 1: 176-191, 1981). Although it is recognized that efferent cholinergic neurons in the FN supply skeletal muscles of the face, the cellular localization and function of muscarinic receptors in this region are unknown. The aim of the present study was to determine whether these muscarinic receptors are associated with the motoneurons. Previous studies have demonstrated that cholinergic efferent neurons in the hypoglossal nucleus and dorsal motor nucleus of the vagus (J. Anat. 107: 197-208, 1970) lose acetylcholinesterase (AChE) in response to axotomy. This change presumably reflects a generalized deficiency in protein synthesis by the damaged neurons. Therefore, receptors normally synthesized by the efferent neurons should likewise be depleted after axotomy. Making this assumption, we examined the effect of facial nerve transection on the localization of AChE and [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding in rat FN.

Male Sprague Dawley rats were anesthetized with pentobarbital, and an incision was made candal to the external auditory meatus. The right facial nerve was isolated and cut in experimental animals. Fourteen days after surgery, animals were decapitated and their brains removed for analysis. Brain stems were frozen on specimen plates and transverse sections were cut for thionin staining, QBN autoradiography, and AChE histochemistry. Sections for autoradiography were incubated in 1 nM [^{3}H]QNB or 1 nM [^{3}H]QNB plus 1 µM atropine for 2 hr at room temperature. After 3 washes in cold PBS, the sections were dried and placed in x ray cassettes with LKB Ultrofilm. After a 4 week exposure period, the autoradiograms were developed and photographic prints made for analysis.

After sectioning the right facial nerve, there was a marked depletion of AChE from most cholinergic efferent neurons located in the ipsilateral FN. Although AChE staining was also decreased in the neuropil, numerous AChE-containing nerve fibers were still present. Specific binding of [^{3}H]QNB was decreased substantially in the ipsilateral FN after axotomy. These data indicate that the bulk of the muscarinic receptors in the FN are associated with the motoneurons. The persistence of AChE-containing nerve fibers in the neuropil of the ipsilateral FN is consistent with the motoneurons receiving a cholinergic innervation.

211.12 EFFECT OF CERVICAL VAGOTOMY ON [^{3}H]QUINUCLIDINYL BENZILATE BINDING, CHOLINE ACETYLTRANSFERASE AND ACETYCHOLINESTERASE IN VAGAL NUCLEI. J. C. Hancock and D. B. Hoover. Dept. of Pharmacology, Quillen-Dishner College of Med., East Tenn. State Univ., Johnson City, TN 37614

The dorsal motor nucleus of the vagus (DNV) and the nucleus ambiguus (NA) contribute to the parasympathetic innervation of the heart and other viscera. Cholinergic neurons and neuropil in these regions stain intensely for cholinesterases (ChEs). Recent autoradiographic studies have shown a high density of muscarinic receptors in NA and low to moderate density in DNV suggesting the presence of cholinergic synapses. In the present study, we examined the effect of cervical vagotomy on muscarinic receptors and choline acetyltransferase (ChAT) in vagal nuclei. An earlier study demonstrated that ChEs are depleted from ipsilateral DNV and NA by unilateral cervical vagotomy (J. Anat. 107: 197-208, 1970). Based on this finding, we expected muscarinic receptors and ChAT associated with vagal efferent neurons would decrease in response to axotomy.

Male Sprague Dawley rats were anesthetized with pentobarbital for right cervical vagotomy or control surgery (neck incision). ChAT activity and autoradiographic localization of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) binding were examined at 14 days after surgery. To label muscarinic receptors, transverse sections of medulla oblongata were incubated in 1 nM [^{3}H]QNB in PBS for 2 hr at room termperature. After several washes in cold PBS, sections were dried, and the localization of label was determined with LKB Ultrofilm. Photographic prints were made from autoradiograms and evaluated in a single blind fashion by two persons. Another series of brains were used for analysis of ChAT activity. Alternate 50 µm and 300 µm sections were cut through the medulla. The 50 µm sections were stained for acetylcholinesterase (AChE) and used to aid in micropunching 300 µm sections for subsequent ChAT determination.

Right vagotomy caused a 52% decrease in ChAT in the ipsilateral DNV/n. tractus solitarius complex and a 42% decrease in the rostral pole of NA. No significant changes in ChAT occured at other levels of NA or in the hypoglossal nucleus. In adjacent sections, AChE was depleted in ipsilateral DNV and rostral pole of ipsilateral NA. Therefore, both enzymes made by vagal efferent neurons are decreased after vagotomy. In contrast, no difference in localization of QNB binding was seen in vagal nuclei of experimental vs control brains. This data suggests muscarinic receptors in these medullary nuclei are not located on efferent cholinergic neurons.

211.13 TOPOCHEMICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IN MOUSE BRAIN, M.N. Gordon* and C.E. Finch (SPON: P.S. Gott). Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089-0191.

The specific activity of choline acetyltransferase (CAT) was measured at neuroanatomically defined loci using the Palkovits punch technique. Young female C57BL/6J mice were ovariectomized 4-7 d before sacrifice. Unfixed frozen brains were sectioned (200 µm) in a cryostat and 1 mm diameter punches were collected from nuclei in the basal forebrain and hypothalamus. Highest activity was seen in the corpus striatum (161±12, n=9), followed by rostral areas of the diagonal band of Broca (88.9±16.4, n=5), the magnocellularis area of the preoptic area (65.6±4.9, n=23) and the n. accumbens (45.8±6.4, n=3). Moderate activities were seen in the medial septal n. (21.7±3.2, n=13) and the bed n. of the stria terminalis (15.7±1.8, n=15). Hypothalamic regions had the lowest CAT specific activity of the areas examined (suprachiasmatic n., 12.4±1.8, n=16; median eminence-arcuate n., 10.9±1.8, n=15; medial preoptic area, 4.53±0.63, n=23; ventromedial n, 4.47±0.27, n=6).

The areas of high specific activity were quite circumscribed. For example, at the level of the preoptic area, lateral punches (magnocellularis area) have high activity (65.6±4.9, n=23), while medial punches less than 1 mm away have 14-fold lower specific activity (4.53±0.63, n=23). In addition, the diagonal band showed a profound rostro-caudal gradient of CAT specific activity. Rostral diagonal band (1.0 mm anterior to bregma) showed highest specific activity (88.9±16.4, n=5). CAT activity then decreased approximately linearly in more caudal sections, decreasing to 22% of anterior values by 0.5 mm anterior to bregma (specific activity = 19.9±3.5, n=5). However, there was no evidence for a rostro-caudal gradient of CAT specific activity in the corpus striatum as has been reported for the rat (Strong et al, J. Neurochem 39:831, 1982).

Since all areas of the basal forebrain examined here have CAT-containing cell bodies, differences in CAT specific activity may reflect the relative density of cholinergic cells in these nuclei.

Subcutaneous injections of 17β-estradiol (at doses up to 0.1 ug/g body weight/day for 7 days) had no effect on CAT specific activity in any nucleus examined here.

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211.14 ANTIBODIES THAT DISTINGUISH BETWEEN MOLECULAR FORMS OF AChE LOCALIZE DIFFERENTIALLY ON TORPEDO ELECTROCYTES. L. B. Howland*, M. H. Ellisman, P. Taylor*, T.J. Deerinck*, B.P. Doctor* and S.J. Camp* (SPON: J.H. Brown). Department of Neurosciences, Laboratory for Neurocytology and Department of Medicine, University of California, San Diego, La Jolla, CA 92093.

Distinct molecular forms of AChE have been isolated from Torpedo electric organs and characterized biochemically. These include an asymmetric "tailed" species (17S+13S) and a dimeric hydrophobic species (5.6S). Monoclonal antibodies (MCAb) were raised against Torpedo AChE and have been characterized for antigenic specificity. Most antibodies show a high degree of cross reactivity between the different molecular forms of AChE, but two demonstrate selectivity for only one form. MCAb 4F3 is specific for the asymmetric species (and is unreactive towards this form of the enzyme following collagenase or trypsin removal of the tail section). MCAb 4E7 exhibits 100-fold selectivity for the catalytic subunit of the 5.6S hydrophobic dimeric form of AChE. Using these MCAbs as probes, we were able to examine the distribution of the different forms of AChE in Torpedo electric organ by immunofluorescence and immunoelectron microscopy. MCAb 4E7 shows a diffuse distribution, staining within the synapse giving the appearance of labeling both the innervated and non-innervated surfaces with approximately equal density. These patterns were confirmed using immunoelectron microscopy. In contrast, polyclonal antibodies raised against the asymmetric form bind both asymmetric and hydrophobic forms with equal avidity in vitro. Although these polyclonal antibodies do not appear to distinguish between molecular forms in vitro, the antigenic sites they localize in situ are less uniformly distributed than those localized by the monoclonal antibody directed against the hydrophobic dimer only. Localization by both light microscopy and electron microscopy reveal the same pattern of staining. (Supported by research grants from the NIH #NS14718 to M.H.E., #GM1860 to P.T.; NMSS to M.H.E.; MDA to M.H.E. and P.T. and predoctoral fellowship support for L.B.H. from USPHS GM07752).

211.15 LAMINAR DISTRIBUTION OF NEUROTRANSMITTERS IN THE RAT VISUAL CORTEX. S. G. Speciale, J. K. McDonald, and J. G. Parnavelas. Depts. of Psychiatry and Physiology, UTHSCD, Dallas, TX 75235, USA and Dept. of Anatomy, Univ. College, London, England.

The mammalian visual cortex (VC) contains a variety of putative neurotransmitters, such as the monoamines (norepinephrine (NE) and serotonin (5HT)), GABA, acetylcholine, asparate, glutamate, and a number of peptides (Parnavelas and McDonald, 1983). With the exception of some established functional properties for NE and GABA, little is known about the function of other putative neurotransmitters in the VC. In the present study, we have examined the laminar localization of NE, 5HT, and choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD), synthetic enzymes and markers for acetylcholine and GABA, respectively, in rat VC. It has been speculated that cortical lamination might have functional significance.

Albino rats were decapitated, the brains rapidly removed, and VC blocked out by razor cuts and frozen on dry ice. The limits of each layer were established from stained coronal sections of VC from animals of the same age. Monoamines were analyzed by HPLC-EC, while ChAT and GAD were measured by radiometric assays (Fonnum, 1975). Tissue concentrations of enzyme activities were calculated on the basis of protein content.

NE concentrations were higher in layers I, II&III, and IV, and lowest in layer V (differing significantly from layers IV and VI, $P<0.05$). 5HT concentrations were highest in layer I and exhibited a gradient, being lowest in layers V and VI (almost 50% that in layers II&III). The 5HT metabolite, 5-hydroxyindoleacetic acid, an index of 5HT neuronal activity, was found in similar concentrations in layers I-V, but was elevated in layer VI, about 50% over the upper layers. Dopamine or its metabolite, dihydroxyphenylacetic acid, was not detectable.

ChAT activity was maximal in layer V, followed closely by layers I, II&III, IV and VI. GAD activity was highest in layer IV, where the GAD immunocytochemically reactive non-pyramidal neurons are concentrated, and tapers off slightly in the layers above and below. In a related study, we examined the development of the laminar enzymatic activity, which was clearly detectable in 8 day and older animals. Both enzymes showed a rapid phase of increased activity between days 8 and 16. GAD activity reached adult-like levels by day 24 in all layers, except layer IV, which showed a continued increase to adult levels. As for ChAT activity, it demonstrated a steady increase in all layers to the adult activity from day 16 onward. The functional role of the differences in the laminar distribution of these neurotransmitters remains for further study.

211.16 LOCALIZATION OF ALPHA$_1$-ADRENERGIC RECEPTORS IN HUMAN CEREBRAL CORTEX. R. T. McCabe*, S. S. Stensaas and J. K. Wamsley. Depts. of Psychiatry, Anatomy, Pharmacology, and Pathology, Univ. of Utah, Salt Lake City, Utah 84132.

Alpha$_1$-adrenergic receptors have been autoradiographically localized in rat cerebral cortex using (^{3}H)-WB4101 as a ligand (Young and Kuhar, Proc. Natl. Acad. Sci. 77:1696, 1980). A fairly unremarkable distribution of these sites was shown within the laminae of individual cortical regions. (^{3}H)-prazosin (New England Nuclear; Boston, MA), a more selective ligand for alpha$_1$-adrenergic receptors, has been used for labeling membrane homogenate preparations (Miach et al., N.S. Arch. Pharmacol. 312:23, 1980). Unnerstall and Kuhar (personal communication) have autoradiographically localized alpha$_1$-adrenergic receptors in particular areas of rat brain. Their procedures have been used to label alpha$_1$-adrenergic receptors with (^{3}H)-prazosin in sections of postmortem human cerebral cortex in order to determine their existence and laminar distribution in different cortical areas.

Several cortical regions were obtained at autopsy from 7 adult male patients. The tissue was rapidly frozen on dry ice and individual sections were cut in a cryostat and placed on microscope slides. Tissue sections were labeled by incubating them at room temp. for 60 min. in 0.17M Tris-HCl buffer, pH 7.4, containing 1nM (^{3}H)-prazosin followed by a 1 min. rinse in fresh buffer (4°C). Areas of nonspecific binding were localized by incubating adjacent sections in media containing 10^{-5}M phentolamine to inhibit the specific binding of the radiolabeled ligand. All tissue sections were rapidly dried and apposed to sheets of LKB Ultrofilm for 5½ months. After this exposure period, the film was developed and examined using computer assisted microdensitometry.

Alpha$_1$-adrenergic receptors were unevenly distributed in each cortical region examined. The highest density of receptors was observed in lamina I of the striate cortex and pre- and postcentral gyri, with a 10% decrease of receptor density in lamina VI when compared with lamina I. Intermediate levels of receptor density were found in laminae II-V of these areas. Superior temporal gyrus, in comparison, had slightly lower specific binding in laminae I and VI, with a marked lack of labeling in laminae IV and V. Cingulate and parahippocampal cortex differ from these areas by having homogeneous labeling throughout all layers.

Thus, alpha$_1$-adrenergic receptors are differentially distributed in regions of the human cortex. Coupling these observations with those of alpha$_2$- and beta-adrenergic receptors should provide insight into the complicated sites of adrenergic action in the cortex.

211.17 DIFFERENTIAL DEVELOPMENT IN THE RAT OF MU$_1$ AND MU$_2$ BINDING SITES AS MEASURED BY QUANTITATIVE AUTORADIOGRAPHY. J. Kent*, L. Recht*, E. Hiesiger* and G.W. Pasternak. Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021.

Previous studies have shown that the high affinity (mu$_1$) opioid binding sites had a different developmental appearance than the lower affinity binding sites of ^{3}H-dihydromorphine (DHM; mu$_2$) or ^{3}H-D-ala-D-leu-enkephalin (DADL; delta; Zhang and Pasternak, Eur J Pharmacol 73: 29, 1981) as well as a different regional distibution (Zhang and Pasternak, Eur J Pharmacol 67: 323, 1980). In an effort to examine the regional development of mu$_1$ and mu$_2$ sites, we utilized quantitative autoradiography (QAR) to measure the density of opiate receptors in rats 2 days old and adults using a fixed concentration of ^{3}H-DHM (1 nM). Previous studies have shown that ^{3}H-DHM alone bound to both mu$_1$ and mu$_2$ sites and unlabeled DADL at 1 nM displaced the binding to the mu$_1$ sites selectively (Wolozin and Pasternak, PNAS 78: 6181, 1981). Therefore, sections (10 microns) were incubated with ^{3}H-dihydromorphine alone or with ^{3}H-DHM and 1 nM DADL. Nonspecific binding was determined using levallorphan (1000 nM). Tissue sections were exposed to LKB Ultrafilm for 3 months. The images were then developed and digitized on a PDP11/23 computer. Optical densities were determined from the computerized images. In brief, mu$_1$ binding appeared to develop later and differently from mu$_2$ binding. This is best illustrated in the medial thalamus. In 2 day old rats, 1 nM DADL displaced only approximately 4-5 % of the specific ^{3}H-DHM binding. By contrast, in adult rats, the same concentration of DADL displaced ^{3}H-DHM binding by approximately 80 %. These studies support the concept of differences in the developmental appearance of mu$_1$ and mu$_2$ binding sites.

211.18 AN IMMUNOCYTOCHEMICAL STUDY OF THE SEROTONERGIC INNERVATION OF THE TRIGEMINAL NUCLEAR COMPLEX IN THE RAT. E. C. Cropper*, J. S. Eisenman, and E. Azmitia. Depts. of Physiology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029.

Serotonin has been implicated in the modulation of information through various motor and sensory nuclei, particularly in those sensory nuclei which receive inputs from small diameter afferents. This study describes the distribution of serotonin immunoreactive fibers in the trigeminal nuclear complex, which includes the motor nucleus and sensory nuclei that receive inputs from both large and small diameter afferents.

Animals were pretreated with pargyline (200 mg/kg, IP) and tryptophan (200 mg/kg, IP)and perfused intracardially with a 0.1 M phosphate buffer solution containing 4% paraformaldehyde, 3% sucrose, and 0.05% magnesium sulfate. Brains were postfixed overnight and sections cut in both the coronal and horizontal planes. Tissue was processed for immunocytochemical staining using a modification of Sternberger's peroxidase-antiperoxidase (PAP) technique. The serotonin antiserum was a generous gift of Dr. J. Lauder.

The marginal and gelatinosa layers of the spinal subnucleus caudalis were found to be densely innervated while the magnocellular portion of caudalis contained few immunoreactive fibers. Innervation of most of the subnuclei oralis and interpolaris was sparse although occasional patches of dense innervation were present in both nuclei; oralis appeared to contain more labeled fibers than interpolaris. Little immunoreactivity was observed in the main sensory nucleus. The motor nucleus was as densely innervated as the marginal and gelatinosa layers of caudalis, and when compared to the caudalis innervation, the fibers in the motor nucleus appeared thicker and less varicose.

In conclusion, labeling within the sensory nuclei of the trigeminal complex is dense only in the spinal subnucleus caudalis, i.e., only in an area which receives small diameter afferents and projects to the posterior thalamus. The main sensory nucleus, which receives large diameter afferents and projects to the VB complex of the thalamus, contains few labeled fibers. This suggests that serotonergic modulation of somatosensory transmission in the trigeminal nuclear complex occurs primarily on activity related to small diameter afferent input. The motor nucleus is densely innervated as are other motor nuclei of the brain stem and the ventral horn of the spinal cord.

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211.19 QUANTIFICATION OF MICROAUTORADIOGRAMS BY SINGLE BIT PLANE IMAGE ANALYSIS. R. A. Pearlstein*, J. E. Simons*, W. F. White, S. O'Gorman*, L. J. Regan* and R. L. Sidman. Image Graphics Laboratory, Dept. of Neuroscience, Children's Hospital and Dept. of Neuropathology, Harvard Medical School, Boston, MA 02115.

Autoradiographic techniques are applicable to problems ranging from measurement of metabolic activity, to fiber tract and synaptic field tracing, cell birthday determinations, and neurotransmitter receptor or other ligand binding site localization. A general solution is proposed for the rapid, accurate quantification of autoradiograms using an automated computer analysis system. The components of the system consist of a three-axis computer-controlled brightfield light microscope, 100X neofluar objective, Chalnicon instrumentation video camera, a single bitplane image analyzer and analog thresholding front end (Omnicon 3000, Bausch & Lomb, Rochester, N.Y.). Stage motion in the X,Y plane, focus, image acquisition and analysis are all under operator-independent computer control. Grain and tissue layers are separated by optical sectioning and counterstain-matching wavelength filters. An analog video thresholding algorithm mediates uniform grain detection by sensing peak height of the video waveform; the binary image of grain features is determined by a constant threshold below this peak height. Small but critical changes in grain layer focal plane are compensated by an autofocus technique which operates to maximize the number of features in the binary image, a condition coincident with true focus. Grain number is derived from measurements of the total area occupied by detected features in the binary image. We have verified repeatedly that a linear relationship exists between grain number and detectable feature area at the constant threshold. Specific activity of radionuclide label is determined by preparation of a stepwedge standard: analysis of grain number in the stepwedge is used to convert feature area directly to disintegrations/minute/dry weight. Counts or discrete specific activities are written to a pen plotter as hidden-line supressed three axis maps in which modulation of the Z-axis expresses count/activity level. A version of this autoradiography program now in preparation will allow quantitative radioactivity levels in a given set of serial histological sections to be spatially represented in arbitrary colors superimposed directly onto reconstructed three-dimensional images of the same sections.

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211.20 AUTORADIOGRAPHIC LOCALIZATION OF GLYCINE RECEPTORS IN SPASTIC AND CONTROL MICE USING A COMPUTERIZED SINGLE BIT PLANE IMAGE ANALYSIS SYSTEM. W.F. White and L.J. Regan*. Dept. of Neuroscience, Children's Hospital, and Neuropathology, Harvard Medical School, Boston, MA, 02115.

The mutant mouse spastic has behavioral and electromyographic abnormalities which suggest a decrease in inhibitory neurotransmitter function. On the basis of both pharmacologic and receptor binding studies this decrease appears to involve inhibition mediated by glycine. Previous studies using receptor binding techniques have demonstrated an 80-90% decrease in the specific binding of ^{3}H-strychnine, a ligand for the postsynaptic glycine receptor, to the spinal cord, brainstem, and midbrain of spastic compared with littermate control mice. This decrease could result from a highly localized decrease in ^{3}H-strychnine binding sites with other areas unaffected, or it could result from a more generalized decrease ^{3}H-strychnine binding sites with all areas affected. In an effort to test these two possibilities, autoradiograms of ^{3}H-strychnine binding sites were prepared from spastic and control lumbar, thoracic, and cervical spinal cord, brainstem, and midbrain using diffusable substance autoradiographic techniques. Background binding was defined in adjacent sections using either 10 mM glycine or 100 uM strychnine to displace specific binding. Analyses of these autoradiograms were performed using the procedures described in "Quantification of microautoradiograms by single bit plane image analysis" (this meeting). Receptor localization in fmoles bound per mg dry weight was determined from two dimensional plots of area occupied by silver grains. High receptor densities were seen in the gray matter of spinal cord and specific brainstem and midbrain nuclei. Receptor densities were significantly lower in all regions of spastic spinal cord, brainstem, and midbrain suggesting that the decrease in glycine receptors is not specifically localized.

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PINEAL GLAND

212.1 DECREASE IN PINEAL MELATONIN CONTENT DURING HIBERNATION IN THE GROUND SQUIRREL, CITELLUS LATERALIS. T. L. Stanton, C. M. Craft and R.J. Reiter. A. I. duPont Inst., Wilmington, DE 19899 and Dept. Anat., Univ. Texas Health Sci. Ctr., San Antonio, TX 78248.

Investigations of the effects of pinealectomy or melatonin administration on the incidence and duration of torpor in hibernating mammals have produced inconclusive results. In this study, the role of the pineal gland in modulating the obligatory hibernation bouts of the golden-mantled ground squirrel was explored by comparing pineal melatonin content in hibernating animals with that of euthermic (not hibernating) animals at the same time of year (early February).

Euthermic animals were housed individually in a colony room under natural light conditions. Hibernating animals were housed at 4°C in dim light (.02-.35 fc) on a 12:12 light:dark schedule. Hibernating animals were divided into two groups in order to look for differences between animals who were in the first day of their respective bouts (early bout = EB) and animals who were near the end of their respective bouts just prior to expected spontaneous arousal (late bout = LB). Individual bout periods, averaging 5-15 days across animals but relatively constant within each animal, were separated by short intervals (less than 24 hrs.) of spontaneous euthermia. All animals were sacrificed between 1400-1600 h. Melatonin was measured in duplicate aliquots by radioimmunoassay in a single assay. The data were analyzed by one-way analysis of variance and Duncan's multiple comparison.

The results showed that pineal melatonin decreased significantly during hibernation. Mean melatonin content (pg./pineal ± SEM) was 560.7 ± 95.7 for euthermic animals (n=5), 351.7 ± 51.3 for EB hibernators (n=6), and 167.0 ± 34.0 for LB hibernators (n=6). Pineal melatonin was significantly higher in euthermic animals compared to EB hibernators ($p < .05$) and LB hibernators ($p < .01$). The decrease in pineal melatonin from the EB to LB period of hibernation was also significant ($p < .05$). Of particular interest was the relationship between individual bout length and pineal melatonin content, wherein animals who exhibited longer bout lengths had higher melatonin values. An examination of this relationship revealed a correlation coefficient of .89 for EB hibernators and .96 for LB hibernators. When bout length was introduced as a covariate, the significant difference between EB and LB hibernation groups became even greater ($F=39.5$; $p<.000001$).

The demonstration that melatonin content of the pineal changes as a function of hibernation provides evidence that the pineal gland may play a role in the cyclic hibernatory behavior of this species. The strong correlation between bout length and melatonin content further suggests that pineal melatonin may be important in determining the duration of individual hibernation bouts. (Supported by NSF grant PCM-8003441 to R.J.R. and A.I. duPont Inst.)

212.2 THE EFFECTS OF INSULIN INJECTIONS ON THE PINEAL MELATONIN SYNTHETIC PATHWAY IN EXPERIMENTALLY-INDUCED DIABETIC AND NON-DIABETIC MALE SYRIAN HAMSTERS. T. H. Champney, A. P. Holtorf* and R. J. Reiter. Dept. Anatomy, Univ. of Texas Health Sci. Ctr., San Antonio, TX 78284.

Decreases in nocturnal pineal melatonin content have been shown previously in Syrian hamsters rendered diabetic by injection of streptozotocin or alloxan. This study examined the effect of insulin injections on pineal serotonin (5HT), N-acetylserotonin (NAS) and melatonin content in diabetic and control hamsters. Pineal serotonin N-acetyltransferase (NAT) activity and hydroxyindole-O-methyltransferase (HIOMT) activity were also determined. Male, Syrian hamsters were maintained under a 14L:10D photoperiod (lights on at 0600h) with food and water available ad libitum. They were injected with either alloxan monohydrate (65 mg/kg in saline, i.v.) or streptozotocin (65 mg/kg in 0.05M citrate buffer, pH 4.5, i.p.). Eleven alloxan-injected animals and 11 non-diabetic animals were injected daily at 1600h for 3 days with insulin (2 IU/kg in saline, s.c.). Ten control hamsters were injected with citrate buffer (i.p.) on the first day and with saline (s.c.) for 3 days. On the fourth day, the hamsters were killed at 0400h. Pineals and trunk blood samples were collected. Blood glucose levels were determined by a colorimetric, enzymatic method (Sigma kit #510). Hamsters with blood glucose values over 300 mg/100 ml were considered diabetic as compared to control values of 100 mg/100 ml. Pineal levels of 5HT and NAS were measured by high performance liquid chromatography. Melatonin content was measured by radioimmunoassay. NAT activity and HIOMT activity were measured by radioenzymatic microassay. All of the above parameters were measured within the same pineal gland. Pineal NAS was not detectable in any of the groups. Pineal melatonin content was significantly decreased ($p<0.05$) in both the alloxan and streptozotocin-induced diabetic groups. Insulin injections in alloxan-diabetic animals had normal melatonin values, while insulin injections in control animals had an apparent, but not significant, increase in pineal melatonin content. Pineal 5HT content was significantly depressed ($p<0.05$) in the alloxan and streptozotocin-diabetic groups and in the group injected with insulin alone. Alloxan-diabetic hamsters injected with insulin had normal pineal 5HT levels. Neither NAT activity nor HIOMT activity were significantly altered by experimentally-induced diabetes or insulin injections. This study confirmed that experimentally-induced diabetes reduces pineal 5HT and melatonin content and that insulin injections restored those values to normal. It appears that diabetes alters substrate availability in melatonin synthesis and not enzyme activity. Further studies are planned to examine this effect in detail. (Supported by NSF Grant No. PCM 8003441.)

212.3 AGING DIFFERENCES IN ALPHA-ADRENOCEPTOR MEDIATED EFFECTS ON RAT PINEAL GLAND N-ACETYLTRANSFERASE ACTIVITY. Robert L. Terry*, David M. Bronstein*, Thomas Motroni*, and Loy D. Lytle (SPON: H.J. Carlisle). Laboratory of Psychopharmacology, Department of Psychology, University of California, Santa Barbara, CA 93106.

The activity of the pineal gland enzyme, N-acetyltransferase (NAT), is controlled at least in part by the action of sympathetic nerve terminal release of norepinephrine (NE) onto pinealocyte beta-adrenoceptors. Recent evidence suggests that postsynaptic alpha-adrenoceptors may also be involved in NE-induced changes in NAT activity in pineal gland organ cultures (Klein et al., Proc. Nat. Acad. Sci. 80:599, 1983) and in pineal cell cultures (Rowe, V. and Parr, J., J. Pharmacol. Exp. Ther. 218:97, 1981).

To assess possible age-related differences in alpha-adrenoceptor mediated effects on pineal gland NAT activity, we incubated pineal glands taken from rats at different developmental time periods (fetal, neonatal, adolescent, or adulthood) in vitro with different concentrations of the alpha-adrenoceptor agonist drug phenylephrine or with the alpha-adrenoceptor antagonist drug prazosin. Following a 4 hr incubation period, the glands were removed and assayed for NAT activity by a modification of the radiometric assay of Deguchi and Axelrod (J. Analyt. Biochem. 50:174, 1972) utilizing the accumulation of N-(^{3}H-acetyl)-tryptamine as a measure of enzyme activity.

Incubation of fetal (19 days post-conception) or adult (50 days old) pineal glands with phenylephrine increased NAT activity in a concentration-dependent manner. Fetal glands required approximately 10-fold greater concentrations of the agonist compared to adult glands in order to achieve significant elevations above basal levels of NAT activity. Phenylephrine also significantly increased NAT activity in pineal glands obtained from neonatal (2, 5, or 10 days postnatal) or adolescent (25 days postnatal) animals. The concentrations of agonist necessary to elevate NAT activity in pineal glands at all ages were between 1-10 μM, doses which may be in excess of those required for selective alpha-adrenoceptor activation. However, we also found that NAT activities in pineal glands taken from neonatal animals are decreased by approximately 70% following their incubations with 10 μM of the highly selective alpha-adrenoceptor antagonist drug prazosin. These and other data suggest that alpha-adrenoceptors may be functionally important in pineal glands throughout development, and also support the notion that these receptors may normally mediate some of the effects of NE and/or other catecholamines involved in the control of pineal gland hormone synthesis. (Supported in part by NIMH grant MH-31134).

212.4 MELATONIN BINDING IN BRAIN MEMBRANES AND EFFECTS ON ADENYLATE CYCLASE, L.P. Niles. Department of Neurosciences, Faculty of Health Sciences, McMaster University, Hamilton, Ontario L8N 3Z5 Canada.

Considerable evidence indicates that the pineal hormone, melatonin, influences brain and endocrine function, however, the underlying mechanisms remain obscure. Binding studies with (^{3}H) melatonin have shown the presence of high affinity binding sites in membrane (Vacas and Cardinali, Neurosci. Lett. 15: 259, 1979) and cytosol (Niles et al., Eur. J. Pharmacol. 55: 219, 1979) fractions from rat brain. Nonetheless, a detailed characterization of binding has been hampered by the instability and poor quality of the radioligand.

Recently, high levels of (^{3}H) melatonin binding have been detected in rat brain membranes subjected to multiple washings. While the binding affinity (K_d=15-20 nM) is comparable to that previously reported, the binding site concentration (B_{max}=1200 - 1400 fmol/mg protein) significantly exceeds that previously observed in either particulate or soluble brain fractions. Regional studies indicate that binding occurs in membranes from diverse CNS areas with the hypothalamus being particularly enriched in binding sites. The pharmacologic features of binding indicate that the 5-methoxy substituent is necessary for high affinity binding.

Preliminary studies of melatonin's effects on adenylate cyclase activity in rat brain and endocrine organ homogenates indicate that it produces a GTP-dependent inhibition of enzyme activity in the rat hypothalamus and pineal gland. These findings suggest that the hypothalamus is a primary target for melatonin whose neuroendocrine effects may involve modulation of cyclic-AMP-dependent mechanisms in this brain region. Moreover, melatonin's inhibitory effect on pineal adenylate cyclase activity suggests that this indoleamine plays a role in regulating pineal function and supports the hypothesis (Niles et al., Int. J. Immunopharmacol. 1: 213, 1979) that melatonin may inhibit its own synthesis via a negative feedback system.

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212.5 THE EFFECTS OF MELATONIN ADMINISTRATON ON HUMAN MOOD AND PERFORMANCE. Harris R. Lieberman, Franz Waldhauser*
Gail S. Garfield* and Richard J. Wurtman.
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It has been suggested that the hormone melatonin, which is released at night by the human pineal gland, has hypnotic properties. Also it has recently been established that the normal nocturnal secretion of this hormone in man can be suppressed by presentation of sufficiently bright light. In spite of these unique properties few investigations of the effects of this hormone on human mood and behavior have been conducted. We therefore investigated the acute effects of oral administration of melatonin on human mood, performance, visual sensitivity and memory. Fourteen healthy male subjects participated in this double-blind placebo controlled study. The subjects received a total of 240 mg. of melatonin, a pharmacological dose, in three 80 mg. capsules over a 2 hour period (12:00 noon to 2:00 p.m.). For 2 hours (10:00 a.m. to 12:00 noon) immediately preceding the initial ingestion of melatonin the subjects were given a battery of psychological tests. Performance was assessed with a simple auditory reaction time (RT) task, four-choice visual RT, a test of sustained motor performance using a grooved pegboard, and the Digit Symbol Substitution Test. Certain aspects of memory and visual sensitivity were also evaluated. All of these tests were then repeated in the same order after melatonin administration (2:00 p.m. to 4:00 p.m. Two self-report mood questionnaries, the Profile of Mood States (POMS) and the Stanford Sleepiness Scale (SSS) were administered every hour.

Melatonin had significant effects on mood and performance but did not alter memory or visual sensitivity. Subjects reported significantly more Fatigue on this scale of the POMS at 3:00 PM after melatonin administration compared to placebo. The Vigor scale of the POMS and the SSS also detected significant hypnotic-like effects of melatonin at 3:00 p.m. and also 4:00 p.m. By 5:00 p.m. their were no significant differences between melatonin and placebo sessions. Two of the performance tests were also significantly modified by melatonin administration in a manner consistent with the hypnotic properties of melatonin detected by the mood questionnaires we administered.

It is concluded that melatonin, administerd orally, has definite hypnotic properties affecting both human self-reported mood and performance. The duration of melatonin's effects were brief even though it was administered over a two hour period of time. (Supported by NIH grant 2RO1-HD11722-04 and NASA grant NAG2-132)

212.6 DEVELOPMENTAL CHANGES IN SYMPATHETIC NERVOUS SYSTEM MECHANISMS FOR THE CONTROL OF RAT PINEAL GLAND N-ACETYLTRANSFERASE ACTIVITY. Loy D. Lytle, Robert Terry*, David Bronstein*, and Anthony Altar*. University of California, Department of Psychology, Laboratory of Psychopharmacology, Santa Barbara, CA 93106.

Manipulations increasing norepinephrine (NE) neurotransmitter release from the postganglionic sympathetic neurons innervating the pineal gland enhance the synthesis and release of the pineal gland hormone, melatonin, by increasing the activity of N-acetyltransferase (NAT), the enzyme which apparently rate-limits overall synthesis of the hormone. The enzyme increases are mediated by release of NE from sympathetic nerve terminals onto pinealocyte β-adrenoceptors, which activates a receptor-linked adenylate cyclase system, causing increases in cyclic AMP concentrations leading to the synthesis of new enzyme protein or to an activation of existing enzyme material (Minneman and Wurtman, 1976). The present experiments were undertaken to characterize the approximate time during development when drugs known to alter pre- or post-synaptic noradrenergic neurotransmission, adrenoceptor, or cyclic AMP mechanisms might reach functional maturation.

Fetal (19 or 20 days post-conception), neonatal (0, 2, 5, or 10 days old), adolescent (25 days old), or adult (50 days old) male or female albino rats were killed during the light portion of the day:night cycle. Their pineal glands were incubated *in vitro* for a 4 hr period in the presence of the 10^{-3} N HCl vehicle or with a single dose of a drug known to enhance noradrenergic neurotransmission by acting presynaptically (l-dopa, amphetamine, veratridine, pargyline, or desmethylimipramine), by acting as an agonist at the pinealocyte postsynaptic β-adrenoceptor (epinephrine, norepinephrine, isoproterenol, terbutaline, dobutamine), or by acting to increase intracellular pinealocyte concentrations of cyclic AMP (isobutylmethylxanthine or dibutyryl cyclic AMP). *In vitro* pineal gland NAT activity was quite low in fetal rats, increased dramatically between birth and the 10th postnatal day, and then declined to adult values by 25 days of age. None of the presynaptically acting drugs tested produced any changes in pineal gland NAT activity in fetal animals but each did so in all postnatal animals tested, with maximal drug enhancement of enzyme activity observed between 5-10 days of age. In contrast, the postsynaptic receptor agonist drugs and those which increased cyclic AMP caused significant elevations in pineal gland NAT activity in fetal as well as in all postnatal animals examined. Taken together, these results indicate that presynaptic mechanisms important for the control of pineal gland NAT activity may not become functionally mature until the time of birth. In contrast, postsynaptic receptors or the receptor-linked adenylate cyclase system appear to function at a time during development when presynaptic mechanisms are still immature.

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212.7 A NEURAL SITE OF ACTION FOR PINEAL DEPENDENT PHOTOPERIODIC GONADAL RESPONSES IN THE GOLDEN HAMSTER. A. Roberts, M. Hastings and J. Herbert*(SPON: European Neuroscience Association). Department of Anatomy, University of Cambridge, Cambridge, UK.

This study reports the identification of a site in the anterior hypothalamus (AHA), destruction of which prevents the photo-periodic response to short photoperiods although such lesions do not interrupt normal circadian or estrous functions.

In the first experiment, adult female golden hamsters maintained in long photoperiod (LD 16:8) received bilateral infusions into the anterior hypothalamus of the selective neurotoxin n-methyl aspartate (NMA, 15 µg in 1 µL) or control vehicle. Histological analysis identified areas of neuronal destruction within the anterior hypothalamic area although the paraventricular, supraoptic and suprachiasmatic nuclei and fibers of passage were spared. Following transfer to short daylengths (LD 8:16), unlesioned vehicle-infused animals showed the expected gonadal regression after 6 - 8 weeks. In contrast, lesioned animals failed to show a photoperiodic response and maintained gonadal activity during long-term exposure to LD 8:16. Integrity of the circadian system in lesioned animals was confirmed directly by recordings of locomotor activity patterns in continuous dim red light (DD) or 24-hr LD cycles, and an intact estrus generating system inferred by the maintenance of regular estrous cycles in females.

A second experiment has utilised the counter-antigonadotrophic effect of continuous melatonin application in the golden hamster maintained in short daylengths. Stainless steel cannulae filled with melatonin, melatonin suspended in beeswax or solely beeswax were implanted bilaterally into the anterior hypothalamus or adjacent areas. (Release rates of melatonin into saline at 37°C were previously determined by HPLC/ECD measurements). Adult male hamsters, following surgery were transferred from LD 16:8 to LD 8:16 and gonadal condition monitored for 8 weeks. Preliminary findings suggest that continuous administration of melatonin to a site within the anterior hypothalamic area prevents the normal gonadal regression response to short photoperiods shown by control animals.

The findings from these two experiments, taken together, indicate that a site within the AHA may be a target for the pineal control of the photoperiodic response to LD 8:16 in the golden hamster, separate from neural centers concerned with circadian or estrous rhythms.

212.8 ELEVATED PINEAL ARGININE VASOTOCIN (AVT)-ACTIVITY IN RATS AND HAMSTERS DURING AUGUST. M.M. Prechel*, T.K. Audhya* and W.H. Simmons* (SPON: G.L. Humphrey). Department of Biochemistry and Biophysics, Loyola University Medical Center, Maywood, IL 60153.

A 14-month study of pineal glands from immature rats has shown that AVT-immunoactivity varied from barely detectable picogram levels most of the year, to levels exceeding one nanogram per gland each August (Prechel, et al., Endocrinology 112:1474,1983). We now report that the dramatic August increase in pineal AVT-activity also occurs in adult rats, and in hamsters.

Male and female rats and hamsters were housed with food and water ad libitum for 2-10 days prior to sacrifice. Rats (60-95 days) were maintained in a 12:12 light:dark cycle. Hamsters (55-90 days) were housed in L:D 14:10. Animals were sacrificed by decapitation. Pineal glands were removed, pooled, homogenized in 0.1 N acetic acid with 10^{-6}M pepstatin (an acid protease inhibitor), and then heat denatured to precipitate large proteins. Supernatants were studied by AVT radioimmunoassay (Fernstrom, et al., Endocrinology 106:243, 1980). Each pineal extract consisted of 3 pooled glands, and 3 replicate pools were prepared at each sampling. Male and female animals were studied separately; each of the four groups was sampled once or twice per week from July until early September, 1982.

Results showed that for all four groups the mean pineal AVT-immunoactivity was uniformly low (<10 pg/gland) until mid-July, then increased significantly by mid-August. For hamsters, maximum values of 1272 ± 49 (mean ± SE: n=3) and 1065 ± 62 pg/ gland were recorded for males and females, respectively. For rats, peak values were 940 ± 12 pg/gland for males and 1040 ± 34 for females. For the rats and male hamsters, peak dates were 8/11 and 8/13. In female hamsters AVT-activity exceeded 1000 pg/pineal from 8/9 through 8/21.

The present results closely resemble those of the earlier study with prepubertal rats, which showed peak levels (>1000 pg/gland) of AVT-activity on 8/16/80 and 8/10/81. Thus it now appears that for two separate species, and regardless of age, gender or light:dark environment, a several hundred fold increase in pineal AVT-activity occurs at a remarkably predictable time of the year.

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212.9 DIURNAL VARIATION IN GLUCOSE UTILIZATION IN THE PINEAL BODY OF THE MONKEY. M. Ito, R. Nakamura, C. Kennedy, L. Sokoloff, Laboratory of Cerebral Metabolism and Laboratory of Psychology and Psychopathology, NIMH, Bethesda, MD 20205.

The concentration of melatonin in blood and CSF of the monkey varies during the twenty-four hour day. The concentrations at night are consistently higher than those during the day (Reppert et al., 1979; Perlow et al., 1979). Whereas light deprivation during the day does not result in a rise in the normally low melatonin levels in the CSF, exposure to light at night suppresses the normally high nocturnal level (Reppert et al., 1981). These diurnal variations in melatonin levels are the result of corresponding changes in the rate of production and elaboration of melatonin in the pineal gland.

In order to determine if the diurnal variations in melatonin levels and pineal function are reflected in corresponding changes in energy metabolism of the pineal gland, we employed the quantitative [^{14}C]deoxyglucose method to measure glucose utilization in the pineal gland of pubescent monkeys housed in an environment in which lighting was automatically controlled to produce a 12-hour day and 12-hour night. Glucose utilization was measured under four conditions: A) in daytime with both eyes open (n=7); B) in daytime after 3 hrs of light deprivation (n=3); C) at night, awake, in total darkness (n=4); and D) at night during slow-wave sleep in total darkness (n=4). Arterial pH, pCO_2, pO_2, mean arterial blood pressure, and hematocrit were monitored. Glucose utilization in the pineal was 80-111% higher in the nocturnal, awake animals compared to the rate of both groups studied during the day (Bonferroni's t test, $p<0.05$). During slow-wave sleep the mean rate was also above the rates of daytime animals, but the differences were not statistically significant. Short term visual deprivation during the day was without effect on pineal glucose utilization.

These results indicate that there is an elevation of pineal metabolic rate at a time when blood and CSF levels of melatonin are elevated. The metabolic increase probably reflects increased activity in sympathetic nerve terminals distributed throughout the gland which stimulate its increase in hormone production.

212.10 EFFECTS OF DIET, PHOTOPERIOD AND MELATONIN ON BODY WEIGHT AND ENERGY BALANCE IN SYRIAN HAMSTERS. T.J. Bartness* and George N. Wade. Department of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

Both endogenous (short-day-induced) and exogenous (afternoon injections) increases in melatonin (MEL) cause a collapse of the reproductive tract in Syrian hamsters. We report the effect of these manipulations and of diet on body weight and energy balance. In Experiment 1, male and female Syrian hamsters housed in long (16:8) or short (8:16) light:dark cycles were fed either Purina #5001 chow pellets (Chow) or a high-fat diet (2:1 chow: shortening). Half the long-day-housed animals received daily afternoon (3 hrs prior to lights-off) s.c. injections of MEL (25 µg) or the ethanolic saline vehicle. Despite roughly equivalent caloric intakes across the 12 wk period, body weight gain (Fig. 1 & 2), carcass lipid (Fig. 3), carcass energy and % calories stored (Fig. 4), interscapular brown adipose tissue (IBAT) wet weight, protein and DNA (Fig. 5) and NE-stimulated thermogenesis (Fig. 6 & 7) were increased in fat-fed hamsters vs chow-fed controls. These effects were enhanced by short days and MEL. Females showed greater diet and photoperiod/MEL effects than males.

Because the effect of short days and MEL on these measures could be due to a functional gonadectomy, in Experiment 2 the protocol of Experiment 1 was repeated using ovariectomized hamsters. As before fat-feeding resulted in increased body weight gain, feed efficiency, and IBAT and parametrial white adipose tissue (PWAT) wet weight. These effects were again enhanced by short days and MEL to an extent of about 80% that seen in intact females (Fig. 8).

To determine the role of the pineal/MEL in the effects of short days seen above, pinealectomized (PINX) and sham-PINX (Sham) female hamsters fed the high-fat diet were housed in long and short days. Fourteen wks later, the short-day-housed PINX group had body weight gains, feed efficiencies, and IBAT and PWAT wet weights equal to that of their sham counterparts. Uterine weight was reduced in sham, but not PINX, animals housed in short days. Both short-day-housed groups exhibited the enhanced effects of this photoperiod on body weight and related measures vs the long day controls (Fig. 9).

Therefore, it appears that short photoperiods exert pineal/MEL- and gonad-independent and dependent effects on body weight and energy balance in Syrian hamsters, effects which were exaggerated by high-fat diets.

212.11 CIRCADIAN CHANGES IN CATECHOLAMINE SYNTHESIS IN THE HAMSTER PINEAL GLAND AFTER SUPERIOR CERVICAL DECENTRALIZATION OR GANGLIONECTOMY OR CONTINUOUS LIGHT. C. M. Craft, W. W. Morgan and R. J. Reiter. Dept. of Anatomy, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284.

The purpose of this study was to examine the effect of superior cervical decentralization (SCDC) or ganglionectomy (SCGX) or continuous light (LL) on the circadian changes in catecholamine (CA) synthesis in the hamster pineal gland. Earlier experiments (Endocr. Abst. #385, 1983) demonstrated for the first time diurnal changes in CA synthesis in the pineal with peak levels of CA synthesis in the hamster pineal two hours after the onset of darkness. Fluctuations in CA synthesis provide a measure of sympathetic neuronal activity which is believed to elicit the well established diurnal rhythm in indoleamines in the pineal gland. With SCDC, SCGX or LL, the circadian fluctuations do not occur in pineal indoleamine production. Therefore, we examined catecholamine synthesis in the pineal under these conditions to determine if CA changes occurred to explain the disappearance of pineal indoleamine rhythms. Male Syrian hamsters were maintained in a 14:10 LD cycle (lights on at 0600 h) for one week prior to and during the experiments except the group in LL which was maintained in 24:0 LD for 10 days after the first week. In the first experiment, animals were SCDC, SCGX or sham (S) operated ten days prior to the experiment. All animals were injected with NSD-1015 (100 mg/kg, i.p.), a dihydroxyphenylalanine (DOPA) decarboxylase inhibitor, 30 min prior to killing. CA synthesis was measured by the accumulation of DOPA. Norepinephrine (NE), DOPA and dopamine (DA) were estimated by high performance liquid chromatography and expressed in ng/pineal. In SCDC and SCGX, DOPA accumulation was measured at 2200 h, 2400 h and 1400 h and was significantly lower than S at 2200 h (1.19 ± .33, S; .32±.15, SCDC; .17 ± .04, SCGX; $p<.005$) and 2400 h (1.29 ± .19, S; .44 ± .14, SCDC; .66 ± .46, SCGX; $p<.001$). NE content was lower at 2400 h in SCGX than S (1.63 ± .17, S; .91 ± .1, SCGX; $p<.05$). In no experimental group was NE completely depleted. In the LL experiment, DOPA accumulation was low throughout the 24-hr study (0200 h, 0400 h, 1000 h, 2000 h, 2200 h and 2400 h)and significantly less than the controls at 2200 h (.22 ± .03 vs .78 ± .17 ng/pineal; $p<.025$) and 2400 h (.24 ± .03 vs .64 ± .03 ng/pineal; $p<.001$). NE content was depressed ($p<.05$) in the LL compared to the LD group. In conclusion, since CA synthesis which is a measure of neuronal activity is depressed in the hamster pineal gland during SCDC, SCGX or LL, the dissappearance of circadian changes in pineal indoleamines could be a result of decreased CA synthesis in postganglionic fibers of the sympathetics. (Supported by NSF grant no. PCM 8003441 to RJR and NIH grant no. DA00755 to WWM.)

212.12 PREVENTION BY TRH OF SHORT PHOTOPERIOD-INDUCED TESTICULAR ATROPHY IN GOLDEN HAMSTER: POSSIBLE PARTICIPATION OF BETA-ENDORPHIN. H.J. Chen, J. Targovnik*, L. McMillan*, and S. Randall*. Barrow Neurological Institute, St. Joseph's Hospital & Medical Center, Phoenix, AZ 85013 and Maricopa Medical Center, Phoenix, AZ 85008.

Daily injection of luteinizing hormone-releasing hormone (LHRH) prevents short photoperiod (SP)-induced testicular atrophy in golden hamsters (H.J. Chen, J. Endocrinology 96: 147-154, 1983). The purpose of the present study was aimed at investigating if TRH, another hypothalamic hormone, could prevent testicular regression in the male golden hamster exposed to SP. 48 male golden hamsters were divided into 8 groups of 6 animals each and given treatments as follows: Group 1, long photoperiod (LP) controls were given vehicles (the remaining groups were in SP of 6L:18D); Group 2, SP controls were given vehicle at lights on (LO) and lights off (LX); Group 3 animals were injected with 1 ug LHRH at LO; Group 4 animals were given 1 ug TRH at LO; Group 5 animals were given 1 ug LHRH and 1 ug TRH at LO; Group 6 animals were given 1 ug LHRH at LX; Group 7 animals received 1 ug TRH at LX; and Group 8 animals were given 1 ug LHRH and 1 ug TRH at LX. The animals were terminated after 8 weeks of treatment and 15 min after last injection of LHRH and/or TRH. Both LHRH and TRH prevented testicular regression if they were injected at LO ($p<0.01$ when compared with vehicle injected controls). LHRH, but not TRH, partially prevented testicular regression if injected at LX. Synergistic effects of LHRH and TRH to prevent testicular regression were observed only when given at LX. LHRH stimulated about a 15-fold increase in LH 36± vs 554± ng/ml) and 2 to 3-fold increase in FSH, but no increase in PRL in response to TRH was observed at this bleeding schedule. Plasma beta-endorphin concentrations in LP controls were 286±49 pg/ml vs 380±31 pg/ml in the SP controls ($p<0.01$), others tending to be higher in groups with atrophic testes and lower without atrophic testes. These results indicate that TRH like LHRH can prevent SP-induced testicular regression in hamsters by some unknown mechanism and that beta-endorphin may be involved in the control of testicular function in hamsters.

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212.13 DESTRUCTION OF THE DEEP PINEAL FAILS TO PREVENT SHORT-DAY-INDUCED TESTICULAR REGRESSION IN THE GOLDEN HAMSTER. Keith D. Anderson* and Fred W. Turek. Dept. Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60201.

The pineal gland of the golden hamster consists of two parts, the superficial pineal (SP), located just beneath the confluens sinuum, and the deep pineal (DP), located in direct contact with the third ventricle between the habenular commissure and the posterior commissure. The pineal gland is innervated by sympathetic nerve fibers that originate in the superior cervical ganglion, many of which terminate in the SP, but some of which pass through the SP and the pineal stalk to terminate in the DP. Bilateral superior cervical ganglionectomy prevents short-day-induced testicular regression that normally occurs in male hamsters transfered from a long day of 14 hrs light, 10 hrs darkness (14L:10D) to a short day (6L:18D). Superficial pinealectomy (SPx) also eliminates the short-day testicular response, indicating that the SP mediates the effect of photoperiod on testicular function. However, since SPx also interrupts sympathetic input to the DP, this result does not rule out the possibility that the DP mediates, at least in part, the effect of short days on reproductive function. To determine if the DP is required for short-day-induced testicular regression, we monitored testis size in hamsters with an electrolytic lesion of the DP. 37 adult male hamsters were placed under 14L:10D for 8 weeks prior to surgery. 24 of these animals were lesioned by passing 2 mA of current through a stereotaxically placed electrode for 15 sec. The remaining 13 hamsters were sham operated (SH). One week later, 12 of the lesioned and 6 of the SH hamsters were transfered to 6L:18D. Testis widths were measured at 2-3 week intervals for 9 weeks. All animals were then sacrificed, testes were weighed, and lesion placements were histologically verified. As expected, the testes of SH hamsters exposed to 14L:10D were large, while the testes of SH hamsters exposed to 6L:18D had completely regressed. No significant difference existed between the mean testes weight of SH hamsters (2843 mg ± 224; N=7) and hamsters with confirmed DP lesions (3189 mg ± 561; N=7) exposed to 14L:10D for 9 weeks, nor between SH hamsters (346 mg ± 54; N=6) and hamsters with confirmed DP lesions (491 ± 129; N=5) exposed to 6L:18D for 9 weeks. Testis width measurements revealed no difference in the rate of testicular regression between SH hamsters and DP-lesioned hamsters exposed to 6L:18D. These results demonstrate that the deep pineal is not required for normal short-day-induced testicular regression in the golden hamster.

(Supported by a grant from the Whitehall Foundation.)

212.14 EVIDENCE FOR MEDIATION OF PHOTOPERIODIC RESPONSES IN THE DJUNGARIAN HAMSTER BY CHANGES IN DURATION OF THE CIRCADIAN PULSE OF PINEAL MELATONIN SECRETION. B.D. GOLDMAN*, Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545

The Djungarian hamster is a species in which the pineal is known to be capable of exerting both stimulatory and inhibitory effects on reproduction, depending on photoperiodic conditions. To study the role of pineal melatonin in reproductive regulation, juvenile males were pinealectomized (pinx) and given timed daily infusions of melatonin. In the first experiments, the animals were raised from birth in a stimulatory (i.e., long day) photoperiod and were pinx at 18 days of age. When 10 ng melatonin was administered over a period of 8h or 10h daily, testis growth was inhibited after 12 days of treatment (testes wts. = 63 ± 7 and 54 ± 9 mg for 8h and 10h melatonin groups, vs. 412 ± 26 mg in saline-infused controls). When the same amount of melatonin was given over a 4h or 6h period each day, testis growth was not impaired (testes wts. = 430 ± 33 and 501 ± 30 mg for 4h and 6h melatonin groups). The time of day at which the infusions were administered did not affect the response. Thus, the duration of the daily melatonin pulse, but not its circadian phase, appears to be the most important parameter. Hamsters must be exposed to daily melatonin pulses of > 6h duration to inhibit testis development. The long duration daily pulse of melatonin must be almost continuous to produce the inhibitory effect, since hamsters which received two 5h infusions each day, separated by a 2h period with no treatment (i.e., 5h on-2h off-5h on) did not show inhibition (testes wts. = 532 ± 30 mg).

In a second set of experiments, juvenile males were raised in an inhibitory (i.e., short day) photoperiod and were pinx at 23 days of age. Daily melatonin infusions were given for 12 days. Animals which received melatonin (0.83 ng/h) for 4h or 6h each day showed testicular stimulation, while those which received melatonin for 8h or 12h daily showed inhibition (testes wts. = 196 ± 16 and 180 ± 21 mg for 4h and 6h melatonin groups vs. 38 ± 6 mg in saline-infused controls; testes wts. = 47 ± 15 and 22 ± 1 mg for 8h and 12h melatonin groups). Thus, in summarizing the results from both studies, exposure to daily melatonin pulses of 4-6h duration was stimulatory to testis growth, while exposure to melatonin pulses of 8-10h duration was inhibitory. In conjunction with other data from this laboratory, these results suggest that the effects of photoperiod on testis growth in the Djungarian hamster are mediated by changes in the duration of the circadian peak of pineal melatonin secretion.

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212.15 PINEAL MELATONIN IN TURKISH HAMSTERS EXPOSED TO DIFFERENT PHOTOPERIODS AND DURING HIBERNATION. J.M. Darrow*, D.S. Carter*, M.J. Duncan*, B.D. Goldman* and L. Tamarkin*. (SPON: R.D. Hall) Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

The rhythmic daily production of melatonin by the pineal gland is known to be regulated by the circadian pacemaker system and to be important in the photoperiodic control of annual breeding cycles in several mammalian species. In order to characterize the 24-hour pattern of melatonin production in long-day breeding Turkish hamsters (Mesocricetus brandti) during different phases of the annual cycle, we measured pineal melatonin content (by (RIA) in adult female hamsters exposed to long days (LD 16:8), short days (LD 10:14) and during hibernation. Both long and short day females show a nocturnal elevation of melatonin beginning several hours after lights-off and ending shortly before the lights-on transition. No significant differences in amplitude of the peaks was detected (mean values ranged from <100 pg/gland during the day to >600 pg at night), but the two groups did differ in the duration of their melatonin peaks (mean values were elevated above 200 pg/gland for 4 hours on long days; 6 hours on short days). This finding may have functional significance, since in the Djungarian hamster (Phodopus sungorus), a similar extension of the pineal melatonin peak occurs in animals exposed to short days and is largely responsible for mediating the effects of photoperiod on reproduction.

When Turkish hamsters are induced to hibernate by exposure to LD 10:14 at 6°C, they undergo alternating bouts of torpor (lasting 5-8 days) and arousal (lasting 1-3 days) over a period of 4-5 months. The torpid state is characterized by a dramatic decrease in core body temperature (by more than 20°C). Most females sacrificed during torpor showed baseline melatonin values (<50 pg/gland), regardless of time of day, suggesting an attenuation of pineal melatonin production during torpor. In contrast, hamsters sampled during the first day after arousal displayed a nocturnal peak of pineal melatonin similar in phase, amplitude and duration to that seen in short day females maintained at room temperature (21°C). These results indicate that during the hibernation season, the Turkish hamster continues to receive a nocturnal pineal melatonin signal on days of arousal. The data also suggest that the circadian clock, which governs the phase of the melatonin peak expressed during arousal, is not seriously affected by the decreased body temperature occurring during torpor.

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POSTSYNAPTIC MECHANISMS II

213.1 ACTION OF SUBSTANCE P AND TELEOST LHRH ON BULLFROG SYMPATHETIC NEURONS. Stephen W. Jones and Paul R. Adams. Dept. of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794.

Jan et al. (PNAS 76:1501-1505, 1979) have shown that M-LHRH [mammalian luteinizing hormone- (or gonadotropin-) releasing hormone] mimicks the late, slow EPSP of bullfrog ganglia, and than an M-LHRH-like peptide is present in these ganglia. However, the endogenous peptide is not identical to M-LHRH, and may be the LHRH of teleost fish (T-LHRH: [Trp^7,Leu^8]M-LHRH; Sherwood et al., PNAS, in press). We have found that T-LHRH is at least 10x more potent than M-LHRH on the bullfrog ganglion. As for M-LHRH, the most obvious and consistent effect of T-LHRH under voltage clamp is inhibition of the M-current, a voltage-dependent K^+ current that is activated by depolarization below -60 mV. The EC_{50} for M-current inhibition by T-LHRH is approximately 0.35 μM, with a Hill coefficient near 1. In some cells, T-LHRH also induces an additional inward current associated with an increased conductance, which (like M-current inhibition) would depolarize an unclamped cell.

Chicken LHRH ([Gln^8]M-LHRH), in contrast, is less active than M-LHRH, as is [Gln^7,Leu^8]M-LHRH. [Trp^8]M-LHRH is less potent than T-LHRH, but more potent than M-LHRH. These results support the hypothesis that T-LHRH is the actual transmitter for the late, slow EPSP of bullfrog sympathetic ganglia.

Substance P (SP) and muscarinic agonists also depolarize bullfrog ganglion cells, and inhibit the M-current. As for T-LHRH, these agents occasionally induce an additional inward current. This effect was typically seen at higher concentrations, and was slower in onset and offset than the M-current inhibition. Cells varied widely in their response to SP, with some cells unresponsive to 10 μM, and others with EC_{50}'s of 10 nM or lower. The effect on a given cell was clearly concentration-dependent. Hill coefficients for inhibition of the M-current by SP were consistently less than 1, which was also observed for muscarine and oxotremorine. The M-current inhibition produced by SP occasionally desensitized during a 1 min application, which has not been observed for other M-current inhibitors. The SP effects are mimicked by other tachykinins (eledoisin, eledoisin-related peptide, and physalaemin). Two proposed SP antagonists, [D-Pro^2,D-Trp^{7,9}]SP and [D-Arg^1,D-Pro^2, D-Trp^{7,9},Leu^{11}]SP, showed weak agonist activity and no clear antagonism of SP on these cells. The muscarinic effect is responsible for the slow EPSP of bullfrog ganglia; it is not yet known whether SP mediates a synaptic potential there.

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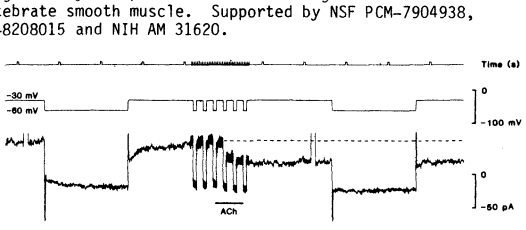
213.2 CHOLINERGIC STIMULATION OF SINGLE VERTEBRATE SMOOTH MUSCLE CELLS IS ASSOCIATED WITH A CONDUCTANCE DECREASE. Stephen M. Sims,* Joshua J. Singer and John V. Walsh. Dept. Physiology, Univ. Massachusetts Med. School. Worcester, Mass. 01605.

Single electrode voltage clamp and current clamp techniques were used to study acetylcholine (ACh) induced responses in freshly isolated gastric smooth muscle cells from the toad Bufo marinus. Application of ACh (50 μM to 1 mM) by pressure ejection from a micropipette caused slow depolarization, sometimes giving rise to action potentials and contractions. ACh-induced depolarization was accompanied by a conductance decrease, although the cells exhibit outward-going rectification in the absence of ACh. ACh application to voltage clamped cells confirmed the conductance decrease and revealed the suppression of an outward current, as illustrated in the inset. Under conditions of elevated $[K^+]_{out}$, the ACh-suppressed current reversed in direction when the potential was more negative than E_K. These observations are consistent with ACh- suppression of a K^+ current.

Further results suggest that the K^+current suppressed by ACh has some characteristics similar to the M-current of sympathetic neurons. Slow current relaxations to steady-state levels, reflecting the closing of ionic channels, occurred in response to hyperpolarizing voltage commands. The reversal potential for the current relaxations was -94 mV, and shifted 51 mV positive per tenfold increase in $[K^+]_{out}$, indicating that K^+ ions carry most of the charge for the current relaxations. The slow relaxations were abolished by ACh, suggesting that ACh acts on the same ionic channel responsible for the relaxations. Cholinergic agents appear to reversibly depress this voltage-sensitive K^+ current. These results can explain the ACh-induced conductance decrease and depolarization observed in the isolated muscle cells. The existence of a cholinergic conductance decrease is of interest because it runs counter to the generally accepted view of cholinergic excitation in vertebrate smooth muscle. Supported by NSF PCM-7904938, PCM-8208015 and NIH AM 31620.



213.3 ROLE OF EPHAPTIC INTERACTIONS IN PAIRED PULSE AND FREQUENCY POTENTIATION OF HIPPOCAMPAL FIELD POTENTIALS. R.W. Turner, T.L. Richardson and J.J. Miller. Department of Physiology, University of British Columbia, Vancouver, B.C. V6T 1W5

Recent studies have suggested that extracellular field potentials in the hippocampal formation elicit an ephaptic depolarization capable of discharging pyramidal neurons. Since both paired-pulse (PP) and frequency stimulation of certain afferent inputs to the hippocampus are known to result in an augmentation of field potential amplitudes, it was of interest to determine the extent to which ephaptic interactions may contribute to these short-term potentiation phenomena.

Orthdromic ground referenced intracellular responses, evoked by stratum radiatum (SR) stimulation, were recorded from CA1 pyramidal neurons in the 'in vitro' slice preparation. The electrode was withdrawn from the cell and the evoked extracellular field potential subtracted from the intracellular response to obtain a measure of the transmembrane potential (TMP). Action potential generation on intracellular ground referenced recordings occurred from the peak of a graded negative-going "notch" on the rising edge of the EPSP at a latency equivalent to the extracellular population spike. Subtraction of field potentials revealed a sharp depolarizing wave on the TMP with an amplitude and latency that varied directly with that of the population spike. With a subthreshold stimulus intensity for intracellular spike generation, PP or frequency stimulation of SR evoked a potentiation of the population spike, a corresponding increase in the amplitude of the TMP depolarizing wave and an increase in the probability of action potential discharge. Action potentials elicited by either a test stimulus or during a frequency train arose from the peak of the TMP depolarizing wave in virtually all cells examined.

It is proposed that ephaptic interactions contribute to potentiation of the CA1 extracellular population spike through recruitment of neighboring cells during repetitive afferent stimulation.

213.4 POSTSYNAPTIC-CELL MEMBRANE EVENTS ACCOMPANYING HABITUATION. C. Larry Keenan and Harold Koopowitz, Developmental and Cell Biology, Univ. of California, Irvine.

Habituation of intracellularly measured vibration evoked responses from polymodal neurons in the brain of Notoplana acticola involves at least three postsynaptic phenomena. Changes that occur concurrently with habituation include decreases in postsynaptic membrane resistances, increased thresholds for firing action potentials, and decreases in cell membrane time constants. Membrane input resistances decreased on the average 11% from a mean of 33.8 MΩ ± 8.9 (n=6). Thresholds for firing as measured by injecting depolarizing current into the cells increased up to threefold in some cases. This increase was further demonstrated by interspersing suprathreshold depolarizing pulses between vibration stimuli (1 stimulus/2 sec). Under these conditions both the number of action potentials evoked by the pulses decreased as habituation proceeded and the latency to the first spike evoked by each pulse increased. Current injections of the same magnitude and frequency but without the vibration stimuli showed no significant decrease in number of spikes evoked. From this we concluded that neither accommodation nor fatigue were contributing factors. When prehabituation resting potentials were compared to posthabituation resting potentials, the posthabituation potentials were 4.4 mV ± 2.1 (mean ± S.D.) more negative than prehabituation resting levels (n=13). Decreases in time constants calculated from a Rall analysis were obtained from all types of vibration-sensitive neurons studied. The results suggest that both cell resistivity and capacitance may be altered significantly in postsynaptic membrane as a consequence of synaptic events accompanying habituation.

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213.5 STRUCTURE-DEPENDENT MODIFICATION OF END-PLATE CURRENT KINETICS BY ALKYL-BIS-GUANIDINES. Stephen M. Vogel*, Jay Z. Yeh* and Toshio Narahashi (SPON: Richard A. Berenberg). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The alkyl-bis-guanidines contain two guanidinium groups, which are positively charged at physiological pH, separated by a linear alkyl chain of 4 to 10 carbons (bis-G-C_4 to bis-G-C_{10}). We examined the influence of several compounds of this series on the end-plate current (EPC). The neuromuscular junction of the frog cutaneous pectoris muscle was voltage-clamped by the two-microelectrode method at 22°C. Clamp steps of 2 sec were made from a holding potential of -40 mV. The EPC was elicited by nerve stimulation 1 sec following the beginning of a clamp step. The clamp potential was varied between -140 and +60 mV in 20-mV increments. All of the derivatives reversibly suppressed the peak EPC in a manner dependent on the concentration. Bis-G-C_4 (10 mM) depressed the EPC in a voltage-independent manner. In contrast, bis-G-C_6 (0.3 mM), bis-G-C_8 (0.003 mM to 0.03 mM), and bis-G-C_{10} (0.02 mM) caused a voltage-dependent block, i.e., the block was intensified with hyperpolarization of the end-plate. The derivatives also affected the time course of the EPC decay phase. Bis-G-C_4 and bis-G-C_6 lengthened the decay time constant of the EPC. With bis-G-C_4, the prolongation was seen at 1 mM, a concentration at which it did not appreciably affect the amplitude of the EPC; this suggests that the closing rate constant, α, could be slowed. Bis-G-C_8 induced a double exponential decay, as reported earlier (Vogel et al., Fed. Proc., 42, 1144, 1983). Bis-G-C_{10} drastically shortened the decay time constant, which remained a single exponential. For bis-G-C_8, kinetic analysis of the EPC using a sequential model gave a forward blocking rate of 0.61×10^8 M^{-1} sec^{-1} at 0 mV and an unblocking rate of 0.63 $msec^{-1}$ at 0 mV; the potential dependence of the rate constants suggested a binding site about 50 through the membrane electric field. A similar analysis suggests that the dissociation rate is slow for bis-G-C_{10}, giving rise to a monophasic decay. The alkyl-bis-guanidines appear to be useful probes for the study of the shape and size of the acetylcholine-activated ionic channel. Supported by NIH grant NS14145.

213.6 MEPROADIFEN ENHANCES ACTIVATION AND DESENSITIZATION OF THE ACETYLCHOLINE RECEPTOR IONIC CHANNEL COMPLEX (AChR): SINGLE CHANNEL STUDIES. Y. Aracava*, S.R. Ikeda,* and E.X. Albuquerque. Dept. Pharmacol. & Exp. Ther., University Maryland Sch. Med., Baltimore, MD 21201.

The quaternary local anesthetic meproadifen is a noncompetitive antagonist of the AChR which increases the affinity of acetylcholine (ACh) for its binding site in Torpedo electric organ membranes (Mol. Pharmacol. 15:294, 1979). At the frog neuromuscular junction, meproadifen causes a voltage- and time-dependent decrease in the peak amplitude of endplate currents and miniature endplate currents and accelerates ACh-induced desensitization. All the effects of meproadifen on these macroscopic events occurred without significant change in channel lifetime or conductance (Mol. Pharmacol. 22:636, 1982). In the present study, we used patch clamp technique on myoballs cultured from neonatal rat muscles to assess directly the effects of meproadifen on ACh-induced single channel currents. The presence of meproadifen at low concentrations (10-50 nM) and ACh (20-300 nM) in the patch pipette resulted in an initial increase in the frequency of channel openings which subsequently decreased. For example, with ACh (250 nM) and meproadifen (10 nM) in the pipette, the frequency increased to 300-400% of control values and after 20-30 min it declined gradually. At a higher concentration of meproadifen (> 200 nM), the duration of increased channel activation was shorter and occasionally not seen. Indeed, at 500 nM, meproadifen produced such a rapid decrease in the frequency of channel opening that the initial period of increased activation was not observed. However, at all concentrations of meproadifen (up to 2.5 μM) applied with ACh in the pipette, no significant change in the channel lifetime or conductance was seen. In addition, during channel openings in the presence of meproadifen, a marked increase in the thickness of the baseline with fast 'flickers' occurred. These events happened simultaneously with 'bursting' activity which appeared between long silent periods, finally culminating in almost complete absence of channel opening. In contrast to other quaternary agents such as pyridostigmine (Akaike et al., unpublished) meproadifen, even at high concentrations (10 μM), did not affect the frequency, lifetime or conductance of ACh channels when applied in the bath to cell-attached or inside-out patches. Thus, in agreement with our previous observations, it is suggested that meproadifen interacts primarily with AChR site located in the extracellular face of the cell membrane. Further, this study indicates that antagonists such as meproadifen can facilitate activation and desensitization without affecting channel lifetime or conductance. (Supported by USPHS Grant NS-12063, U.S. Army Medical Research and Development Command Contract DAMD 17-81-C-1279 and FAPESP, Brazil.)

213.7 EFFECT OF GEPHYROTOXIN (GyTX) ON THE ACETYLCHOLINE (ACh) RECEPTOR IONIC CHANNEL COMPLEX: OPEN CHANNEL BLOCKADE AND ENHANCEMENT OF DESENSITIZATION. C. Souccar*[1], W. Varanda*[1], Y. Aracava*[1], J. Daly[2] and E. X. Albuquerque[1] (SPON: R.G. Grenell). [1]Dept. Pharmacol. and Exp. Ther., University of Maryland Sch. of Med., Baltimore, MD 21201 & [2]Lab. Bioorganic Chemistry, NIADDKD, NIH, Bethesda, MD 20205.

GyTX is a tricyclic alkaloid, identified as [1S,3aS,5aS,6S(Z),-9aR,10R]dodecahydro-6-(2-penten-4-yl)pyrrolo[1,2-a]quinoline-1-ethanol, found in skin secretions of the Colombian poison arrow frog Dendrobates histrionicus. As previously reported by our laboratory (Fed. Proc. 41:1299, 1982), the action of GyTX on the frog neuromuscular junction is characterized by a combined effect both as an open channel blocker and as a desensitization-enhancing agent. We have found that the toxin decreases the peak amplitude of the endplate current (epc) as well as the epc decay time constant (τ_{epc}). These effects were concentration dependent but only slightly voltage- or time-dependent. The plot of $1/\tau_{epc}$ versus concentration of GyTX was linear in accordance with the sequential model for open channel blockade. Noise analysis data showed that GyTX shortens the channel lifetime without affecting its conductance. To assess more directly the effects of GyTX at the microscopic level, we used patch clamp technique in tissue-cultured myoballs obtained from neonatal rat muscles. This allowed us to measure both lifetime and conductance of ACh-activated ionic channels. GyTX was either superfused into the bath solution or present in the patch pipette together with ACh. Recordings were obtained both from cell-attached and inside-out patches. GyTX (1-50 μM) decreased the channel lifetime in a concentration-dependent manner, but single channel conductance remained unchanged at all concentrations studied. Quantitatively similar effects were seen by adding the drug to the bath in both cell-attached and inside-out patches. However, when GyTX was present in the pipette, the onset of the drug effect was faster and its potency higher. For example, at 20 μM , GyTX decreased channel lifetime by about 35% of control values and this effect appeared 20-30 minutes after the toxin superfusion. On the other hand, at the same concentration, GyTX present inside the pipette shortened channel lifetime by about 75% almost immediately after the gigaseal was achieved. In addition to blocking the ACh ionic channel in its open conformation, GyTX also seemed to induce an increase in affinity of ACh for its receptor. This was seen as an enhancement of the desensitization rate as indicated by studies using double-barrelled pipette technique and also by an increase in the binding of agonists to the ACh recognition site in Torpedo electroplax membranes. (Supported by USPHS Grant NS-12063, U.S. Army Research Office Grant DAAG 29-81-K-0161 and FAPESP and CNPq, Brazil.)

213.8 ALTERATION OF GANGLIONIC FAST EXCITATORY POSTSYNAPTIC CURRENTS BY BARIUM. E.A. Connor and R.L. Parsons. Dept. of Neurobiology, Stanford Univ.,Stanford, CA 94305 and Dept. of Anatomy & Neurobiology, Univ. of Vermont, Burl., VT 05405.

Barium is used as a pharmacological tool in many studies of membrane ion channels. It is a potent blocker of most voltage-dependent potassium channels and often has been used as a calcium substitute to investigate the kinetics of voltage-gated calcium channels. We have found that barium also has a direct post-synaptic action on the nicotinic fast excitatory postsynaptic current (EPSC) recorded from amphibian postganglionic sympathetic neurons. In the present study, barium-induced alterations in fast EPSCs have been studied in voltage-clamped bullfrog sympathetic ganglion B cells (22-23°C). In the presence of barium (2-8 mM) EPSC decay was prolonged and in many cells, the EPSC decay phase deviated from a single exponential function. The decay phase in these cases was more accurately described as the sum of two exponential functions. The prolongation and frequency of occurrence of a complex decay increased both with increasing barium concentration and hyperpolarization. For 8 control cells voltage-clamped to -50mV, the EPSC decay τ was 4.7 ± 0.1ms (mean ± SEM) whereas for 11 other cells voltage-clamped to -50mV and exposed to 8mM barium, the decay time course, fitted as the sum of two exponential components, had time constants of 5.1 ± 0.2 and 17.9 ± 1.0ms. The decay time course of spontaneous miniature excitatory postsynaptic currents (MEPSCs) was prolonged to an extent very similar to that of evoked EPSCs recorded in the same barium-treated cells. In control cells, the EPSC decay τ increased with hyperpolarization; the coefficient of voltage dependence being $-0.0027 \pm 0.0003 mV^{-1}$. Both decay components in 8mM barium-treated cells increased with hyperpolarization; the coefficient of voltage-dependence being $-0.0049 \pm 0.0011 mV^{-1}$ for the initial component and $-0.0086 \pm 0.0022 mV^{-1}$ for the second, slower component. At -50mV, EPSC amplitude was not markedly influenced by barium (2-8mM) whereas at -90mV there was a progressive increase in peak size with increasing barium concentration. As a consequence, although the EPSC-voltage relationship was linear between -20 and -100mV in control cells, in barium-treated cells this relationship became progressively non-linear with membrane hyperpolarization. The EPSC reversal potential was shifted from -6.3 ± 1.6mV to -11.4 ± 2.1mV in the presence of 8mM barium. There was a voltage-dependent increase in charge movement during the EPSC in barium-treated cells which was not present in control cells. We conclude that barium alters the kinetics of receptor-channel gating in amphibian post-ganglionic sympathetic neurons.

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213.9 DENDRITIC SPINE EPSPs IN CA1 PYRAMIDAL CELLS - ACTIVE OR PASSIVE TRANSFER FROM SPINE TO SOMA? D.A. Turner. Inst. of Neurophysiology, Oslo, Norway and VAMC, Univ. Minn., Minneapolis, MN. 55417

Andersen et al (J. Physiol. 307:273, 1980) observed that proximal and distal synapses onto CA1 hippocampal pyramidal cells are equipotent and similar in time course. Their explanations for this anomalous finding include the effect of dendritic spines and 'active' enhancement of dendritic EPSPs. Passive electrotonic spread of EPSPs may be analyzed by computing EPSP shapes and magnitudes for varying input regions of a dendritic tree. Differences between modeling predictions and observations may indicate either net enhancement or rectification, implying 'active' EPSP transfer from a spine to the soma.

To predict passive EPSP characteristics in CA1 pyramidal cells I have used a segmental cable model derived from HRP anatomy (Neurosci. Abstr. 8:945, 1982). Spine EPSPs were simulated with a transient conductance change (α =50 and integrated magnitude of $12*10^{-10}$ S-msec). This input was applied to anatomically chosen proximal (PX) and distal (DS) sites on CA1 pyramids. The resulting soma waveforms were computed and compared (n=36 sites each):

	X_{in} (λ)	R_{spine} ($10^8\Omega$)	Spine Peak (mV)	Soma Peak (μV)	Rise Time (τ)	Half-Width (τ)	Charge Loss (%)	Charge Transfer (%)
PX	0.32	12.8	21.3	82.1	0.10	0.59	30.4	57.1
DS	1.00	17.4	23.2	16.1	0.40	1.45	33.5	22.5

The PX and DS parameters varied significantly, except the spine peak and the charge loss due to the reduced driving potential. These values are also close to those observed by Andersen et al: rise time of 0.25τ and halfwidth of 1.0τ. Thus, this passive cable model suggests the proximal and distal inputs to be different in terms of both synaptic efficacy (soma peak and charge transfer) and waveform parameters (rise time and halfwidth).

In this model, dendritic spines did not significantly delay or attenuate the simulated EPSPs. The spine neck contributed less than 100 MΩ of longditudinal resistance, compared to the relatively high input resistances into the dendrites. Other possible explanations for Andersen et al's observations include a diffuse spread of inputs and an 'active' enhancement of the EPSPs. Preliminary physiological evidence suggests that small EPSPs from proximal and distal sources (less than 1 mV) display a difference in rise time. Thus, the larger EPSPs recorded by Andersen et al (5-10 mV) may be above threshold for some active process, such as a slow inward current. Clearly, though, the model predictions differ from the observations on larger EPSPs, suggesting 'active' EPSP transfer in these neurons. NIH Grant #NS 06792

213.10 **DIFFERENT SYNAPTIC CHANNEL KINETICS IN FAST AND SLOW CONDUCTING SYMPATHETIC NEURONS OF THE FROG.** **L.M. Marshall* (SPON: J.N. Weakly). Dept. of Physiol. University of North Carolina, Chapel Hill, NC 27514.**

Lumbar sympathetic ganglia of the frog have two populations of principal neurons that are innervated by two distinct classes of cholinergic preganglionic nerve fibers. The rapidly conducting B-neurons are innervated by rapidly conducting preganglionic B-fibers, whereas the slowly conducting C-neurons receive synaptic input from the slowly conducting C-fibers (Nishi et al., J. Cell. Comp. Physiol. 66:19, 1965). This study examined and compared the properties of nicotinic acetylcholine channels that produce fast excitatory postsynaptic currents (EPSCs) in these two types of sympathetic neurons.

Neurons in the 9th and 10th paravertebral ganglia of adult bullfrog (Rana catesbiana) were identified according to preganglionic conduction velocity and voltaged-clamped at -50 mV (22 deg. C). The EPSC, evoked by preganglionic stimulation, decayed as a single exponential for both cell types, but the decay time constants differed considerably; 5.5 ± 1.1 msec (mean ± s.d.) for B-neurons and 10.2 ± 2.3 msec for C-neurons.

Spectral analysis was performed on membrane current fluctuations produced by the steady application of acetylcholine, in the presence of 0.5 μM atropine (Anderson & Stevens, J. Physiol. 235:655, 1973). Power density spectra fitted single Lorentzian relations and gave estimates of the mean channel open-time of 5.2 ± 0.9 msec (mean ± s.d.) for B-neurons and 9.8 ± 2.0 msec for C-neurons.

This close agreement between the EPSC decay time constant and the mean channel open-time supports the idea that, in frog sympathetic neurons, the decay of synaptic current is controlled by the closure of membrane channels, as proposed for the frog end-plate (Katz & Miledi, J. Physiol. 231:549, 1973). Further, the clear differences in EPSC decay rate for B- and C-neurons is most likely due to the differences in the mean open-time of their synaptic channels. (Supported by NIH grant NS 17203).

213.11 CALCIUM CHANNEL ANTAGONISTS DISPLACE [^{3}H]PHENCYCLIDINE BINDING TO TORPEDO ELECTRIC ORGAN MEMBRANE. P.M. Epstein*, J.J. Lambert*, S. Moraski Jr.*, and E.G. Henderson. Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032. (SPON: S. Pfeiffer)

The specific binding of 2nM [^{3}H]phencyclidine ([^{3}H]PCP) to electric organ membrane isolated from Torpedo californica, measured in the presence of 10μM carbachol, was displaced by $CaCl_2$ and several structurally different classes of calcium channel antagonists, including verapamil, diphenylmethylalkylamines, and the 1,4-dihydropyridines. The IC_{50}'s for displacement of [^{3}H]PCP binding by these compounds was: $CaCl_2$ - 2.3mM, verapamil - 4.8μM, flunarazine - 2.7μM, cinnarazine - 1.5μM, nicardipine - 1.4μM, nimodipine - 2.5μM, and nifedipine - 9.8μM. At the concentrations at which they produced 50% inhibition of [^{3}H]PCP binding, none of these antagonists inhibited the initial rate of [^{125}I]-α-bungarotoxin binding to these membranes. These results suggest an association between the [^{3}H]PCP binding site and an ion channel that can conduct calcium in Torpedo membranes. PCP has been shown to interact with the ion channel associated with the nicotinic acetylcholine receptor (AchR) in Torpedo membranes (Eldefrawi et al., Proc. Natl. Acad. Sci. USA 77:7458, 1980). Since this channel is not ion specific and can conduct Ca^{2+} in addition to Na^+ and K^+ (Lambert et al., Ann. Rev. Pharmacol. Toxicol. 23:505, 1983), it is possible that Ca^{2+} antagonists may interact with this channel in Torpedo membranes. Effects of these antagonists on this channel lifetime were, however, not evident. Verapamil (25μM) and nicardipine (10μM) produced no change in channel lifetime (τ) in voltage-clamped, transected frog cutaneous pectoris muscle when end-plate currents were elicited 500 msec after jumps to potentials ranging from -130 to +50mV. Hence, if these antagonists interact with the ionic channel associated with the AchR, they may interact with an agonist activated, but nonconducting state of the channel. (Supported in part by Miles Laboratories, a Wellcome Research Travel Grant to J.J.L., and U.S. Army contract DAAG29-81-K-0165.)

213.12 WAVEFORM ANALYSIS OF DISCRETE INHIBITORY SYNAPTIC POTENTIALS IMPINGING ON MOTONEURONS DURING THE ATONIA OF ACTIVE SLEEP. F.R. Morales, P.A. Boxer* and M.H. Chase. Depts. of Physiology and Anatomy and the Brain Research Institute, School of Medicine, University of California, Los Angeles, CA 90024.

We have reported, in the chronically prepared cat, the presence of spontaneous discrete IPSPs in high gain intracellular recordings of alpha motoneurons during the atonia of active sleep (Exp. Neurol. 78:471, 1982). In the present study we performed a quantitative analysis of the waveform parameters of these potentials in order to understand the wide variability in IPSP amplitudes observed within given motoneurons. These inhibitory potentials were found to exhibit large variations in amplitude, not only within a given cell, but also across cells. Their amplitudes ranged from 0.2mV (resolution limit) to 3.5mV; 10-90% rise-times ranged from 0.3 to 1.6 msec and half-widths from 0.5 to 6 msec. The relationships among these waveform parameters were then evaluated within the conceptual framework developed by Rall based on a mathematical neuronal model (J. Neurophysiol. 30:1138, 1967).

For all cells (n=31) a statistically significant correlation was found between the rise-time and potential amplitude. Correlation coefficients (r) ranged from 0.48 to 0.73. The slopes of these correlations were all positive and extended from 0.2 to 0.65. For theoretical synaptic potentials that differ only in their location, Rall has shown that a negative correlation should be expected between rise-time and amplitude. Consequently, the differences in amplitude in the IPSPs cannot be explained purely on the basis of different synaptic locations.

A positive correlation also existed between the rise-time and half-width of these potentials; however, the importance of this correlation, vis-a-vis their synaptic location, is reduced due to an unexpected positive correlation between the potential's amplitude and its half-width. A statistically significant negative correlation was found between the rate-of-rise normalized by the amplitude and the amplitude of the IPSPs (r: from -0.49 to -0.65; slopes from -0.25 to -0.8), indicating that the rising phases of large IPSPs are relatively slower than those of the small IPSPs.

These data can be tentatively explained by postulating that the presynaptic inhibitory neurons which are responsible for the large IPSPs provide relatively greater numbers of synaptic boutons to alpha motoneurons compared with the presynaptic neurons generating small IPSPs. The variability in IPSP size may reflect a need for a highly tuned pattern of inhibition to accord with the fluctuating levels of neuronal activity during active sleep.

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213.13 ACTIVATION OF AN ELECTROGENIC SODIUM PUMP IN HIPPOCAMPAL PYRAMIDAL NEURONS. Scott M. Thompson* and David A. Prince. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Application of glutamate to CA1 hippocampal pyramidal cells produces a brief depolarization which is followed by a prolonged hyperpolarization. The mechanisms by which this post-glutamate hyperpolarization (PGH) is produced were examined. Transverse slices of guinea pig hippocampus were prepared and maintained in vitro in the usual manner at 37°C. Applications of glutamate (5-25 mM in normal bathing medium) were made from broken pipettes (5-10 μm tips) placed in stratum radiatum or stratum pyramidale within 200 μm of the recording electrode. Neuronal responses consisted of a depolarization of up to 30 mV, followed by a rapid repolarization and a prolonged hyperpolarization which gradually returned to resting levels. The amplitude and duration of the PGH were proportional to the size of the depolarization evoked by the glutamate application. The PGH could be as large as 15 mV and last as long as 2.5 min. Experiments were performed to distinguish between two possible explanations for this post-glutamate hyperpolarization: 1) stimulation of an electrogenic sodium pump by sodium accumulation during the depolarization, or 2) a prolonged increase in calcium-activated potassium conductance.

The PGH was found to be unaffected by perfusion with media containing manganese (4-5 mM) and low concentrations of calcium (0-1 mM) which effectively blocked voltage-sensitive calcium channels and synaptic events. Furthermore, the PGH did not reverse polarity when the cells were polarized beyond the potassium equilibrium potential with direct current injection. However, the amplitude and duration of the PGH were reduced by lowering the temperature as little as 5°C. In addition, local application of strophanthidin (0.1 mM) completely abolished the PGH. This evidence therefore supports the hypothesis that the post-glutamate hyperpolarization is produced by the activity of an electrogenic sodium pump which is stimulated by sodium accumulation during the depolarization. Preliminary data suggest that the electrogenic sodium pump is also activated by prolonged stimuli, such as direct depolarizing current injection, which evoke a steady train of action potentials. These data make it likely that the pump would be strongly activated during sustained epileptiform discharges. Steady state pump activity may also directly contribute to the resting potential. Post-glutamate hyperpolarization provides a useful physiological measure of sodium pump activity in hippocampal neurons.

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213.14 DIRECT EFFECTS OF NEOSTIGMINE ON THE END-PLATE RECEPTOR CHANNEL COMPLEX. J. F. Fiekers. Dept. of Anatomy and Neurobiology, Univ. of Vermont College of Medicine, Burlington, VT 05405.

Most effects of the anticholinesterase agents at cholinergic synapses are considered primarily indirect as a result of the inhibition of the acetylcholinesterase enzyme (esterase). However, additional direct actions of many of the anticholinesterase agents have also been reported. In the present study the effects of a carbamate antiesterase agent, neostigmine, (Neo) on the MEPC amplitude and decay kinetics and agonist induced end-plate current fluctuations were studied in voltage-clamped snake costocutaneous neuromuscular junctions. Neo, in concentrations up to 10^{-5}M, produced a concentration-dependent increase in both the amplitude and the time constant of MEPC decay (τ_{MEPC}) without altering the voltage dependence of τ_{MEPC}. Power density spectra obtained with either acetylcholine (ACh) or carbachol (CCh) were well fitted by a single Lorentzian with a characteristic frequency unchanged by neostigmine. Concentrations of Neo greater than 10^{-5}M produced a concentration dependent decrease in MEPC amplitude and a corresponding increase in τ_{MEPC}. In addition, the spectra of fluctuations produced by ACh could only be fitted with two Lorentzian components. Two component spectra were also obtained with CCh-induced current fluctuations indicating that the effects were not a result of esterase inhibition. Additional experiments were performed on muscles pretreated with collagenase (2 hours, 0.2%) to inactivate the esterase. Power density spectra obtained with either ACh or CCh-induced fluctuations also exhibited multiple time constants. In each case, the second, slower time constant was increased with either membrane hyperpolarization or increasing concentrations of Neo. The effects of Neo were reversible with washing. These results indicate that Neo produces direct effects on the receptor-channel complex which are unrelated to inhibition of esterase. Further, the voltage and concentration dependencies of each spectral component do not appear to support an open channel blocking mechanism. It is suggested that Neo, in addition to its actions on the esterase, has direct actions to (1) block the cholinergic receptor and (2) to produce drug-induced alterations in the gating kinetics of the receptor-channel complex at the neuromuscular junction.

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213.15 EFFECTS OF SOMAN ON ACETYLCHOLINE MEDIATED CONDUCTANCES IN APLYSIA CALIFORNICA NEURONS. Margaret G. Filbert* (SPON: J.F. Glenn) Neurotoxicology Branch, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Acute intoxication by organophosphorus anticholinesterase compounds produces cholinergic crises resulting in convulsions, ataxia, respiratory paralysis, coma and death. Treatment for exposure to these compounds is based on the premise that the toxic properties of the inhibitors are due to the persistence and accumulation of acetylcholine. Classical acetylcholine antagonists, however, such as atropine, d-tubocurarine and mecamylamine do not entirely reverse the effects nor significantly increase the LD50. A number of investigators have suggested that while the primary effects of the organophosphorus compounds can be attributed to the inhibition of cholinesterase, an additional action on effector organs may also be present.

Using current- and voltage clamp techniques, the effects of soman were examined on neurons of Aplysia californica. Acetylcholine and carbachol were applied by iontophoresis from double barrel micropipettes. Soman was dissolved in artificial sea water and applied by superfusion into a recording chamber which was continually perfused with artificial sea water. Membrane potential, input resistance and current-voltage relationship were monitored before and after application of soman. At 10^{-5}M and higher concentrations of soman, a marked increase in input resistance was observed. The response to acetylcholine usually showed an initial increase in amplitude and in duration while the response to carbachol was decreased in spite of the increased input resistance. The acetylcholine response subsequently decreased in amplitude. When the responses to acetylcholine and carbachol were normalized to constant input resistance a dose dependent inhibition by soman on response amplitude was apparent. Upon washout of soman by perfusion with soman-free sea water, recovery to control amplitude of both acetylcholine and carbachol responses was seen but the prolonged duration of the acetylcholine response, resulting from inhibition of acetylcholinesterase, remained unchanged. These results suggest that a direct effect of soman on passive properties of the membrane and a blocking action at the receptor-channel complex may occur in addition to the inhibition of acetylcholinesterase.

213.16 ROLE OF CYCLIC AMP IN ADENOSINE-INDUCED POTENTIATION OF MUSCARINIC HYPERPOLARIZATION IN NEUROBLASTOMA CELLS. Akinobu Tsunoo* and Toshio Narahashi (SPON: S.-C. Cheng). Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

We have previously reported that adenosine, 2-chloroadenosine, and prostaglandin E_1 (PGE_1) potentiate the muscarinic hyperpolarization but not dopamine depolarization in neuroblastoma cells, and proposed that this effect is produced by the receptor-mediated increase in cyclic AMP (Tsunoo, A. and T. Narahashi, Biophys. J. 41, 66a, 1983). The present study was carried out to substantiate the notion that adenosine and PGE_1 activate adenyl cyclase which in turn increases cyclic AMP producing its physiological effect.

Intracellular recordings were made from cultured cells of neuroblastoma N1E-115 line. Acetycholine and dopamine were iontophoretically applied. Other drugs were given by bath application or intracellular pressure injection through a second micropipette.

Injection of 10 mM cyclic AMP for 0.2-0.4 sec potentiated the muscarinic hyperpolarization to 173 ± 19% of the control (mean ± SEM, n=4) without changing the resting membrane potential, nicotinic depolarization and dopamine depolarization. Three-second injection caused a further potentiation up to 287 ± 59% of the control (n=4). However, injection of the carrier solution containing 150 mM KCl and 5 mM HEPES (pH 7.4), or 10 mM adenosine 5'-monophosphate did not affect the muscarinic hyperpolarization. It has been shown that activation of various receptor-related adenyl cyclase is GTP-dependent. Therefore, 3 mM GTP-γ-S, a GTP analog resistant to hydrolysis, was injected. This potentiated the muscarinic hyperpolarization to 162 ± 17% of the control (n=5) without application of adenosine and PGE_1. Morphine, which is known to inhibit adenyl cyclase, was examined whether it depressed the 2-chloroadenosine-induced potentiation of the muscarinic hyperpolarization. Bath application of 75 μM 2-chloroadenosine increased the amplitude of the muscarinic hyperpolarization to 273 ± 32% of the control, and 2 μM morphine decreased the 2-chloroadenosine-induced potentiation to 170 ± 16% of the control (n=4). Naloxone antagonized the inhibition by morphine. Potentiations by 75 μM 2-chloroadenosine in the absence and presence of 2 μM morphine and 8 μM naloxone were 260 ± 38% and 252 ± 32% of the contral (n=4), respectively.

The present results provide further evidence that cyclic AMP mediates the adenosine- and PGE_1-induced enhancement of the muscarinic hyperpolarization and that morphine inhibits such potentiation by depressing adenyl cyclase through activation of specific opiate receptors. Supported by NIMH grant MH36183.

213.17 MODELLING OF GLUTAMATE RECEPTORS IN THE DISTAL RETINA OF THE MUDPUPPY. M.M. Slaughter*, B. Kalman*, and R.F. Miller. (SPON: P. Simmons). Dept. of Ophthalmology, Physiology, and Biophysics, Washington University School of Medicine, St. Louis, Mo. 63110

Photoreceptors in the vertebrate retina are believed to use an excitatory amino acid neurotransmitter. Biochemical release measurements using HPLC techniques and pharmacological experiments using an electrophysiological approach indicate that glutamate is the cone transmitter in the mudpuppy retina. Although ultrastructural data indicates that these cone photoreceptors synapse onto three classes of neurons, our experiments have shown that each class of these second-order neurons contains a different type of glutamate receptor. We were interested in characterizing these three distinct glutamate receptors by comparing the molecular structures of compounds that selectively interacted with each receptor type. Experiments were performed in the superfused retina eyecup preparation using intracellular recording procedures. Conductance measurements and synaptic blocking agents were used to evaluate the mechanism and site of action of bath-applied drugs. Glutamate agonists and antagonists all have three apparent binding sites: 1) an alpha amino group, 2) an adjacent alpha carboxyl group, and 3) a more distant, terminal carboxyl group. When the spatial relationship between the first two binding sites was kept constant, the three types of glutamate receptors found in the distal retina could be distinguished by the distance between these two groups and the third, terminal carboxyl group. The ON bipolar synaptic receptors were selectively activated by L-2-amino-4-phosphonobutyrate, L-O-phosphoserine, ibotenate, and amino dicarboxycyclopentane. Comparisons of the three dimensional molecular structures of these compounds indicate that the ON bipolar receptor binds to an extended conformation of glutamate in which the intercarboxyl distance $\geq$ 4A. The synaptic input to the horizontal cells was antagonized by cis 2,3 PDA and selectively by D-O-phosphoserine. Matching the active sites of these two molecules suggests that the horizontal cell synaptic receptor binds to a partially folded configuration of glutamate with an intercarboxyl distance $\approx$ 3.5A. The OFF bipolar was less sensitive to large, conformationally restricted glutamate agonists such as kainic acid and the synaptic input was blocked by cis 2,3 PDA but not by D-O-phosphoserine. Although this does not delineate the precise spatial relationship of the binding sites for the OFF bipolar receptor, it does indicate that this receptor is activated by glutamate in a folded conformation in which the intercarboxyl distance is $\leq$ 3A. Three dimensional molecular models of the preferred ligands for each receptor will be presented.

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213.18 DESENSITIZATION OF THE GABA RECEPTOR IN HIPPOCAMPAL NEURONS ISOLATED FROM THE ADULT GUINEA PIG. Randy Numann* and R.K.S. Wong (SPON: L.Y. Huang). Dept. of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Individual neurons were isolated from the adult guinea pig hippocampus by using an enzymatic dispersion procedure. The GABA action on the pyramidal type neurons were studied using a suction electrode voltage clamp technique. GABA at 10^{-4}M was pressure ejected from a 10-15 MΩ pipette and found to elicit a hyperpolarizing-depolarizing response in these neurons. The reversal potentials were -70mV and -30mV for the hyperpolarizing and depolarizing responses respectively. The depolarizing response was minimally activated and often non-detectable when GABA concentrations were reduced to 10^{-5}M. To examine the desensitization of the hyperpolarizing response neurons were voltage-clamped to -30mV. GABA pulses applied then induced only outward currents. It was observed that when long duration (1-3 sec.) GABA pulses (10^{-5}M) were applied, the outward current rapidly attenuated with a time constant of 1.7 sec. Double pulse experiments also showed that the amplitude of the outward current induced by the GABA test pulse was reduced compared to the conditioning pulse. The time constant for recovery of the test pulse response was 5.0 sec.

This type of fading of the GABA induced hyperpolarization is commonly observed in mammalian central neurons. It has been considered that such fading can be attributed to at least three causes. (1) The gradual development of a separate depolarizing response, (2) accumulation of intracellular Cl^- and (3) true receptor desensitization. Since we can now clamp the membrane voltage to the reversal potential of the depolarizing response; contribution of (1) to our observed fading can be exlcuded. To evaluate the contribution of Cl^- accumulation upon desensitization we carried out paired pulse experiments using 10^{-5}M GABA with the conditioning pulse applied at two different clamped potentials (-30mV and -70mV). In a typical example from 15 experiments the conditioning pulse applied at -30mV elicited a large outward current (660pA). The test response applied 3 sec. later was 280pA. When the cell was briefly clamped to -70mV, the conditioning pulse applied at this potential produced no significant outward current yet the following test pulse applied 3 sec. later at -30mV still showed attenuation (260pA). Since attenuation of the test response occurred even in the absence of outward current flow during the conditioning pulse; we suggest that fading of the GABA response in hippocampal pyramidal cells cannot be attributed to intracellular chloride accumulation and that GABA receptor desensitization is the primary mechanism underlying this phenomenon.

213.19 KINETIC PROPERTIES OF CHOLINERGIC DESENSITIZATION IN VOLTAGE CLAMPED APLYSIA NEURONS (SPON: C. D. Anderson). A. F. Hall*, N. T. Slater* and D. O. Carpenter, NYS Dept. of Health, Albany, NY 12201

The influence of agonist concentration and membrane potential on the kinetics of desensitization onset of excitatory acetylcholine (ACh) responses in Aplysia RB neurons have been studied. Neuronal cell bodies were mechanically isolated from abdominal ganglia and voltage clamped with two microelectrodes. ACh was rapidly microperfused over the cell for periods of several minutes, followed by 30 minute recovery periods, and the time course of the decline of the ACh-induced current at varying membrane potentials or agonist doses was recorded. Computer simulations of the current decline best fit the experimentally observed responses as the sum of two exponentials plus a constant (C):

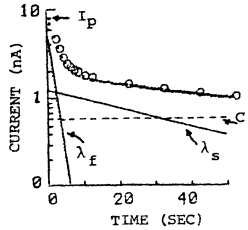

Fig. 1 Desensitization of ACh-induced inward current. Open circles represent experimental results, dotted lines through the circles represent the current decline according to the form:

$$I = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} + C$$

where $\lambda_f = 1/\alpha$, and $\lambda_s = 1/\beta$

With increasing agonist dose the peak ACh-induced current extrapolated to time zero (I_p) was increased and the time constants of both exponential components were reduced, resulting in an acceleration of the normalized rate of desensitization and sigmoidal relationship between I_p and ACh dose (1 to 50 μM).

For a fixed agonist dose (50 μM), increasing membrane potential resulted in a linear increase of I_p; however, no change in the rate of either exponential component was observed over the range of membrane potentials studied (-50 to -110 mV).

Desensitization is currently viewed as a cyclic scheme in which the activated but closed state of the receptor-ionophore complex may switch to form an agonist-bound but inactivated ('desensitized') complex. The ACh dose dependency and voltage independence of desensitization onset of this response is consistent with this scheme. (Supported by USPHS NS 18435).

213.20 CALMODULIN INHIBITORS ACCELERATE CHOLINERGIC DESENSITIZATION IN VOLTAGE CLAMPED APLYSIA NEURONS. N. T. Slater*, A. F. Hall* and D. O. Carpenter (SPON: J. A. Ramaley). New York State Department of Health, Albany, NY 12201.

The excitatory response of Aplysia RB neurons to acetylcholine (ACh) is pharmacologically similar to the vertebrate nicotinic response. In isolated, voltage clamped cell bodies of Aplysia abdominal RB neurons, the decline of the ACh-induced inward current is the sum of two exponential components plus a constant (Hall et al., Neurosci. Abst., 1983). This preparation allows known concentrations of agonists and other compounds to be applied by rapid microperfusion, and the kinetics of desensitization to be studied in a quantitative fashion.

We have studied the effects of the calcium antagonists D-600, SKF 525A and the neuroleptic trifluoperazine (TFP) on the kinetics of desensitization onset to microperfused ACh. Over the concentration range 0.1 to 20 M these compounds produce a dose-dependent acceleration of the rate of desensitization, the order of potency being TFP>SKF 525A>D-600. This acceleration of desensitization is associated with a dose-dependent increase in the relative contribution and rate of the fast exponential component and a decrease in the ratio C/Ip, where Ip is the peak ACh-induced current and C is the steady-state current to which the response declines. This effect does not appear to be related to actions of the compounds such as channel blockade, as the acceleration of desensitization is often maximal at concentrations which have little effect on Ip. Also channel blockers such as hexamethonium have no effect on desensitization.

Previous investigations of the mechanisms underlying cholinergic desensitization at the vertebrate neuromuscular junction have demonstrated that the rate of this process is accelerated by increases in intracellular free calcium (Chesnut, J. Physiol. 336:229, 1983). A diverse number of pharmacological agents, including neuroleptics and calcium antagonists, also accelerate desensitization by a mechanism generally attributed to some local anesthetic action. However, these compounds share an ability to inhibit the calcium-binding protein calmodulin, TFP being the most potent calmodulin inhibitor described (Volpi, et al., PNAS, 78:795, 1981). In light of these actions and the proposed calcium dependence of desensitization, we suggest that the acceleration of desensitization observed in these experiments may result from an inhibition of calmodulin, thereby increasing intracellular free calcium levels. It remains to be determined whether calcium ions act directly to accelerate the rate of inactivation of the ACh receptor protein, or whether a calcium-dependent protein kinase is involved. (Supported by USPHS grant NS18435).

VESTIBULAR SENSORY ORGANS

214.1 PERILYMPHATIC AND ENDOLYMPHATIC ACTION OF OUABAIN ON THE MEMBRANE POTENTIAL OF THE AXOLOTL (AMBYOSTOMA MEXICANUM) VESTIBULAR NEUROEPITHELIAL CELLS. Enrique Soto E.*, R. Budelli*, M.T. González Estrada* y H. Bracho*.(SPON: Maria Mirta Rodriguez Budelli).Depto. Investigaciones Biomédicas, Instituto de Ciencias de la Universidad Autonoma de Puebla, y Unidad de Investigaciones Cerebrales del Instituto Nacional de Neurología y Neurocirugía.México.

The epithelium of the inner ear separates two fluids of different ionic composition: the endolymph, which has a high potassium content (above 100 mM) and the perilymph, with low potassium content (below 5 mM).

The mechanism responsible for this high endolymphatic K^+ concentration is unknown. The stria vascularis has been proposed as responsible for this high K^+ concentration.Since the Axolotls have no stria vascularis, neuroepithelial vestibular cells may be involved in endolymph production. In order to mantain this ionic distribution, it is necesary to actively transport K^+ from the perilymph. We propose that this transport is achieved by the concerted action of two pumps. One, located in the perilymphatic (basolateral) membrane would transport K^+ into the cell. The Na^+-K^+ dependent ATPase may be responsible for this.Another pump located in the endolymphatic (apical) membrane would transport K^+ form the inside of the cell to the endolymph. In this study we test if the Na^+- K^+dependent ATPase is located in the basolateral membrane, by recording membrane potentials of neuroepithelial cells when perfusing with ouabain (10^{-4} M) either the basolateral or the apical membrane in an in vitro preparation of the Sacular epithelium of the Axolotl.

While ouabain placed at the endolymphatic side exerted practically no effect, a clear depression of membrane potentials was observed when the drug acted on the perilymphatic basolateral side.

Our results suggest that Na^+- K^+dependent ATPase is located only at the basolateral membrane of the neuroepithelial vestibular cells.

214.2 COMPARATIVE STUDIES OF OTOCONIAL GROWTH AND DEVELOPMENT IN BIRDS, AMPHIBIA AND REPTILES. J. Ballarino* and H.C. Howland (SPON: R. Hoy). Sections of Physiology and Neurobiology and Behavior, W204 Mudd Hall, Cornell University, Ithaca, NY, 14853.

Otoconia are crystals of high specific gravity which form the weight of the gravity receptors of the inner ear. Each otoconial crystal consists of an organic matrix and crystalline calcium carbonate. In birds, mammals and some reptiles the calcium carbonate is crystallized in the form of calcite, while in amphibians and some reptiles, it is crystallized in the form of aragonite. Previously, we have investigated otoconial morphology in developing chicks using scanning electron microscopy and polarized light microscopy and have found various forms of otoconia (Ballarino, J. and H.C. Howland, Anat. Rec. 204:83, 1982). Based on the time of appearance and frequency of occurrance of the various forms, we have proposed a normal growth sequence for otoconia in the chick. Our studies of chick otoconial development indicate that the organic matrix may play some role in guiding the formation of calcium carbonate into a stable calcite crystal. This raises the question of whether the same is true of aragonite formation in amphibia and many reptiles. Is there a similar sequence of events for aragonite and calcite formation? In particular, how do some reptiles form both calcite and aragonite otoconia? What is the pattern of distribution of calcite and aragonite otoconia during the development of these reptiles? Based on scanning electron microscopy and polarized light microscopy of developing amphibian and reptilian otoconia, we will present data on the sequence of events in the formation of otoconial aragonite and calcite in amphibia and reptiles and compare their development to that of the chick.

214.3 QUANTITATIVE ANALYSIS OF HAIR CELL AND GANGLION CELL PROLIFERATION IN THE FISH SACCULE. A. N. Popper and B. Hoxter*. Dept. of Anatomy, Georgetown University Schools of Medicine and Dentistry, Washington, D. C. 20007.

The complete complement of sensory hair cells in the ears of amniotic vertebrates appears to arise during embryonic development. The only post-embryonic change involves loss of sensory cells. In contrast, there is evidence that sensory hair cells continue to be added well into post-embryonic stages in some anamniotes (Corwin, J. T., J. Comp. Neurol., 201:541-553, 1981; Platt, C., J. Comp. Neurol., 172, 283-297, 1977). However, evidence for continuous proliferation has not been quantified, and so its extent has heretofore been unclear. In order to obtain data on sensory hair cell proliferation we have investigated the number of sensory hair cells and eighth nerve ganglion cells in the saccule of the cichlid fish Astronotus ocellatus (the oscar) using animals from 2.0 cm to 19.0 cm in standard length.

The smallest animals (2.0 cm) had about 5,000 hair cells while larger specimens (over 16 cm long) had over 150,000 sensory cells. The increase in number of sensory cells in animals from 2 to 15 cm in length was correlated (r^2=0.7) with increases in both fish length and body weight. The density of sensory hair cells was greater at the margin of the epithelial region than in the more central region. However, the densities in any one selected region did not vary between fish, even when the overall number of hair cells in the saccule increased substantially.

In contrast to the steady increase in sensory hair cell number in animals up to 15 cm long, all larger animals had about the same number of hair cells even though the sensory epithelium itself grew somewhat. This was further reflected in differences of sensory cell density between the animals above 15 cm and the animals smaller than 15 cm. While the density of cells was similar in the peripheral regions of the epithelium for both groups, the larger group had a density of hair cells in the central region that was significantly smaller than the density in the smaller animals.

Ganglion cells also increased with the size of the animal. The smallest fish had approximately 150 ganglion cells, while the largest animals had about 750 cells. If we make the assumption that all sensory cells are innervated by afferent fibers, it becomes apparent that the ratio of sensory cells to ganglion cells in large fish is much greater than in small fish. Thus, each afferent fiber innervating the saccule receives input from over 200 sensory cells in large animals and about 30 cells in small animals.

The increase in number of sensory cells in Astronotus is the first detailed quantitative demonstration of substantial post-embryonic hair cell proliferation in the ear of any vertebrate. The implications of this increase for the function of the saccule in fishes in not yet clear, nor do we have any understanding of the functional significance of the substantial change in the sensory cell to ganglion cell ratio in different sized animals. (Supported by grants from NSF and ONR.)

214.4 GROWTH OF TWO DISTINCT POPULATIONS OF HAIR CELLS IN THE LAGENA OF THE GOLDFISH INNER EAR. Christopher Platt. Dept. Biological Sciences, Univ. Southern California, Los Angeles, CA 90089.

The lagena in the goldfish ear has an unusual macula because it contains not one, but two populations of hair cells having large ciliary bundles. The caudal patch is separated from the rostral patch by a zone of hair cells with small ciliary bundles; the caudal patch is covered by the otolith, but the rostral patch is covered only by otolithic membrane. During development of many frogs, the amphibian papilla fuses from two patches into a contiguous strip with the two ends showing different acoustic sensitivities (Lewis, E. R., Brain Res., 219:149, 1981). To see how the two separate patches in goldfish develop, scanning electron microscopy (SEM) was used on lagenar maculae from goldfish ranging in length from 20-100 mm and mass from 0.2 to over 20 g. After conventional fixation, dehydration and critical-point drying, tissues were photographed using SEM for measurements primarily on three size classes of fish.

From the tiniest through the largest fish, the rostral and caudal patches of hair cells with large bundles remained distinctly separate. Total macular area was as small as 5.2 x $10^4 \mu m^2$ for fish of 20-30 mm, 26 x $10^4 \mu m^2$ for 40-50 mm fish, and 39.8 x $10^4 \mu m^2$ for 70-80 mm fish. The rostral patch occupied 20-30% of this total area in all three classes, with larger relative areas in larger fish; the caudal patch occupied 15-20% of the total area in all three classes. At a pair of consistent sites of sampling in the lagenar patches, the populations showed a density of 500-600 hair cells/$10^4 \mu m^2$, in all size classes. The linear distance between the patches increased from 30 µm in the smallest size, 90 µm in the middle size, to over 160 µm in the largest.

The results show that the area of the macula grows at a rate that initially may be near the square of linear growth proportion for body length, but slows with increasing size. Since the patches with large bundles maintain both a similar proportional area and similar cell density during growth, the expansion of the patches and macula is not simply spreading of a fixed number, but must involve addition of cells. Also, since the inter-patch gap expands, either there must be two growth zones in the macula, or else cells would need to metamorphose from a type with a large bundle to a type with a tiny bundle. The second alternative is unlikely on the basis of present knowledge.

These data suggest that in goldfish, the lagenar macula expands by adding new hair cells in at least two areas: 1) not only around the periphery, as usual, but also 2) between the margins of the rostral and caudal patches of hair cells with large bundles.

214.5 RECEPTOR-CALYX JUNCTION IN THE CRISTA AMPULLARIS OF THE DEVELOPING CHICKEN. K.D. Peusner, N.H. Lindberg* and P.F. Mansfield*. Dept. of Anatomy, Geo. Washington Univ. Sch. of Med., Wash., D.C. 20037 and Jefferson Med. Col., Phila., Pa. 19107.

Vestibular ganglion cells form colossal fibers that centrally innervate the principal cells of the avian lateral vestibular nucleus (tangential nucleus) by large spoon endings. Peripherally the colossal fibers synapse with hair cells of the cristae ampullaris by means of large calycine endings. The spoons undergo developmental changes in synapses as studied in 15 day embryos, hatchlings and 3 year old chickens (Peusner, '82). To determine if a relationship exists between developmental events in the endorgan and the central connection, calyces and their synaptic contacts were examined at corresponding ages.

The linear surface of hair cells and their membrane specializations apposed to calyces were measured on electron micrographs. Profiles of type I hair cells (HCI) are enclosed by a calyx except at the cell's apex. Type II hair cells (HCII) are not surrounded by calyces, but do juxtapose outer calycine surfaces where synapses occur. Membrane specializations measured include: ribbon synapses, asymmetric membrane densities and agranular reticulum (AR). Membrane densities may be partial sections through ribbons. AR closely parallels portions of the hair-cell plasmalemma. The results are tabulated below:

	Embryo	Hatch.	3 Y.O.
HCI-Calyx no. of profiles:	49+	146	132
total linear surface measured	932um	4,266um	3,707um
a. ribbon synapses	0.64%	0.22%*	0.48%
b. membrane densities	2.42%*	0.18%*	0.48%*
c. AR	7.98%*	10.64%	10.54%
linear surface covered by a,b,c	11.04%	11.04%	11.50%
HCII-Calyx no. of profiles:	28+	49	49
total linear surface measured	197um	624um	343um
a. ribbon synapses	4.57%	2.02%*	3.26%
b. membrane densities	7.22%	6.68%	11.08%*
c. AR	0	0	0
linear surface covered by a,b,c	11.79%	8.70%*	14.33%

* Statistically significant from other two ages; + preliminary.

In conclusion, hatchlings show a significant decline in ribbons at the calyx junction with HCI and HCII. There was no diminution of ribbons at the calyx junction with increased age, up to 3 years. Interestingly, there is a constancy in amount of linear surface of HCI-calyx junction covered by membrane specializations during development. From this and previous studies, it appears that birth or thereabouts marks a time for major developmental changes peripherally and centrally in the vestibular system, notably a decline in "chemical" transmission sites. (Supported by USPHS grants 2 R01 NS18108 and 5 R01 NS15633).

214.6 PERIPHERAL INNERVATION PATTERNS OF INDIVIDUAL VESTIBULAR NERVE AFFERENTS IN THE CHINCHILLA. R.A. Baird, C. Fernandez* and J.M. Goldberg. Depts. Pharmacol. and Physiol. Sci. and Surgery (Otolaryngology), Univ. Chicago, Chicago, IL 60637

The detailed innervation patterns of individual afferents have not been described, nor have these patterns been related to afferent physiology. To study these innervation patterns, extracellular injections of HRP were made into the superior vestibular nerve in four chinchillas. Peripheral arborizations of 175 fibers, located in the superior or horizontal cristae or in the utricular macula, were reconstructed and were presumed to be the processes of afferent, rather than of efferent, neurons. The terminal fields can be divided into calyceal, bouton-like, or dimorphic patterns, depending, respectively, on whether they only have calyx endings, only have bud-shaped endings, or have both types of endings. Typically, all of the endings are located within 50 um of the parent process. All three patterns are seen both in the cristae and in the macula. Dimorphic patterns are more commonly seen in the macula, whereas calyceal and bouton-like patterns are more frequently seen in the cristae.

Calyceal patterns. These patterns have been found only in the apex of the cristae and in the striolar region of the macula. A fiber can run undivided to terminate in a single calyx ending or else can divide into two branches, each terminating in a calyx ending. The calyx ending can be simple, apparently containing a single hair cell, or complex, apparently embracing several hair cells. So far, complex endings have been seen only in the macula. Bouton-like patterns. In the canals, these patterns are found only in the base of the cristae. Only one example of this pattern has been seen in the macula, where it was located in the extrastriolar region. Typically, there are 15-30 bud-shaped endings, with a maximum of 50, per fiber. Dimorphic patterns. These patterns occur in all regions of the sensory ephithelia, including the base, slope and apex of the cristae and the striolar and extrastriolar regions of the macula. Usually, 1-3 calyx endings and 5-25 bud-shaped endings are seen for each fiber, although macular afferents with up to 8 calyx endings are found. Both canal and macular afferents can have as many as 50 bud-shaped endings.

Intracellular injections of HRP into physiologically characterized afferents have been started. Fibers with calyceal patterns are irregularly discharging, whereas fibers with dimorphic patterns can be either regularly or irregularly discharging. To date, no physiologically characterized afferent has had a bouton-like innervation pattern. (Supported by NIH grants F32 NS06679 and NS01330 and NASA grant NAG2-148.)

214.7 FUNCTIONAL AND MORPHOLOGICAL CHARACTERISTICS OF GRAVITY-SENSITIVE PRIMARY CANAL AFFERENTS. A.A. Perachio, M.J. Correia, and G.A. Kevetter (SPON: A.R. Eden). Depts. Otolaryngol., Physiol. & Biophys., Anat., Marine Biomedical Institute, Univ. Texas Medical Branch, Galveston, TX 77550-2778.

In the anesthetized gerbil a large percentage of physiologically identified lateral (71%) and anterior (48%) canal afferents respond to static changes in head tilt position (Perachio et al., Neurosci. Abstr. 7: 148; 1981). Since fluctuations in anesthetic level may alter the characteristics of the sensory elements of the labyrinth and/or of vestibular afferents, the experiment was replicated in unanesthetized, decerebrated gerbils. First the spontaneous activity of lateral and anterior canal afferents was measured while the head was held in the standard position (lateral semicircular canals co-planar with earth's horizontal plane). The average discharge rate (87.6 ± 44.2 imp/s, n = 91) was significantly faster than that measured in the canal afferent sample in anesthetized animals (66.2 ± 33.0 imp/s, n = 84). In both types of preparations, a significant inverse correlation was obtained between mean firing rate and the coefficient of variation (CV); decerebrate r = −0.48; unanesthetized r = −0.83. In decerebrated animals irregularly firing afferents (CV > 0.5) constituted a smaller percentage of the sample (13.2%) than that of the anesthetized sample (25%). In decerebrated preparations, significant changes in firing rate (> 10%) were measured when the head was tilted ± 10 deg. about the pitch (left/right) axis: lateral 57% (n = 17/30), anterior 53% (n = 19/36). The magnitude of the response was significantly correlated to the CV (standard position) except for decerebrate anterior afferents. Preliminary studies have been conducted to determine whether these response characteristics are related to afferent cell morphology. Afferents have been injected (n = 50 to date) with HRP. As previously reported for cat Scarpa's ganglion neurons (Chat and Sans, Neurosci. 4: 651; 1979), multipolar neurons have been identified with branching axonal or peripheral (dendritic) processes. These results suggest a possible mechanism for converging inputs from different receptors or interconnections between primary afferent neurons.

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214.8 ACTIONS OF ATROPINE AND BICUCULLINE ON THE ACTIVITY OF AFFERENT FIBERS IN THE XENOPUS LAEVIS LATERAL LINE. R. P. Bobbin, S. Bledsoe, Jr., G. L. Jenison, S. Winbery,* and G. Ceasar*. L.S.U. Medical School, Dept. of Otorhinolaryngology and Biocommunication, Kresge Hearing Research Lab. of the South, New Orleans, LA 70119 and Kresge Hearing Research Institute, Univ. of Michigan Medical School, Ann Arbor, MI 48109.

An excitatory amino acid (e.g., glutamate, aspartate) has been proposed as an afferent excitatory transmitter and acetylcholine has been proposed as an efferent inhibitory transmitter in the lateral line of Xenopus laevis. On the other hand, GABA has recently been shown to supress, and both acetylcholine and carbachol to increase the spontaneous activity in the lateral line (Chihal et al., ARO Midwinter Abstr., 1980, Winbery et al, ARO Midwinter Abstr., 1983). Therefore, to further define these responses we examined the effects of the exogenous application of atropine sulfate, and bicuculline methiodide on afferent stimulation and the action of various agonists.

The compounds were dissolved in a frog ringer solution and then applied to the serosal surface of an isolated frog lateral-line stitch utilizing techniques previously described (Bledsoe and Bobbin, Exp. Brain Res., 32:315-320, 1980).

Atropine (1-10 μM) blocked the response to carbachol (5 μM), but did not affect the respose to L-glutamate (1 mM), and had only very little suppressant affect on spontaneous activity and excitation induced by water motion. Bicuculline, at high concentrations (100μM), blocked GABA (1.25-5mM), did not affect the excitation to carbachol (5μM), and exerted only a slight suppressant effect on spontaneous activity and excitation induced by water motion .

Atropine and bicuculline had little effect on excitation induced by water motion at doses which blocked their respective agonists. Therefore, these results indicate that neither acetylcholine nor GABA are probably the excitatory afferent transmitter in the lateral line of Xenopus laevis. Future experiments with further explore the site of action of charbachol and GABA.

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214.9 TERMINATION OF VESTIBULAR AFFERENTS IN THE VESTIBULAR NUCLEAR COMPLEX IN THE GERBIL. G.A. Kevetter and A.A. Perachio. Depts. Otolaryngol., Anat., Physiol. & Biophys., Marine Biomed. Inst., Univ. of Texas Medical Branch, Galveston, TX 77550-2778.

Injections of horseradish peroxidase (HRP) were made into individual ampullae of the peripheral vestibular apparatus in gerbils to determine the terminal distribution of each receptor organ. Transganglionic transport allowed visualization of the central course and terminals of the vestibular nerve in the medial (MVN), descending (DVN), superior (SVN), or lateral (LVN) vestibular nuclei. Ganglion cells in either the superior or inferior division of Scarpa's ganglion were labeled. Both small and large ganglion cells (diameters 12.2-24.75 μm) were labeled. Occasionally multipolar cells in the ganglion were labeled.

Some animals received peripheral injections that spread to include many of the structures innervated by the anterior or posterior division of the vestibular nerve. The anterior division of the nerve had cell bodies located in the superior ganglion. Descending fibers coursed in the DVN, leaving to travel and terminate medially in MVN. Terminals were also identified ventrally in DVN and LVN, particularly at the junction between these nuclei. Other terminals were found rostrally in SVN and the vermis of the cerebellum. After injection of receptors whose afferent fibers travel in the posterior division of the vestibular nerve, labeled ganglion cells were present in the inferior Scarpa's ganglion. Terminals in MVN were found more lateral than the terminal distribution after injection of structures innervated by the anterior nerve. Terminals were located ventrally in DVN, especially along the MVN/DVN border. Fibers and terminals were also seen in cell group y, SVN, and the cerebellar vermis. These studies have confirmed differences in the terminal distribution of the anterior and posterior divisions of the vestibular nerve. After ionophoresis of HRP into the lateral ampulla, ganglion cells were labeled rostrally in the superior division of Scarpa's ganglion. Terminals were found in the medial ventral part of MVN. Terminals and fibers were seen in the ventral part of LVN as the fibers traveled toward MVN. Fibers also traversed LVN in a dorsal direction, many terminating in SVN. When the ampulla of the posterior canal was injected, labeled ganglion cells were located in the inferior Scarpa's ganglion. Heavy fascicles of fibers descended through DVN. Terminals were found caudally in MVN and DVN and also more rostrally in SVN and in MVN, adjacent to the ventricle. This study has identified differences and similarities in the areas of the vestibular nuclei where primary afferent input from orthogonally oriented canals (horizontal vs. posterior) is received and processed.

(Supported in part by NAG2-26 (AAP) and NAS9-14641 (MJC).)

214.PO APPLICATION OF A STOCHASTIC VERSION OF AN AFTERHYPERPOLARIZATION MODEL TO THE STEADY-STATE DISCHARGE OF VESTIBULAR-NERVE AFFERENTS. Jay M. Goldberg and Charles E. Smith*, Dept. Pharmacol. and Physiol. Sci., Univ. Chicago, Chicago, IL 60637 and Dept. Statistics, North Carolina State Univ., Raleigh, NC 27650

An afterhyperpolarization (AHP) model, modified from Kernell [Brain Res. 11, 685-687 (1968)], simulates many features of the steady-state responses of vestibular-nerve afferents to natural stimulation of the end organs and to galvanic stimulation of the afferents themselves. Major assumptions are: a) There is a single spike-trigger site and firing occurs whenever the transmembrane depolarization there reaches a fixed critical level. b) Postsynaptic voltage fluctuations, due to the random times of occurrence of quantal synaptic inputs, are entirely responsible for interspike-interval variability. c) Following each spike, there is a time-dependent increase in potassium conductance (g_K) that leads to an AHP. d) The g_K's left over from previous spikes add linearly with that triggered by each spike. e) Galvanic currents act only at the trigger site. Many differences between regularly and irregularly discharging afferents can be explained if the units differ both in their synaptic noise and in the prominence of their AHPs. The more irregular the discharge of a unit, the greater is the assumed synaptic noise and the smaller and briefer is the assumed AHP. Of the two factors, variations in AHPs appear to be four times more influential in determining the relative discharge regularity across units. The model accounts for the interspike-interval statistics obtained during natural stimulation and their modification by galvanic stimulation; the positive proportionality between discharge regularity, measured by a normalized coefficient of variation, and galvanic sensitivity, the latter measured in spikes•sec^{-1}/μA; and the lengthening of the mean interspike interval following an interposed, shock-evoked spike. (Supported by NIH Grant NS 01330, NASA Grant NAG 2-148, NSF Grant ISP 80-11451, and the South Carolina State Appropriation for Biomedical Research.)

215.1 ARACHNOIDITIS CHANGES PAIN SENSITIVITY IN MICE. B.T. Lipman and V.M. Haughton*. Allen Bradley Laboratory, Medical College of Wisconsin, Milwaukee, Wisconsin 53226.

The possibility that arachnoiditis causes increased pain sensitivity has been suggested by clinical studies but the effects of arachnoiditis on pain threshold has not been studied experimentally.

Tail-flick latencies were compared in 2 groups of mice, one with arachnoiditis after intrathecal injection of iocarmate, and a control group, after intrathecal injection of artificial cerebrospinal fluid (CSF). In both groups the lumbar subarachnoid space was punctured via a 27 G needle and 30 ul of artificial CSF or iocarmate injected by a Hamilton syringe with the animal under halothane anesthesia. Intrathecal injection of iocarmate was verified radiographically. Sensitivity of tail-flick latency to intraperitoneal morphine sulfate was tested in each group. The arachnoid was studied histologically in both groups after necropsy to verify arachnoiditis. The threshold intensity of thermal stimulation producing a tail-flick response was measured in both groups.

Tail-flick latencies were significantly decreased at 2, 3, and 4 weeks after iocarmate myelography. No significant changes in tail-flick latency were measured in the control mice. The data suggests that arachnoiditis causes hyperalgesia in mice.

215.2 STUDIES ON THE PHARMACOLOGY OF SPINAL OPIATE RECEPTORS WHICH MODIFY THE RAT'S RESPONSE ON CUTANEOUS THERMAL AND VISCERAL CHEMICAL EVOKED RESPONSES: TEST SELECTIVITY AND A DISASSOCIATION OF μ, δ AND κ RECEPTORS. C. Schmauss*, T.L. Yaksh*, Y. Shimohigashi*, T.S. Jensen*, G. Harty* and D. Rodbard* (SPON: B.F. Westmoreland). Dept. of Neurosurgical Res., Mayo Clinic, Rochester, MN; NIH, Bethesda, MD; Max Planck Institut, Munchen, Germany; Aarhus Univ., Aarhus, Denmark.

We have examined the pharmacological characteristics of spinal opiate receptors which modify the animal's response to a strong stimulus. Dose response curves were carried out with a variety of putative opiate receptor selective agonists administered intrathecally in the rat. Three measures were examined, hot plate (HP), tail flick (TF) in the same group, and the acetic acid evoked writhing response (AAEWR) in a separate group.

	ED_{50} (nM)[2]		
	TF	HP	AAEWR
Metkephamid (μ/δ)	0.018	0.020	0.6
β-Endorphin (μ/δ)	0.075	0.091	0.7
Morphine (μ)	3.9	3.1	1.8
d-ala^2-d-leu^5-enkephalin (DADL,δ)	1.6	2.5	>18
DPE_2 (dimeric enkephalin pentapeptide,δ)	0.36	0.13	>20
DTE_2 (dimeric enkephalin tetrapeptide,δ)	2.3	0.9	>19
U50488H (κ)	>430	>430	17

[1]All drugs administered in 15 μl. [2]Each ED_{50} based on least squares regression analysis of 3-5 doses and 6-36 animals/dose.

To determine whether the effects of putative δ ligands on cutaneous thermal tests are mediated by spinal receptors distinguishable from those acted upon by morphine, rats with intrathecal catheters received one 75-mg morphine pellet, twice during 5 days. Dose response curves were carried out in morphine tolerant and drug naive animals.

	TF ED_{50}-Naive	TF ED_{50}-Tolerant
Morphine	1.9	18.4
DADL	1.3	1.1

These data suggest that: μ ligands are equally active in all tests, but δ ligands are selectively effective in cutaneous thermal but not visceral chemical tests. κ ligands show the reverse efficacy. Mixed μ/δ ligands are exceedingly potent on cutaneous thermal tests, but lose a significant proportion of their actions on the AAEW. The failure to see cross tolerance suggests that morphine and δ ligands act upon distinguishable spinal receptors to block cutaneous thermal evoked responses. These data are consistent with the existence of 3 discriminable populations of spinal receptors and a differential association with spinal substrates modulating the response to cutaneous thermal and visceral chemical stimuli. (DA02110 and Mayo Foundation)

215.3 STUDIES ON THE ACTION OF MORPHINE IN THE PERIAQUEDUCTAL GRAY (PAG), RAPHE MAGNUS (RM) AND THE N. RETICULOGIGANTOCELLULARIS (NRGC). EFFECTS OF INTRATHECAL PHENTOLAMINE, METHYSERGIDE, NALOXONE AND FLUPHENTIXOL. T.S. Jensen* and T.L. Yaksh* (SPON: J.F. Poduslo). Section of Neurosurgical Research, Mayo Clinic, Rochester, MN; Aarhus Univ., Aarhus, Denmark.

To determine whether opiate receptor linked systems in the brainstem produce their antinociceptive effects by the activation of spinopetal systems, the receptor antagonist for the proposed descending systems must be administered intrathecally (IT), near the spinal terminal fields of these systems. To carry out these experiments rats were stereotaxically implanted with guide cannula in the PAG, NRM or NRGC and a lumbar intrathecal catheter passed 7.5 cm caudally through the cisternal membrane. Rats in which morphine (5 μg/0.5 μl) produced at least a 2 sec increase in the tail flick (TF) latency and 20 sec increase in hot plate (HP) latency within 15 min of the microinjection were given either phentolamine (15 μg), methysergide (15 μg), fluphentixol (10 μg) or naloxone (10 μg). These antagonist doses were shown to block the changes produced by doses of exogenous agonists which completely blocked the TF. The inserted table presents the percent maximum possible reversal of the effect, as compared to the response latency measured immediately prior to the IT injection.

		Sac	Phen	Methy	Phen + Methy	Flu	Nal
TF	PAG	103	28*	61*	12*	101	82
	NRM	100	64*	33*	19*	96	52*
	NRGC	101	36*	80*	28*	100	54*
HP	PAG	102	74*	64*	40*	98	97
	NRM	108	104	86	91	106	98
	NRGC	98	84	100	84	101	96

* $p < 0.05$; each cell is based on results obtained of 5-15 brain sites; none of the antagonist treatments produced a change in baseline latencies.

Opiate linked brainstem receptor systems produced an effect on the TF which was uniformly mediated by spinal 5-HT/NE receptors. NRM/NRGC, but not the PAG showed a significant spinal naloxone-sensitive link. Perhaps most surprising was the failure of any of the treatments to produce a reversal of the HP effects of brainstem opiates comparable to that observed on the TF, even with higher spinal doses. These observations suggest that: 1) many of the effects observed on TF may only be relevant to motor processing; 2) that brainstem opiates either activate a pharmacologically novel spinopetal system or produce their "analgesia" (as opposed to antireflexia by a supraspinal mechanism). (NS16541 and Mayo Foundation)

215.4 EFFECT OF MORPHINE, FENTANYL AND NALOXONE ON SPONTANEOUS AND EVOKED NEURONAL ACTIVITY IN THE INTACT AND DEAFFERENTED LUMBAR DORSAL HORN. J. Ovelmen-Levitt, B. Johnson, P. Bedenbaugh, and B.S. Nashold, Jr. Div. of Neurosurgery, Duke Univ. Med. Ctr., Durham, N.C. 27706

The activity of lumbosacral dorsal horn neurons in laminae III-VII was studied before and after IV administration of morphine SO_4 (3mg/kg), fentanyl (.05 mg/kg), and naloxone HCl (.16mg/kg) in eleven control cats and in six animals with unilateral avulsions of the dorsal roots L_4 - S_1. Recordings were made using 3M KCl filled capillary micropipettes. The cats were lightly anesthetized with sodium pentobarbital at the time of the recordings, and arterial blood pressure and end tidal CO_2 were monitored. Spontaneous activity and receptive field properties were determined. In control animals, morphine was not found to alter responses in cells driven by light cutaneous stimulation only. Morphine produced a decrease in the spontaneous activity in wide dynamic range (WDR) multimodal cells and also in the evoked response to cutaneous noxious stimulation. This was also the case with IV fentanyl in two neurons, while in another two cells there was a decrease in the response but not in the spontaneous firing. These effects were reversed by naloxone. By one month cells can be found in the avulsed dorsal horn which can be easily influenced from the ipsilateral flank and/or the contralateral flank and limb. Morphine was found to depress the spontaneous firing of several cells one month after avulsion injury. One year after avulsions, fentanyl altered the firing pattern without decreasing the level of spontaneous firing in a cell in L_6-L_7 which was driven by strong pinch to the skin of the groin, bilaterally. The evoked response was more fatigueable after fentanyl. These effect were also reversed by naloxone. In both the control and the deafferented preparation spontaneous activity and evoked responses were affected differentially. Because they were administered systemically, these subsances may be acting directly on the cells recorded, indirectly at the level of the substantia gelatinosa, at a supraspinal level on descending modulatory systems, or they may be acting presynaptically.

215.5 ANALGESIC EFFECTS AND BINDING PROPERTIES OF A FLUORINATED ANALOG OF DYNORPHIN(1-13). J.M. Walker*, E.A. Young*, D.H. Coy*+, and H. Akil (SPON: S. Berent). Mental Health Res. Inst., Univ. of Michigan, Ann Arbor, MI 48109; Dept. of Medicine, Tulane Univ., New Orleans, LA.

Despite the high affinity of dynorphin for opiate receptors, its analgesic effects have been difficult to establish. In our hands dynorphin fails to produce analgesia upon micro-injection in either the periaqueductal gray or the lateral ventricle. We also failed to observe analgesic effects with D-Ala^2-dynorphin. We do confirm the findings of Herman et al that C-terminal shortened analogs (D-Ala^2-dynorphin(1-11)-NH$_2$) produce analgesia. These results are consistent with the previous suggestions that two active sites occur within the dynorphin sequence.

Generally, for enkephalin-containing peptides the aromatic character of position 4 (Phe) must be maintained for opiate activity. This limits the types of substitutions that can be made. One approach to the development of a potent analog is the substitution of a pentafluorophenylalanine residue which was found to greatly enhance the analgesic activity of methionine-enkephalin. Presumably, fluorination increased the lipophilicity and perhaps the stability of the compound which underlies its greater biological activity. In view of the poor analgesic effects of dynorphin(1-13) this approach had considerable appeal.

Consequently, a fluorinated compound, D-Ala^2-F$_5$-Phe^4-dynorphin(1-13)-NH$_2$, was synthesized by one of us (DHC) and tested for its analgesisc effects. Upon micro-injection into the lateral ventricle it produced short duration (30 min or less) analgesia in the tail-flick test and "wet-dog shakes." These effects were highly consistent, and dose related.

Surprising preliminary results suggest that high (10 mg/kg s.c.) doses of naloxone do not prevent this response. However, we do find that this analog displaces [^{3}H]dynorphin from its binding sites in rat and guinea pig brain homogenates. These results are difficult to explain in the context of the present literature and studies are underway to further address the mechanisms and binding properties of this analog.

215.6 THE ANTINOCICEPTIVE EFFECTS OF SELECTED OPIOID PEPTIDES DERIVED FROM BOVINE PRE-PRO ENKEPHALIN A ADMINISTERED ALONE OR CONCOMITANTLY WITH CAPTOPRIL, MK-422 OR THIORPHAN IN MICE. B.S. Barbaz* and W.L. Autry* (SPON: D.L. Cheney). Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

[Met^5]enkephalin Arg^6-Gly^7-Leu^8 (YGGFMRGL), [Met^5]enkephalin Arg^6-Ph^7 (YGGFMRF), [Met^5]enkephalin Arg^6 (YGGFMR), [Met^5]enkephalin (YGGFM) and [Leu^5] enkephalin (YGGFL) are opioid peptides derived from bovine pre-pro enkephalin A. Biochemical studies (Benuck et al., Neurochem. Intl., 4,5:389, 1982; Benuck et al., Biochem. Biophys. Res. Commun., 107:1123, 1982) indicate the cleavage of YGGFMRF is mediated primarily by the angiotensin converting enzyme, a dipeptidyl carboxypeptidase. Thiorphan, an enkephalinase inhibitor (EI), is specific for the metalloendopeptidase that cleaves the Gly^3-Phe^4 bond of YGGFM in purified enzyme preparation but has no inhibitory effect on the dipeptidyl carboxypeptidase that cleaves the Gly^3-Phe^3 bond of YGGFMRF in synaptosomal plasma membranes. These peptides with the exception of YGGFMR have been reported to show transient and weak analgesic activity using the tail flick procedure (Inturrisi et al., Proc. Natl. Acad. Sci., 77:9, 1980). The purpose of our experiments was 1) to assess the antinociceptive effects of these peptides using the hot plate test (55 ± 0.5° C) in mice and 2) to determine whether the angiotensin converting enzyme inhibitors (ACEI) captopril and MK-422 and the EI, thiorphan enhance the activity of these opioid peptides. All test agents were dissolved in distilled water and administered intracerebroventricularly (ICV) in a volume of 10 µl. Fifteen minutes after drug administration, jump latencies were measured. Each trial was terminated after the first jump response or after 240 sec if no response occurred. All the peptides, except for YGGFMR produced naloxone reversible analgesia. YGGFMRF was the most potent with a minimum effective dose of 1.9 µg. YGGFMRGL, YGGFM and YGGFL were 100-fold less potent. YGGFMRF was inactive (7.5 µg) and doses of 15, 30 and 60 µg produced neurotoxicity in the form of clonic seizures. When subanalgesic doses of captopril (100 µg) or MK-422 (7.5 µg) were concomitantly administered ICV with subanalgesic doses of YGGFMRGL (60 µg), YGGFMRF (0.94 µg) or YGGFM (60 µg) significant increases in jump latencies occurred. Concomitant ICV adminstration of a subanalgesic dose of thiorphan (0.94 µg) with the peptides YGGFM (60 µg) or YGGFL (60 µg) significantly increased jump latencies, but thiorphan was ineffective when co-administered with YGGFMRGL or YGGFMRF. The results of our in vivo studies indicate that the ACEIS enhance the antinociceptive activity of YGGFMRGL, YGGFMRF and YGGFM. However, the facilitatory effects of thiorphan are restricted to the pentapeptides YGGFM or YGGFL.

215.7 THE OPIOID RECEPTOR MECHANISMS FOR SPINAL AND SUPRASPINAL ANALGESIA IN THE MOUSE DIFFER. G.W. Pasternak and G.S.F. Ling. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, N.Y. 10021, USA

Opioid binding studies have suggested the existence of a common high affinity (mu_1) binding site for opiates and opioid peptides. Although the decrease in the analgesic potency of opiates and opioid peptides by the selective mu_1 antagonist naloxone implied an important role for mu_1 sites, the markedly different shifts in their analgesic ED$_{50}$ values suggested the existence of a second receptor analgesic mechanism. For example, morphine was shifted 12-fold while D-ala^2-D-leu^5-enkephalin (DADL) and metkephamid were shifted only 3.6-fold and 2.5-fold, respectively. If mu_1 sites were still mediating analgesia in naloxonazine-treated mice, the ED$_{50}$ Shifts for the different drugs by naloxazone should have been the same. Correlations of these analgesic compound shifts in vivo with the ability of the compounds to displace the binding of a variety of ^{3}H-opioids showed an excellent correlation (r2=0.95) only with ^{3}H-DADL binding. Equally important, the ability of all the opiates studied to displace ^{3}H-ethylketocyclazocine binding correlated well (r2=0.95) with their ability to displace ^{3}H-DADL binding. Only unlabeled DADL could discriminate between the two ^{3}H-ligands. Thus, distinguishing between kappa and delta actions using morphine, ethylketocyclazocine, ketocyclazocine, SKF10047, cyclazocine and nalorphine was not possible without including DADL. Spinal transection in mice had effects very similar to those of naloxazone. Transection shifted morphine's analgesic ED$_{50}$ 17-fold while metkephamid was shifted less than 3-fold. The greater analgesic potency of an enkephalin compared to morphine in transected mice suggested delta involvement in spinal analgesia in the mouse while the similar effects of transection and naloxazone implied a supraspinal localization of mu_1 analgesia. However, caution should be used in extrapolating these findings to other species of animals or man.

215.8 EFFECT OF INTRATHECAL THIORPHAN, A PUTATIVE ENKEPHALINASE INHIBITOR, ON FOOTSHOCK INDUCED ANALGESIA (FSIA) & CLASSICALLY CONDITIONED ANALGESIA (CCA). R.M. Fay*, L.R. Watkins, I.B. Kinscheck* & D.J. Mayer (SPON: K. Corley). Dept. Physiol. & Biophys., Med.Coll. Va./ Va. Commonwealth Univ., Richmond, VA 23298.

Thiorphan, a putative enkephalinase inhibitor, has been shown to potentiate the analgesic effects of exogenous enkephalin. Since front paw (FP) FSIA & CCA are attenuated by morphine tolerance & by intrathecal (IT) naloxone, it is possible that enkephalins are involved in these phenomena at the level of the spinal cord (Sci., 216:1185). Hind paw (HP) FSIA, on the other hand, appears to be nonopiate since it is not attenuated by high doses of systemic naloxone, IT naloxone, or morphine tolerance. In order to determine whether spinal enkephalins are involved in any of these phenomena, 2 doses of either 100 µg thiorphan or equivolume vehicle (1 µl 5% sodium bicarbonate in 0.5 M phosphate buffer pH 7.4) were injected IT onto the lumbosacral cord of rats at 15 & 5 min prior to 90 sec exposure to either front paw shock, hind paw shock, or the conditioned stimulus (Sci., 216:1185). Analgesia was then assessed through 45 min using the tail flick (TF) test. Thiorphan potentiated FP FSIA throughout the 45 min timecourse ($p<0.0001$,ANOVA). CCA, however, was not reliably affected. Assuming that thiorphan is a highly specific enkephalinase inhibitor our results may be evidence that CCA involves an endogenous opiate the effects of which are antagonizable by naloxone yet not affected by thiorphan. Alternatively, the tissue permeability of thiorphan is not presently understood; CCA may involve an enkephalinase at deeper dorsal horn laminae which are unaffected by our IT drug regimen. As such, these data would support the idea that differences exist in the microcircuitry of FP FSIA & CCA (Brain Res., in press). HP shock produced maximal analgesia in both groups until 25 min; from 30-45 min, potentiated analgesia was observed in thiorphan-treated rats ($p<0.0001$,ANOVA). Since enkephalinase inactivates peptides besides enkephalin, the question of thiorphan's specificity must be carefully addressed before firm conclusions can be reached. IT thiorphan had no effect on TF latencies in the absence of an analgesia-producing manipulation. The observed potentiations also cannot be accounted for by diffusion of the drug to the brain since neither FP FSIA nor HP FSIA was potentiated when thiorphan was injected onto thoracic rather than lumbosacral cord.

This research was supported by a grant from Eli Lilly & by PHS Grant DA 00576.

215.9 **OPIOIDS IN THE MOUSE ZINGERONE TEST FOR ANALGESIA.** Peggy J. K. Dobry, CNS Research, The Upjohn Company, Kalamazoo, MI 49001.

The zingerone test for analgesia in guinea pigs (Miller et al., 1981, Neurosci. Abst. 7:504) was adapted for use in mice. Zingerone is the pungent principle of ginger. A drop of 1% zingerone placed into a mouse's eye elicited an immediate, vigorous, repetitive wiping of the eye. The response was measured as the number of continuous forepaw wiping motions in the initial, intense phase. Groups of 10 female CF-1 mice, 20-24 g, were injected subcutaneously (s.c.) with drugs 20 min before the test. Capsaicin was injected s.c. 24 hrs before the test. Groups were compared by the Mann-Whitney U-test.

Morphine sulfate, pentazocine lactate, and codeine phosphate suppressed the wiping in a dose-dependent way. Nalorphine HCl was a weak analgesic. Naloxone HCl was not analgesic; 3 mg/kg actually increased the response by 58%. SKF-10047 also increased the response at a dose which caused ataxia and sedation.

Ketazocine did not cause analgesia, although ethylketocyclazocine methanesulfonate was the most potent analgesic tested. Nefopam HCl caused dose-dependent analgesia, and aspirin was modestly analgesic. Ketamine, diazepam, chlorpromazine HCl, and d-amphetamine sulfate were not analgesic. Ketamine and diazepam actually increased the vigor of the response. However, imipramine HCl suppressed responding at 30 mg/kg, a dose causing ataxia.

Capsaicin caused a dose-dependent analgesia. At the time of testing, all mice looked normal, although the highest doses had caused prostration immediately after injection.

In summary, the mouse zingerone test for analgesia is sensitive to aspirin, narcotic analgesics of the mu type, capsaicin, imipramine, and nefopam, and slightly sensitive to nalorphine. It does not show analgesia for the sedative compounds diazepam and chlorpromazine, the stimulant compound d-amphetamine, the sigma prototype SKF-10047, the dissociative anesthetic ketamine, the narcotic antagonist naloxone, or the kappa agonist ketocyclazocine (ketazocine).

215.10 THE INTESTINAL ANTISECRETORY ACTION OF OPIATES IS MEDIATED AT CNS SITES. D.R. Brown and R.J. Miller*, Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Opiates such as morphine have long been used to arrest diarrhea. Although this antidiarrheal action has been attributed to decreases in gut motility, recent evidence indicates that it might also result from an opiate-induced reduction in active anion secretion mediated through enkephalin-selective (δ) receptors situated in the intestinal mucosa. The possibility that these antisecretory effects could be mediated at sites within the CNS has not hitherto been addressed. Therefore, we examined the action of the stable enkephalin analog [D-Ala^2-Met^5]enkephalinamide (DAMA) administered intravenously (i.v.) and intracerebroventricularly (i.cv.) on intestinal fluid secretion induced by cholera toxin (CT). Male Sprague-Dawley rats (250-350 gm) were food-deprived, anesthetized with pentobarbital, and laparotomized. Ten cm loops of jejunum (J; 13-23 cm from pylorus), proximal ileum (PI; 43-53 cm from pylorus) and distal ileum (DI; 10-20 cm from ileocecal junction) were filled with 0.5 ml saline containing 1 µg CT. DAMA or saline (SAL) was administered 3 h after surgery and rats were sacrificed 1 h later. Loops were weighed, emptied of residual fluid and reweighed; results, expressed as µl fluid absorbed/cm length of loop/4 h, were:

	J	PI
SAL, 5 µl/rat i.cv.	- 33 ± 12	- 92 ± 13
DAMA, i.cv. 0.1 µg	- 34 ± 15	-109 ± 22
0.3 µg	- 2 ± 4**	- 68 ± 19
1.0 µg	+ 8 ± 8**	- 18 ± 20*
3.0 µg	+ 13 ± 5**	- 53 ± 13*
SAL, 1 ml/kg, i.v.	- 51 ± 21	-124 ± 34
DAMA, i.v. 100 µg/kg	- 34 ± 15	-175 ± 12
300 µg/kg	- 9 ± 16	-129 ± 32
1000 µg/kg	- 12 ± 21	- 39 ± 7*
3000 µg/kg	- 51 ± 24	-151 ± 32

(Two tailed t test; *$p < 0.05$; **$p < 0.01$)

I.cv. DAMA decreased CT-induced secretion in J and PI but had no consistent effect in DI or in any segment when given i.v. The antisecretory actions of i.cv. DAMA were blocked by the opiate antagonist, naltrexone, 1 mg/kg, s.c. 15 min before DAMA. Moreover, they were eliminated after peripheral sympathectomy by guanethidine (20 mg/kg, i.p. for 2 days) or α-adrenergic receptor blockade by phentolamine (1 mg/kg, s.c. 1 h before DAMA). These results suggest that the antisecretory effects of DAMA and possibly other opiates are mediated at CNS sites and are manifested by an increase in peripheral sympathetic activity in the upper small intestine. (Supported by NIH grants DA-02121 and MH-14274).

215.11 EFFECTS OF INTRAVENOUS B-ENDORPHIN ON INSULIN SECRETION IN INTACT AND ADRENALECTOMIZED RATS, C.Landau, R.M.Palmour*and C.Chang*. Dep't of Physiology/Anatomy Univ. of Cal-Berk. CA 94720

This study was designed to test the possibility that the opioid peptide B-endorphin, plays a role in the regulation of glucose in times of stress. In an attempt to recreate the effects of stress-secreted endorphin without the other confounding variables of a stress response, B-endorphin was administered intravenously through indwelling jugular catheters. To test the possibility that B-endorphin has a dual effect on the glucoregulatory apparatus through its stimulation of a hyperglycemic agent from the adrenal, and insulin from the pancreas, Long Evans female rats were adrenalectomized or sham operated and then given saline, morphine, B-endorphin, or naloxone. Intravenously administered endorphin resulted in the stimulation of insulin secretion in all animals tested. At high doses (4mg/kg) insulin levels rose and remained high for 25' fropping towards baseline by 60' postinjection. Intermediated doses (1mg/kg) produced a biphasis response with the first peak at 5' followed by a second peak at 25'. Low doses (0.5mg/kg) produced an insulin response resembling the first peak of the intermediate dose.

The experiments also demonstrate that B-endorphin does affect blood glucose concentrations. Intact and sham operated animals responded to intravenous endorphin with elevations in plasma glucose concentrations up to 40% over baseline levels. The hypoglycemic response to endorphin seen in adrenalectomized animals is consistent with the reported effects on insulin secretion.

The effects of B-endorphin, morphine and naloxone on insulin and plasma glucose levels in intact and adrenalectomized rats will be reported. In addition an hypothesis concerning the role that B-endorphin plays in physiological adaptation will be presented.

215.12 COMPARISON OF CHRONIC VS. ACUTE MORPHINE INDUCED CHANGES IN DISTRIBUTION OF CEREBRAL BLOOD FLOW. Law, W.R*, K.A Steece, J. Lee, M.A. Kapin*, J.L. Ferguson*, and R.F. Ritzmann. Univ. of Ill. at Chicago, Health Science Center. Chicago, IL 60680

Changes in regional cerebral blood flow (rCBF) have been shown to correlate with changes in neuronal activity in specific brain regions and with various regional metabolic activities. Changes in regional neural activity correlate with the uptake of 2-deoxyglucose. In addition, morphine has been shown to have both stimulatory and inhibitory effects on neuronal activity. However, it has been assumed that morphine does not alter cerebral blood flow (CBF). Due to recent advancements in techniques available for measurement of rCBF, we thought it important to reassess the effects of both chronic and acute administration of morphine on rCBF. Male guinea pigs were surgically prepared by left ventricular cannulation for measurement of rCBF using tracer microspheres. In the chronic study morphine pellets (65 mg/pellet; 1.5 pellets/100 g body weight) were implanted subcutaneously immediately after surgery. The percent cardiac output (%CO) to brain was assessed just prior to and 48 hours after pellet implantation as well as 30 minutes after i.p. naloxone (10 mg/kg) administration. Chronic administration of morphine resulted in a significant increase of 40.8% in the %CO received by the brain, the greatest increases being in the cerebellum (71.8%) and pons (60.9%). When withdrawal was precipitated by naloxone, the %CO to the brain decreased toward control values. Naloxone alone had no significant effects on %CO to the brain. Brain morphine levels were found to be 9.00 ± 2.1 µg morphine/gm tissue at the 48 hour time period. Measurement of brain levels 15 minutes after a series of acute doses of morphine indicated that intravenous administration of 200 mg/kg resulted in brain morphine levels of 9.9 ± 2.35 µg/gm tissue. Another group of guinea pigs were surgically prepared as before sans pellet implantation. Following surgery (48 hours), the animals were administered 2, 20, or 200 mg/kg morphine i.v. %CO to brain was assessed just prior to, 15, and 60 minutes post morphine. None of the doses produced any change in heart rate or mean ventricular pressure at the time periods studied. Lower doses of morphine did not significantly alter the %CO to the brain at the 15 min. time period. However, at 60 min post morphine, using 2 mg/kg, there was a significant decrease in %CO to the brain which is in agreement with the findings of Buchweitz, et al (Fed. Proc. 42(3):306, 1983).At 15 min. post 200 mg/kg morphine, there was a significant increase in %CO to brain (66.5%) which was greater than that observed in the 48 hour chronically treated animals. In contrast to the chronic study, the areas of greatest increase with acute administration of 200 mg/kg were the midbrain (77.3%) and cortex (74.9%). (Supported by CHA grant C82-13).

215.13 DOSE EFFECTS OF NALOXONE ON REGIONAL CEREBRAL GLUCOSE METABOLISM IN MORPHINE DEPENDENT RATS. GF Wooten and WA Geary, Dept of Neurology, Univ. of VA, Charlottesville,VA and Dept of Pharmacology, Wash. Univ., St. Louis, MO.

We have simultaneously studied regional cerebral glucose utilization (RCGU) and behavior during naloxone precipitated morphine withdrawal (MW). For RCGU studies, 25 brain regions were analyzed that previously had been shown to participate in MW (Wooten,et al PNAS 79:3360-3364,1982; Geary and Wooten,BRN.RES., in press,1983). Animals were treated for 11 days with twice daily subcutaneous injections of increasing doses of morphine-sulfate (Mallinckrodt, St.Louis, MO). Just prior to the final morphine dose, polyethylene catheters were inserted in the right jugular vein. The MW was produced by intravenous injection of variable doses (.0005-5.0 mg/kg) of naloxone-HCl (Endo Labs, Garden City,NY) in normal saline. A 25uCi pulse of 14C-2-deoxyglucose(2DG;Pathfinder Labs,St.Louis,MO) was injected IV 60 seconds after naloxone. Four behavioral indices of MW were recorded: wet shakes,jumps,acute weight loss(WL), and autonomic signs(ANS). Animals were killed 50min after 2DG injection and brains were removed,frozen,and processed for autoradiography. Control rats were treated with chronic saline and injected with variable naloxone doses. Four rats were used for each dose in all experiments; all experiments were conducted 11-13hrs after the final morphine dose(100 mg/kg).

No behavioral signs of MW were observed with .0005mg/kg of naloxone. There was no dose effect of naloxone on wet shakes. Jumps were maximal at .005mg/kg. In contrast, WL and ANS were naloxone dose dependent. Changes in RCGU showed 3 classes of response to increasing naloxone dose in fixed dependence rats: Class I structures (paraventricular, ventromedial, and lateral hypothalamus) exhibited their largest RCGU increases between .005-0.05mg/kg naloxone; Class II structures (pre-optic areas,basal ganglia,anterior and intralaminar thalamic nuclei,mammillary nuclei,and some midbrain areas) showed gradual RCGU increases across the 10^4 naloxone dose range; Class III structures (diagonal band, the medial and lateral septum, and the central amygdaloid nucleus) displayed largest RCGU increases between 0.50-5.0mg/kg naloxone. Regression analyses of RCGU vs behavior showed high correlations between Class I & ANS ($r=.992; p<.001$),Class I & WL ($r=.964; p<.001$),Class II & WL ($r=.979; p<.001$), and Class III & WL ($r=.918; p<.05$). No correlations were found between any RCGU response class and jumps or wet shakes.

Thus, changes in RCGU show a graded, dose dependent response to naloxone precipitated MW. RCGU may be a more useful index of MW than behavior because of anatomical specificity, objectivity, and the relative freedom from state-dependent variables. The functional relationship between changes in RCGU and behavior during MW remains to be determined.

215.14 THE EFFECTS OF SPINAL OPIATES ON MICTURITION IN UNANESTHETIZED ANIMALS. C. Ronald Brent*, Gail Harty* and Tony L. Yaksh* (SPON: F. W. L. Kerr). Section of Neurosurgical Research, Mayo Fndn., Rochester, MN 55905.

Urinary retention has been frequently reported in the clinical use of spinal and epidural opiates for analgesia. A preparation was devised to examine the spinal pharmacology of opiates in unanesthetized rats and cats. Indwelling polyethylene catheters were placed in the urinary bladders of rats (PE-50) and cats (PE-90) and tunneled subcutaneously to exit through the scalp, allowing both ureters and urethra to remain intact. These animals were previously fitted with polyethylene (PE-10) intrathecal (IT) catheters directed to the lumbosacral enlargement. Rats were placed in a restraining cage, cats in a small circular chamber, over a collecting device attached to a strain gauge for measurement of urine volumes. The bladder catheters were attached to a transducer and pump for measurement of intravesicular pressure during continuous infusion of normal saline. At a constant rate of infusion (200 µl/min in the rat; 1.5 ml/min in the cat), bladder contractions (54 ± 22 cm H_2O, $n = 8$ in rat) were observed at regular intervals and were associated with simultaneous and efficient expulsion (no residual volume) of discreet volumes of urine (mean volume = 0.84 ± 0.12 ml, $n = 8$, in rat). The significance of using unanesthetized animals in studying this volume-evoked micturition reflex (VEMR) was demonstrated by the complete inhibition of this reflex in rats anesthetized with pentobarbital (50 mg/kg, i.p.), chloralose (130 mg/kg, i.p.), ketamine (100 mg/kg, i.p.) and halothane (by inhalation). In unanesthetized animals, a complete inhibition of the VEMR was produced by IT administration of opiates. In the rat, 5 out of 5 were inhibited by a µ agonist (morphine, 15 µg) and 4 out of 5 were inhibited by a δ agonist (d-ala^2-d-leu^5-enkephalin: DADL, 0.8 µg). This dose-dependent effect was reversed with IT naloxone (15 µg). The κ agonist, U-50488H (50-100 µg) failed to inhibit the VEMR. Systemic morphine (1 mg/kg, i.p.), IT naloxone (15 µg), and IT thiorphan (70-200 µg) administered individually had no effect on volume-evoked micturition. IT morphine (15 µg) was also observed to cause a naloxone-reversible inhibition of volume-evoked contractions in the automatic bladders of chronic (>7 days) spinal-transected (high thoracic) animals. Similar results were observed in parallel experiments carried out in cat. We conclude that volume-evoked micturition is modulated through µ and/or δ, but not κ opiate-receptor systems at the lumbosacral spinal level. Failure of naloxone or thiorphan to have any significant effect in the unanesthetized and spinally intact animal suggests that spinal opiate systems have little or no intrinsic activity during normal micturition. (Mayo Foundation)

215.15 CLONIDINE REVERSES THE BEHAVIORAL AND RESPIRATORY EFFECTS OF CONTINUOUS NALOXONE INFUSION. D.H. Malin, A.G. Hempel*, R.J. Exley* and S. Addington*. Univ. of Houston-Clear Lake. Houston, Tx. 77058.

Blockade of the endorphin system by continuous infusion of .75 mg/kg/hr naloxone produces behavioral symptoms and respiratory hyperactivity reminiscent of opiate abstinence syndrome. Clonidine (an alpha-2 adrenergic agonist) has been reported to potently reverse many morphine abstinence symptoms in human patients and in rats. An experiment was performed to determine whether clonidine would also reverse the "endorphin blockade syndrome" produced by continuous naloxone infusion.

Twenty male 135 g rats were implanted with Alzet model 2001 osmotic minipumps. Ten rats received pumps filled with saline. Ten rats received pumps filled with 50 mg/ml naloxone. These rats received 0.75 mg/kg/hr naloxone s.c. on a continuous basis.

Prior to implantation, all rats were habituated to the chamber of an oxygen consumption apparatus, and baseline oxygen consumpmeasures were taken. Twenty eight hours after implantation, all rats were retested for oxygen consumption and were observed for 15 min in a clear plastic chamber for morphine-abstinence-like behaviors, such as wet-dog shakes, abdominal writhes, hind foot scratches, etc.

The naloxone-infused rats had significantly, $p < .001$, higher oxygen consumption as percentage increase above baseline (48.4% vs 3.6%). They also showed significantly, $p < .001$, more abstinence-like behaviors (13.2 vs. 1.8). Following the s.c. injections, the saline-infused rats remained near baseline oxygen consumption and near zero behavioral symptoms, regardless of whether they were injected with saline or clonidine. The naloxone-infused rats injected with saline retained their elevated O_2 consumption (40.7% over baseline), while clonidine reversed O_2 consumption to 9.7% below baseline. The naloxone-infused rats injected with saline also continued to display a high level of behavioral symptoms (11.2) while clonidine injection reduced the symptoms to zero. In each case, the difference between the saline and clonidine injected rats was significant, $p < .01$.

Clonidine reversal of the "endorphin blockade syndrome" suggests a further point of resemblance to the opiate abstinence syndrome. It is also consistant with the hypothesis of hyperactive brain adrenergic mechanisms in both syndromes.

Supported by U. Houston-Clear Lake Organized Research Fund and Melrose-Thompson Fund.

215.16 POSTRADIATION MOTOR PERFORMANCE OF RATS PRETREATED WITH NALOXONE. C. J. Gossett-Hagerman*, V. Bogo*, S. A. Simpson* and L. G. Cockerham. Physiology and Behavioral Sciences Departments, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814.

Naloxone, an opiate antagonist, has been shown to diminish the degree of hypotension and increase the survival time of rats subjected to either endotoxic or hypovolumic shock. Since blood pressure falls dramatically following radiation, this has been postulated as a possible explanation of postradiation behavioral, early transient incapacitation and/or performance decrement. Behavior was assessed with the accelerod, a test of rodent motor performance in which rats perform on an elevated rod that accelerates at 1 rpm/sec to determine if pretreatment of rats with naloxone would alter radiation induced performance decrement. Forty-eight male Sprague-Dawley rats (200-400 gm) were trained to a stable level of performance on the accelerod, randomly assigned to one of four groups, surgically implanted with osmotic mini-pumps, and then allowed to restabilize after surgery. The osmotic pumps were implanted 6 days prior to radiation exposure. The pump delivered naloxone at a dose rate of 0.5 mg/kg/hr or an equal volume of saline. Sham radiation runs were conducted for 5 days. The four groups were as follows: saline/sham radiated, saline/radiated, naloxone/sham radiated, and naloxone/radiated. The subjects were bilaterally exposed to 10,000 rads of gamma radiation in a ^{60}Co source at 5000 rad/min. All groups were accelerod tested at 5, 10, 20, 30, 45, 60, and 90 min after radiation or sham exposure. Performance of the naloxone and saline treated radiation-exposed subjects were significantly different from controls during the initial two test periods, but they did not differ significantly from each other, which indicates that naloxone delivered with a mini-pump at a dose rate of 0.5 mg/kg/hr did not act as a radioprotectant for motor performance. In addition, neither radiation exposed group returned to baseline performance levels during the 90 min of testing, as has been suggested by other investigators.

215.17 SELECTIVE μ & δ RECEPTOR ANTAGONISTS AND NEUROENDOCRINE RESPONSES TO MORPHINE: EVIDENCE FOR μ RECEPTORS IN PROLACTIN RELEASE. J.W. Holaday, L. Pennington*, and S.J. Ward[1], Neuropharm. Br., Dept. Med. Neurosci., Div. Neuropsych., Walter Reed Army Institute of Research, Wash., DC 20307 and [1]Dept. Pharmacol., Sterling Winthrop Research Institute, Rensselaer, NY 12144.

Following injection of opioid agonists, variable neuroendocrine responses have been reported, including elevations of plasma corticosterone (CS) and prolactin (PRL). The purpose of these studies was to characterize the possible involvement of central μ or δ opioid receptors in CS and PRL responses following morphine administration in conscious rats.

Male S.D. rats (250-300g) were surgically prepared with catheters in the external jugular vein as well as an intracranial guide tube for right lateral intracerebroventricular (icv) injections (20 μl over 20 sec). β-funaltrexamine (β-FNA, a long lasting antagonist at μ sites) was administered icv (5 nM) 18 hrs prior to intravenous morphine sulfate (MS) challenge, whereas 200 nM icv ICI 154,129 (ICI; a putative δ antagonist) or saline was injected 5 min after MS doses. Previously, these doses and treatment times for antagonists were shown to have selectively different effects upon antinociceptive, cardiovascular, and respiratory responses to MS (Soc. Neurosci. Abstr. Vol. 8, p 388-389, 1982). One day following surgery, venous blood was withdrawn from these conscious, unrestrained rats. One hour following intravenous MS administration in rats treated with β-FNA, ICI or saline, a second blood sample was obtained. Plasma CS and PRL were measured by radioimmunoassay.

β-FNA by itself was without effect upon resting levels of CS (10-15 μg %) or PRL (5-15 ng/ml). ICI resulted in a doubling of resting CS levels, but was without effect upon basal PRL. Unexpectedly, at MS doses between 2 and 160 mg/kg, CS values remained within control ranges (< 20 μg %) regardless of treatment group. By contrast, MS evoked a dose related increase in PRL, with threshold response (30 ng/ml) occuring at a dose of 8 mg/kg MS, and a maximum PRL response (> 1000 ng/ml) at 40 mg/kg MS. ICI resulted in a twofold rightward shift, and β-FNA, a fourfold rightward shift of the PRL response to MS.

Unlike prior reports, we were surprised to find that MS doses up to 160 mg/kg were without effect upon CS levels, indicating that MS is not a potent stimulus for CS release in the conscious rat. Based upon this evidence, the doubling of resting CS levels produced by ICI may or may not be related to opioid-specific responses. With regard to PRL release, MS resulted in a dose-related increase in PRL which was antagonized to a greater degree by β-FNA than by ICI. Parallel to earlier findings with the effects of these selective antagonists upon nociceptive responses to MS, evidence indicates that μ receptors play a dominant role in both the antinociceptive and PRL-releasing effects of MS.

216 EXCITATORY AMINO ACID NEUROTRANSMITTERS. C. Cotman, Chairman Univ. Calif., Irvine; R. Altschuler and J. Fex, NIH-NINCDS; R. Wenthold, Univ. of Wisconsin Med. Sch.; R.F. Miller, M.M. Slaughter, S.C. Massey, and E. Dicks, Washington Univ. Sch. of Med.; J. Coyle, Johns Hopkins Univ. Sch. of Med.; M. Ito*, University of Tokyo; R. Miledi*, I. Parker*, and K. Sumikawa*, Univ. College London.

New evidence derived from several lines of experimentation points toward glutamate aspartate and perhaps related derivatives as major excitatory transmitters in the CNS. Acidic amino acid receptors are highly localized, exist as multiple types and differentially interact with various potent antagonists of excitatory amino acid synaptic transmission. Glutamate appears to be a transmitter at major projection pathways and several of these appear to be highly plastic. Cotman will summarize current information on the classes of receptors for acidic amino acids and present autoradiographic data on their distribution in the CNS. Autoradiographic data shows receptor classes for ^{3}H-glutamate are topographically organized and can be resolved into distinct classes. NMDA sensitive sites, for example, are particularly concentrated and differentiated in hippocampus, cortex, basal ganglia and thalamus. Altschuler will describe data showing that antisera against glutaminase or aspartate amino transferase, two enzymes involved in glutamate and aspartate metabolism, may serve to identify acidic amino acid neurons. Major suspected aspartate/glutamate pathways are labelled with one or both antisera. Miller will discuss the properties of acidic amino acid mediated neurotransmission in the vertebrate retina. Results are compatible with the idea that glutamate mediates the synaptic action of photoreceptors and at least some bipolars. The second order neurons have at least two types of specialized synaptic receptors which are segregated according to the polarity of the light response. Coyle will discuss the presence of endogenous brain neuropeptides as candidates for excitatory neurotransmitters. Ito will discuss the plasticity of parallel fibers. These fibers appear to use glutamate as their transmitters, and a decrease in glutamate sensitivity appears to underlie the long-lasting depression in parallel fiber-Purkinje cell transmission after simultaneous activation of parallel fibers and climbing fibers. Therefore it may play a key role in the cerebellar plasticity. Finally, Parker will present new data on the induction of neurotransmitter receptors in Zenopus oocytes. This approach allows the study of complex receptor types in a simple system.

217 SYMPOSIUM. APPLICATIONS OF NEUROSCIENCE TO HUMAN PROSTHESES. F. T. Hambrecht, NINCDS, National Institutes of Health (Chairman); Richard B. Stein*, Univ. of Alberta; P. Hunter Peckham*, Case Western Reserve Univ.; Michael M. Merzenich, Univ. of California, San Francisco.

Rapid advances in technology and in the neurosciences are being combined to develop clinically useful human prostheses based on direct interfaces with the nervous system. This symposium will attempt to inform the members of the audience, who represent many disciplines, about some of the progress that is being made and to alert them to problem areas that may be in their fields. Stein will review the use of electromyographic signals to control powered artificial limbs and the current status of multi-joint prostheses for both the upper and lower extremities. Methods of controlling multiple powered movements will be outlined such as multi-state control and pattern recognition techniques. The use of signals from neurons and deep muscles, transmitted by means of telemetry, will be described. In addition to prostheses, robots can be adapted to do a variety of tasks for severely handicapped individuals. The problems of controlling wearable, multi-movement prostheses and free-standing robots will be compared. Peckham will demonstrate how the paralyzed muscles of a spinal cord injury victim can be functionally activated and placed under the individual's voluntary control. He will discuss the future of totally implanted, closed loop feedback control systems utilizing artificial sensory transducers. Merzenich will describe recent technological developments in multichannel cochlear prostheses and neurophysiological and psychoacoustical experiments in individuals with sensory deafness. Hambrecht will give examples of the important role that fundamental neuroscience and technology research have played in the development of state-of-the-art neural prostheses and will discuss problems that need to be solved by future research efforts. If time permits, there will also be a general discussion between the symposium participants and the audience.

218 SYMPOSIUM. THE NEUROGENETICS OF IDENTIFIED CELLS. R.J. Wyman, Yale (Chairman); H.R. Horvitz, MIT; L. Buck (Columbia U. CPS); L.B. Salkoff, UCSF; R.J. Mullen, Utah.

Many of the most interesting properties of neurons are those that inhere in only a single cell or cell type (morphology, connectivity) or those that vary from cell to cell (transmitters or ion channels used). The application of genetic techniques to the study of identified cells has allowed a wholly new approach to these properties.

In the nematode (C. elegans) the lineage, morphology and synaptic connections of every nerve cell is known. Horvitz et al. are studying the genes that establish these properties. He will describe lineage mutants which cause cells normally present to undergo programmed cell death, and other mutants which cause the production of supernumerary copies of cells. Other mutants affect function, morphology or transmitter systems.

Recombinant DNA technology is being used to examine neuron specific gene expression in Aplysia. The genes for the peptides secreted by the bag cells and the atrial gland cells in Aplysia have been cloned and sequenced. Now Buck et al. have isolated messenger RNA from single cells (R2 and R15) and have identified a message coding for a peptide that appears to be expressed in R15 but not in any other cell in the abdominal ganglion.

In Drosophila, Wyman et al. are seeking genes which code for signals required for the specificity of synaptic connection. They have isolated mutants in which single branches of identified cells grow abnormally and do not make their normal synapses. A second copy of these signals, in the wrong ganglion, can be created in bithorax flies. The giant fiber then grows two identical branching trees. Thus homeotic genes transform not just the cuticle, but also affect the CNS.

In Drosophila, Salkoff et al. have studied the DLM muscle cells. The cells were voltage clamped to identify the ion channels present. The different currents develop sequentially during metamorphosis; the first to develop is the fast transient K^+ current (I_A). Mutants at the Shaker locus were found to either alter the kinetics properties of the current, or eliminate the current altogether. The ground work has now been completed for a molecular characterization of this channel.

Although the mammalian cerebellum contains millions of cells, there are only a few neuronal and glial cell types (e.g. Bergman Glia, Purkinje Granule Cells) arranged in a simple and highly repetitious fashion making it a suitable subject for neurogenetic study of identified cells. Numerous mutations affecting specific cell types have been discovered. By producing mouse chimeras, Mullen et al. have defined the primary site of gene action and delineated cell-cell interactions.

PEPTIDES: BIOSYNTHESIS AND METABOLISM I

219.1 MOLECULAR CLONING OF THE R3-14 NEUROPEPTIDE GENE IN APLYSIA. John R. Nambu* and Richard H. Scheller. Dept. of Biological Sciences, Stanford University, Stanford, CA. 94305.

We are interested in elucidating the molecular mechanisms involved in mediating extracellular communication within the nervous system. This necessarily requires a characterization of the chemical messengers involved. The abdominal ganglion of the gastropod mollusc Aplysia californica is a large asymmetric cluster of about 2000 neurons, many of which are phenotypically distinct. In order to examine the role of this tissue in modulating behavior and physiology we have taken an identifiable peptidergic neuron, cell R14, and characterized its neuropeptide precursor and the corresponding gene.

50 Aplysia abdominal ganglia were dissected and individual neurons collected. Poly(A)+ RNA prepared from various cells was used to direct in vitro translation analysis, which indicated a predominant low molecular weight protein product (ca. 14,000 daltons) in the R14 neuron. cDNA was synthesized and utilized as a probe to screen for homologous sequences in an abdominal ganglion cDNA library. Nucleotide sequence analysis of R14 specific cDNA clones provided information on the primary structure of the protein product. The 108 amino acid protein contains both acidic and basic regions separated by a pair of internal dibasic residues (lys-arg, arg-arg), potential sites for proteolytic cleavage. The bioactivities of the various cleavage products are currently under investigation.

The corresponding gene was isolated from a total genomic library and characterized via restriction enzyme mapping and electron microscopy heteroduplex analysis. This has permitted us to determine the orientation of the mRNA transcript on the gene as well as to localize two intervening sequences (introns) within the coding regions.

We are currently pursuing similar studies using identifiable neurons from the abdominal and other Aplysia ganglia. It is hoped that the information generated, including the characterization of the neuropeptide products from individual neurons, will contribute to our understanding of the mediation of the cell-cell interactions which are basic to nervous system function.

219.2 REGULATION OF EXPRESSION OF PROENKEPHALIN mRNA IN CULTURED CHROMAFFIN CELLS OF BOVINE ADRENAL. Joan P. Schwartz, T.T. Quach*, P. Panula* and E. Costa, Lab. Preclinical Pharmacology, NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

Biochemically and histochemically medullary chromaffin cells have been shown to contain not only catecholamines but also enkephalin-like peptides. Previous studies have shown that both the catecholamines and the opioid peptides can be released in vivo following splanchnic nerve stimulation and in vitro by acetylcholine and histamine. Since cyclic AMP participates in the regulation of tyrosine hydroxylase biosynthesis, we have now examined the effect of cyclic AMP on the regulation of enkephalin peptide synthesis. We have used a cDNA probe for human pheochromocytoma proenkephalin (Comb et al., Nature 295:663, 1982) to detect proenkephalin mRNA in bovine adrenal medulla. Total RNA was prepared by guanidine thiocyanate extraction of tissue or cells, followed by centrifugation on a cesium chloride gradient. Poly $(A)^+$-RNA (mRNA) was prepared by two passages over oligo (dT)-cellulose. RNA was fractionated on a formaldehyde-agarose gel, blotted to nitrocellulose and hybridized to a ^{32}P-labeled probe. Northern blot analysis of poly $(A)^+$-RNA from bovine adrenal medulla revealed the presence of 3 RNA species; the predominant mRNA was approx. 1400 bases, as has been previously described while the other 2 species represented less than 15% of the total proenkephalin-positive RNA and were much larger (approx. 3.8 kb and 7.3 kb). Analysis of total RNA prepared from chromaffin cells which had been in culture for four days revealed the presence of the same three species of RNA. Treatment of the cells with 1mM 8-Br-cyclic AMP for 24 hrs led to an increase in the amount of all three RNAs (quantitated by densitometric scans of the autoradiograms). The largest RNA (7.3 kb) increased 3.7 fold, the 3.8 kb 2.0 fold and the 1.4 kb species 1.9 fold. The results suggest that the larger MW RNAs may be nuclear precursors to the cytoplasmic 1400 base mRNA. The effect of 8-Br-cyclic AMP on proenkephalin mRNA content is both time-and dose-dependent and is not reproduced by 8-Br-cyclic GMP. Three days of treatment with 8-Br-cyclic AMP results in a 10-12 fold increase in proenkephalin mRNA; however, at this time total RNA has also increased 3-5 fold, although there is no change in protein synthesis or cell number. In situ hybridization of biotinylated probe in chromaffin cell cultures, detected by streptavidin-horseradish peroxidase staining (Enzo Biochem), has demonstrated that approx. 30% of the chromaffin cells stain for proenkephalin mRNA. Those same cells exhibit PNMT- or met^5-enkephalin-arg^6-gly^7-leu^8-like immunoreactivity, thus demonstrating the coexistence of a catecholamine enzyme(s), an enkephalin peptide(s) and proenkephalin mRNA. This technique will allow us to examine the possibility of coordinate regulation of these cotransmitters by cyclic AMP.

219.3 HYPOTHALAMIC OXYTOCIN BIOSYNTHESIS: EFFECT OF DEHYDRATION. M. Morris*, G.S. Gallimore* and D.K. Sundberg* (SPON: P.B. Smith). Dept. of Physiol. & Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103

Oxytocin is synthesized as part of a larger precursor molecule which is then cleaved during transport to yield the nonapeptide. These experiments were designed to investigate the biosynthesis of oxytocin (OT) in various brain regions. Male rats were prepared with chronic third ventricular cannula. 40 μCi (4 μl volume) of ^{3}H-proline (102 Ci/mM) was injected intraventricularly and the animals were decapitated 3, 6, 12 and 24 hrs later. The tissues examined were the neurohypophysis (NP), median eminence (ME), and paraventricular (PVN) and supraoptic nuclei (SON). The tissues (pooled from 4 animals) were homogenized in 0.2 N acetic acid, purified by an initial Sep-Pak C18 separation (Waters Inst) and then subjected to reverse phase HPLC separation. The fractions were monitored for U.V. absorbance, radioactivity and in some cases immunoreactivity. There was a time dependent increase in neurohypophyeal ^{3}H-OT reaching 825 dpms (total peak) at 24 hrs. The radioactive peak co-eluted with OT as determined by both immunoreactivity and U.V. absorbance. Although there was incorporation of ^{3}H-proline into proteins in the other brain regions, the label was not associated with OT. Thus, most of the label in the PVN, SON and ME is probably associated with precursor molecules. To determine the effect of a stimulus to this system, OT biosynthesis was measured after 48 hrs of water deprivation. There was a reduction in both peptide content and in the absolute amount of neurohypophyseal ^{3}H-OT (187.3 ± 7.9 vs 121.6 ± 9.2 dpms/NP; control vs exp). However, when expressed as specific activity (^{3}H-OT/OT content), dehydration resulted in an increased labeled pool of this neurohormone (.5 ± .03 vs .95 ± .03 dpm/ng).

Thus, there is significant incorporation of a labeled amino acid precursor into a peptide which co-elutes with oxytocin. Dehydration stimulates both the secretion and de novo synthesis of this peptide. The fact that the newly synthesized peptide first appears in the neurohypophysis suggests that precursor processing occurs during transit from the nuclei to the neurohypophyseal axons.

(Supported by NIH grants HD-10900 and HL-22411)

219.4 EFFECT OF CYSTEAMINE ON THE BIOSYNTHESIS IN VIVO OF VASOPRESSIN, OXYTOCIN AND SOMATOSTATIN. R.E. Franco-Bourland*. (SPON: F. Antón-Tay). Departamento de Bioquímica, Instituto Nacional de la Nutrición, Salvador Zubirán, 14000 México, D.F. ºCONACYT-México: PCCBBNA-001716.

The systemic administration in the rat of cysteamine, a thiol reagent, diminishes somatostatin-like immunoreactivity in the hypothalamus, without a concomitant rise of its levels in plasma. The mechanism of action of cysteamine in this instance is not known. The decrease in hypothalamic somatostatin (SRIF) levels might be the consequence of an accelerated degradation and/or an inhibited biosynthesis. Here I report the effect in the rat of cysteamine on the in vivo incorporation of L-(35S)Cys into hypothalamic SRIF, and compare it to that into the other hypothalamic cysteine-containing peptides, vasopressin (AVP) and oxytocin (OT).

Guide cannulas, directed to the III ventricle, were implanted in male rats weighing 250g. Ten days after surgery, paired rats were given subcutaneously 30 and 300 mg/kg of cysteamine-HCl in neutralized H_2O. Controls received 1M NaCl. Four hours after the administration of the drug, the rats received 40μCi of L-(35S)Cys via the III ventricle and were decapitated 4h thereafter. Labeled AVP, OT and SRIF were extracted from individual hypothalami and neurohypophyses into 2M HOAc, and were purified simultaneously by chemoadsorption to octadecyl-silane coupled to silica and 2 sequential HPLC steps. At the end of this procedure the labeled peptides were at least 90% radiochemically pure, as determined by derivatization. The recoveries of the carrier peptides and the endogenous neurohypophyseal content of AVP and OT were measured by absorbance at 206nm. Radioactivity was determined by liquid scintillation.

Compared to controls, the mean values of incorporation of L-(35S)Cys into AVP, OT and SRIF in the hypothalami and neurohypophyses of rats which had received 30 mg/kg of cysteamine were significantly reduced. The incorporation of the label into OT and SRIF in animals which had received 300 mg/kg of cysteamine was undetectable, whereas that into AVP was reduced but measureable. It thus appears, that one mechanism through which cysteamine reduces the hypothalamic content of SRIF is by inhibiting its biosynthesis. It also appears to have a greater effect on OT rather than on AVP biosynthesis.

219.5 SUBSTANCE P BIOSYNTHESIS AND AXONAL TRANSPORT IN THE RAT STRIATONIGRAL SYSTEM: EVIDENCE FOR RAPID PRECURSOR PROCESSING AND FAST AXONAL TRANSPORT FROM PULSE-CHASE EXPERIMENTS. J.E. Krause and J.D. White, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The in vivo production and axonal transport of neural peptides to their respective terminal fields represent important aspects in the functioning of neuronal networks. We have approached these questions by using as a model the striatonigral substance P (SP) projection. SP positive neuronal perikarya in the rostral corpus striatum project ipsilaterally via the ansa lenticularis to an extensive terminal field within the substantia nigra. In the present studies, we have examined the kinetics of in vivo biosynthesis and axonal transport of radiolabeled SP along the trajectory of this peptidergic projection using a pulse-chase paradigm and analytical procedures for the quantitation of SP biosynthesis as previously described (Krause et al., 1982).

The right corpus striatum of rats was pulsed for 2 hrs with ^{35}S-methionine (500 µCi/rat) via 2 indwelling cannulae using an Alzet osmotic minipump delivery system. Rats were killed 2,4,8, 12,16 and 24 hrs after the start of amino acid infusion. Radiolabeled SP was extracted from the striatum, its striatonigral fasciculus and the substantia nigra and was purified by sequential High Performance Liquid Chromatography (HPLC). Chemical sulfoxidation of radiolabeled SP and further HPLC demonstrated the homogenity of the radiolabeled undecapeptide.

The present results suggest that SP is processed from its putative precursor protein at the site of its cell bodies in the corpus striatum. Thus, 2 hr after the start of amino acid infusion, relatively large amounts of ^{35}S-SP can be isolated from the striatum. ^{35}S-SP can be harvested from the striatonigral fasciculus and the ipsilateral substantia nigra from 2 to 4 hr after the start of amino acid infusion. These observations suggest that either ^{35}S-SP or its putative precursor protein is transported to these sites via a fast component of axonal transport (i.e. ≥ 2 to 4 mm/hr). The greatest amount of ^{35}S-SP within the substantia nigra is observed from 4 to 8 hr after the start of amino acid infusion. The radiolabeled SP within the striatum may be used locally, or may be axonally transported to other target sites. These studies demonstrate the feasibility of studying the in situ regulation of SP production and transport, and the kinetics of SP turnover in individual unrestrained animals.

(Supported by NS 18942 to JEK.)

219.6 FURTHER STUDIES ON AN ADRENAL ENKEPHALIN-GENERATING ENZYME: SUBSTRATE SPECIFICITY. I. Lindberg*, H.-Y.T. Yang and E. Costa (SPON: J. Dahl). Lab. Preclinical Pharmacol., NIMH, Saint Elizabeths Hospital, Washington, D.C. 20032

We have previously reported the presence of a serine protease in chromaffin granules which is capable of cleaving high molecular weight enkephalin-containing peptides to generate met^5-enkephalin and other related peptides. This enkephalin-generating enzyme exhibits a pH optimum of 8.0 and an apparent molecular weight of 20,000 by gel filtration. The substrate specificity of this enzyme was examined using 1) small synthetic fluorogenic peptides containing basic residues; 2) the heptapeptides met^5-enk-arg^6-arg and met^5-enk arg^6-phe^7; 3) peptides E and F and 4) the 8.6 kdal N-terminal fragment of proenkephalin (which contains met^5-enkephalin at its carboxyl terminus). The enzyme exhibited little reactivity toward the heptapeptides and the fluorogenic substrates; the Km's for Boc-glu-lys-lys-MCA and Boc-gln-arg-arg-MCA were 0.7 and 2.0 mM, respectively. In contrast, the Km of the enzyme for the 8.6 kdal fragment of proenkephalin was 0.5 μM. Analysis of the enzymatic products of the 8.6 kdal fragment by HPLC followed by RIA revealed the generation of met^5-enkephalin and another immunoreactive peptide (probably lys^1-met^5-enkephalin). Enzymatic digestion of peptide F yielded arg^1-met^5-enkephalin, met^5-enkephaline, and lys^6-met^5-enkephalin. Digestion of peptide E yielded leu^5-enkephalin, an unidentified leu^5-enkephalin-immunoreactive peptide, and arg^6-arg^7-met^5-enkephalin. These results indicate that this adrenomedullary protease is capable of cleaving adrenal high molecular weight enkephalin-containing peptides at the paired basic sites. In addition, the Km for the 8.6 kdal (an endogenous high molecular weight peptide) was considerably lower than that for the synthetic fluorogenic substrates. Taken together, these results suggest that this adrenomedullary protease represents a possible candidate for a proenkephalin processing enzyme.

219.7 ENKEPHALIN CONVERTASE: PURIFICATION AND CHARACTERIZATION OF BOTH THE SOLUBLE AND THE MEMBRANE BOUND FORM OF THE ENKEPHALIN SYNTHESIZING CARBOXYPEPTIDASE. L.D. Fricker, S. Supattapone* and S.H. Snyder. Johns Hopkins University, Depts. of Neuroscience, Pharmacology and Psychiatry, School of Medicine, Baltimore, Maryland 21205.

Enkephalin convertase, the enkephalin synthesizing carboxypeptidase B-like enzyme, is present in brain, pituitary and adrenal medulla chromaffin granules in both a soluble and a membrane bound form. The membrane bound enzyme which constitutes 20-50°/° of the total enkephalin convertase activity in these tissues can be solubilized in high yield with 0.5°/° triton X-100 in the presence of 1 M NaCl. Both forms of enkephalin convertase have been purified to apparent homogeniety utilizing affinity chromatography on p-aminobenzoyl-L-arginine/Sepharose 6B as one of the steps. Purified enkephalin convertase shows a single band on SDS polyacrylamide gel electrophoresis with an apparent molecular weight of 50,000 daltons for the soluble enzyme, and 52,500 daltons for the membrane bound form.

The regional distribution of soluble enkephalin convertase in rat brain and pituitary shows a 30-fold variation whereas the membrane bound enzyme is more homogeneously distributed. The specific activity of both forms of the enzyme is high in the anterior pituitary and low in the cerebellum and brain stem. With the exception of the adrenal medulla, enkephalin convertase activity was not detectable in any other tissues screened.

Enkephalin convertase purified from both soluble extract and membranes of bovine brain, pituitary, and adrenal medulla behaves similarily with respect to substrates, inhibitors, pH and divalent cations. Dicarboxylic acid analogs of arginine and lysine are potent inhibitors of enkephalin convertase. Two of these active site directed inhibitors (guanidinoethylmercaptosuccinic acid and guanidinopropylsuccinic acid) inhibit enkephalin convertase with K_i's of 7-8 nM, which is several hundred fold more potent than their influence on other carboxypeptidase B-like enzymes.

These results suggest that enkephalin convertase activity represents the same enzyme in the brain, pituitary and adrenal chromaffin granules. The only detectable difference between the two forms of enkephalin convertase is that the membrane bound form is 2-3000 daltons heavier than the soluble enzyme. This difference may be due to an additional fragment on the membrane bound enzyme which anchors it to the membrane.

219.8 ANTIBODIES AGAINST BOVINE PITUITARY CARBOXYPEPTIDASE B-LIKE PROCESSING ENZYME FOR HORMONE PRECURSORS, V.Y.H. Hook*, L.D. Fricker, M.J. Brownstein*, and S.H. Snyder. (SPON: J. Axelrod). Lab. of Cell Biology, NIMH, Bethesda, MD. 20205, and Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD.21205.

Peptide hormones such as the enkephalins, β-endorphin, ACTH, vasopressin and others are synthesized as large peptide precursors which are cleaved to yield smaller biologically active forms. Characteristic pairs of basic amino acids (Lys, Arg) flank the N- and C-terminal ends of the hormone within the precursor, suggesting that trypsin- and carboxypeptidase B-like peptidases are required to process the precursor. Carboxypeptidase B-like enzymatic activity involved in processing of proenkephalin has been identified in bovine adrenal medullary chromaffin granules (Hook et al., Nature, 295:341, 1982; Fricker and Snyder, PNAS, 79: 3886, 1982) (previously referred to as enkephalin convertase) and appears to be a thiol metallopeptidase stimulated by Co^{++} (Fricker & Snyder, PNAS, 79:3886, 1982; Hook & Eiden, submitted). Carboxypeptidase B has also been detected in granules from anterior, intermediate, and posterior lobes of rat pituitary. The pituitary enzyme(s) seem to be thiol metallopeptidases similar to the chromaffin granule species (Hook & Loh, submitted). The carboxypeptidase B-like activity in pituitary granules may be involved in processing of proopiomelanocortin and pro-vasopressin.

The carboxypeptidase B-like enzyme from bovine pituitary has been purified to homogeneity by following Co^{++}-stimulated activity throughout the purification procedure (Fricker & Snyder, J.Biol.Chem., in press), and appears to be a glycoprotein of 50,000 mw. Monoclonal and polyclonal antibodies against the purified enzyme were prepared. Monoclonal antibodies were produced by an in vitro immunization method. Spleen cells from Balb/c mice were incubated in thymocyte conditioned RPMI (15% fcs) medium containing 1 ug/ml purified enzyme for 5 days at 37° C, 10% CO_2 - 90% air atmosphere. Spleen cells were then fused with NS-1 mouse myeloma cells and hybridomas producing antibody against the purified enzyme were screened by ELISA using ^{125}I-sheep anti-mouse immunoglobulins. Hybridomas were cloned by the limiting dilution technique. Mouse antisera were prepared by injecting mice with 3 ug purified enzyme in complete Freund's adjuvant, three times each 2 weeks apart. Antiseras were screened by the ELISA method as used for screening monoclonal antibodies. These antibodies will be used to obtain information on the structure and cellular localization of the carboxypeptidase B-like processing enzyme for hormone precursors.

219.9 GLYCINE-DIRECTED PEPTIDE AMIDATION: PRESENCE IN RAT BRAIN OF TWO ENZYMES WHICH CONVERT P-GLU-HIS-PRO-GLY-OH INTO P-GLU-HIS-PRO-NH_2 (TRH). J.S. Kizer*, W.H. Busby, Jr.*, W.W. Youngblood* (SPON: M.A. Lipton). Lab. of Developmental Neuroendocrinology, Biological Sciences Research Center, UNC School of Medicine, Chapel Hill, NC 27514.

The biosynthetic pathway for thyrotropin-releasing hormone (TRH) is currently unknown. To study the possibility of glycine-directed amidation in rat brain and its relevance to the mechanisms of the biosynthesis of TRH, we synthesized the model substrate P-glu-his-pro-gly-OH. Both neonatal rat brain and adult rat pituitary were homogenized, sonicated and fractionated by gel permeation chromatography, and fractions from each tissue assayed for enzymatic activity capable of converting this model substrate into TRH. Addition of N-ethylmaleamide (0.5 mM) to the assay inhibited the degradation of TRH and facilitated the observation of two enzymes in both brain and pituitary catalyzing conversion of P-glu-his-pro-gly-OH into TRH. We conclude that these enzymes are of considerable importance to the post-translational processing of hormones into C-terminally amidated daughter peptides.

In addition, we also synthesized the model peptide, gln-his-pro-gly-OH and coupled it to BSA through an N-terminal γ-amino butyric acid bridge. Antisera raised were against this immunogen and a RIA developed for the model sequence. Neonatal rat brain was found to contain a protein which contained this amino acid sequence. This protein may represent an extended precursor form for TRH.

219.10 HISTIDYL-PROLINE DIKETOPIPERAZINE (His-Pro DKP) BUT NOT TRH IS SECRETED BY FETAL HYPOTHALAMIC AND CORTICAL CELLS IN CULTURE: EVIDENCE THAT His-Pro DKP MAY NOT BE DERIVED FROM TRH. R. Preston Lamberton*, Ronald Lechan and Ivor M.D. Jackson*. Division of Endocrinology, Department of Medicine, Tufts-New England Medical Center, Boston, MA 02111.

In order to study the physiologic role of His-Pro DKP, a cyclized dipeptide, which is a putative metabolite of thyrotropin releasing hormone (TRH), we have established a specific radioimmunoassay (RIA) for His-Pro DKP which does not recognize TRH or related peptides. Cortical and diencephalic segments from Day 17 fetal rats were dissociated in monolayer culture in flasks - 5 x 10^6 diencephalic or 10 x 10^6 cortical cells per dish - containing 4 ml Minimum Essential Medium, horse serum (10%) or fetal calf serum and antibiotics. The cells have been maintained in culture up to 21 days free of infection.

The time course of His-Pro DKP release into the culture medium was as follows:

	Day 5	Day 8	Day 14	Day 18	Day 21
Cortical	2,020	15,276	19,404	16,816	18,464
Diencephalic	2,160	15,320	20,352	15,808	17,008

(pg/flask per day:mean of 3 or 4)

Evidence for authenticity of immunoreactive (IR) His-Pro DKP in the medium was shown by a retention time similar to that of synthetic His-Pro DKP on high performance liquid chromatography (HPLC) (C_{18} μ-Bondapak:30 cm x 3.9 mm:solvent 2% CH_3CN/0.1% TFA) following affinity chromatographic purification on an anti-His-Pro DKP Biorad-affigel column.

Despite the high level of His-Pro DKP in the culture media, no TRH was detected in a RIA with a sensitivity of <1 pg. DKP and TRH were also assayed in various regions of the nervous system (NS) in the Day 17 fetal rat. While TRH was detectable only in the hypothalamus (85 pg/mg protein), His-Pro DKP was found throughout the NS in relatively high concentrations (spinal cord 816 pg/mg, brain stem 485 pg/mg and hypothalamus 375 pg/mg).

We conclude that: 1) His-Pro DKP is an authentic neural peptide found throughout the mammalian NS; 2) His-Pro DKP is synthesized by neuronal cells in culture; 3) TRH may not be a prohormone for His-Pro DKP in some systems.

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219.11 INHIBITION OF PHENOL SULFOTRANSFERASE IN RAT BRAIN AS A POTENTIAL TOOL FOR STUDIES OF CHOLECYSTOKININ TURNOVER.
O. Giorgi and J. L. Meek Lab. Preclinical Pharmacol. NIMH St. Elizabeths Hosp. Washington DC 20032

Cholecystokinin octapeptide (CCK-8) is present in brain as a sulfate conjugate. It is likely that a precursor of CCK-8 is sulfated during post-translational processing. If sulfation of CCK-8's precursor could be blocked pharmacologically, then estimation of CCK-8 turnover would be possible. The enzyme responsible for sulfation is phenol sulfotransferase (PST), which catalyses the transfer of $SO_4^=$ from phosphoadenosine phosphosulfate to a wide variety of phenolic acceptors. Since little is known about PST in rat brain, we investigated whether the best available inhibitor of PST (dichloronitrophenol, DCNP) was capable of inhibiting in vitro the sulfation of several phenols. Synthetic substrates were used since the endogenous precursors for CCK-8 have not been isolated. We also examined the pharmacokinetics of DCNP to determine its suitability as an in vivo inhibitor of brain PST. PST was assayed in whole rat brain homogenates (Foldes and Meek, J. Neurochem, 1974). K_m values were determined from Eadie-Hofstee plots. IC_{50}'s for DCNP were calculated by log-probit analysis at saturating substrate concentration.

Substrate (μM)	V_{max} pmol/min/mg	K_m μM	IC_{50} μM
p-nitrophenol(4)	22	.31	12
Phenol (100)	19	12	-
Dopamine(4,000)	22	1,300	14

DCNP was thus capable of completely inhibiting the sulfation of different phenols in vitro with similar IC_{50} values. For in vivo studies, the concentration of DCNP in plasma and brain was determined by HPLC after an injection at 100 μmol/kg, i.p. A peak concentration in brain of 25 μM was achieved 30 min after injection and decayed with a half life of 8 and 6 h in plasma and brain cortex, respectively. To establish if DCNP can inhibit sulfation in vivo, rats were injected with 150 μmol/kg, i.p. of the drug 30 min before intracortical administration of $^{35}S\text{-}SO_4^=$. Rats were decapidated 4.5 h later. DCNP caused a 50% inhibition of ^{35}S-CCK-8 formation as measured by HPLC (Meek, Iadrola, Giorgi, Br. Res. in press). DCNP concentration at decapitation was 13 μM. To determine if prolonged treatment with DCNP could induce a decrease in CCK-8 content in brain, rats were injected with an initial dose of DCNP (100 μmol/kg) followed at 8 h intervals with doses of 50 umol/kg for 2 and 4 days. No change in CCK-8 content was detectable, probably due to incomplete inhibition of PST. Higher doses of DCNP could not be used due to toxicity. Our results show that DCNP can only produce a partial inhibition of PST in vivo. The drug is not adequate for studying the turnover rate of brain peptides with a long half life. Development of a better, preferably irreversible, PST inhibitor is required.

219.12 PROCESSING OF DYNORPHINS 1-8, 1-13 and 1-17 BY A PURIFIED METALLOENDOPEPTIDASE. M. Benuck*, M.J. Berg* and N. Marks. Center for Neurochemistry, Rockland Research Institute, Ward's Island, N.Y. 10035

Previous studies have shown that N-terminal inactivation of enkephalin containing peptides is inversely related to chain length. This would suggest that for longer peptides, such as the dynorphins-(1-13) and -(1-17), other mechanisms for inactivation or conversion are dominant. Among the enzymes of interest is a membrane bound metalloendopeptidase recently purified from rat brain or kidney. The enzyme cleaves Leu-enkephalin (dynorphin-1-5) and Met-enkephalin-Arg-Phe at the Gly-Phe bond, a reaction inhibited by thiorphan (Ki 100 nM) and phosphoramidon (Ki 4 nM). For studies on the dynorphins-(1-8), -(1-13) and -(1-17), peptides were incubated with enzyme and breakdown monitored by an HPLC procedure as described elsewhere (Benuck et al., Biochem. Biophys. Res. Commun. (1982) 107, 1123) or by dansylation and detection of end-groups by chromatography on polyamide plates or by HPLC. In the case of dynorphin-(1-8), results indicate cleavage at the Gly-Phe bond to release Tyr-Gly-Gly and Phe-Leu-Arg-Arg-Ile; for dynorphin-(1-13) and -(1-17), cleavage occurred at Gly-Phe and Arg-Ile sites. Cleavage at Arg-Ile was more rapid than at the Gly-Phe bond. Studies with the various dynorphin fragments indicate a decreased vulnerability of the Gly-Phe bond with increase in chain length, suggesting that cleavage at the Arg-Ile bond becomes significant with respect to conversion processes.

In studies on metabolism by purified synaptosomal membrane fragments, different patterns of degradation were observed. For Leu-enkephalin (dynorphin 1-5) action of the aminopeptidase and metalloendopeptidase predominated, while with dynorphin-(1-13), rapid C-terminal release of lysine occurred (carboxypeptidase) followed by sequential removal of C-terminal dipeptides by an ACE-like enzyme. Similarly, for dynorphin-(1-17) C-terminal modification occurred leading to the formation of dynorphin-(1-8) as one of the intermediate products.

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219.13 ISOLATION AND CHARACTERIZATION OF TWO PEPTIDES DERIVED FROM PROSOMATOSTATIN IN RAT BRAIN. R. Benoit*, P. Böhlen*, F. Esch*, N. Ling* and B. Alford* (Spon: J.H. Morrison). Salk Institute, La Jolla, Ca. 92037.

Three peptides derived from prosomatostatin have already been characterized in mammalian brain: somatostatin-14 (SS14), somatostatin-28 (SS28) and somatostatin-28(1-12). Using a C-terminally directed antiserum to SS28(1-12), we have recently shown that two N-terminally extended SS28(1-12)-like peptides are present in acid extracts of rat hypothalamus, extra-hypothalamic brain and spinal cord. We describe here the isolation and partial characterization of these two new neuropeptides.

518 fresh female rat brains were homogenized in hot (95°C) 2 M acetic acid. The extract, after defatting and lyophilization, was subjected to carboxy methyl cellulose cation-exchange chromatography: three peaks of immunoreactive material eluted at 0.0025 M, 0.025 M and 0.15 M ammonium acetate (pH4.6) representing 16.1 (peak I), 75.5 (peak II) and 8.4 (peak III) percent of the total SS28(1-12)-like immunoreactivity. Peak I, when chromatographed on sephadex G75, yielded 7.3 nmol SS28(1-12)-like material eluting in a zone compatible with a 4,500 mol.wt. peptide (5 K). Peak II contained two major immunoreactive components after gel filtration: one co-eluting with a SS28(1-12) standard and another one equivalent to 6.3 nmol of a 7,500 mol wt. SS28(1-12)-like material (8 K). Purification to homogeneity of the 5 K and 8 K peptides was achieved with 3 reverse phase HPLC systems using C18 columns. The mobile phases consisted of pyridine formate/n-propanol, triethyl ammonium phosphate/acetonitrile and finally trifluoroacetic acid/acetonitrile. 1.5 nmol pure 5 K and 2.1 nmol 8 K peptides were isolated.

Microsequencing revealed that the N-terminal sequence of the 5 K peptide is LSEPNQTENDALEPEDL. These data, the amino acid composition and the specificity of the antiserum (S320) used during isolation together with information on partial cDNA sequence encoding rat prosomatostatin (Goodman, R.H. et al., J. Biol. Chem. 257:1156, 1982) demonstrate that the 5 K peptide contains 44 amino acids and has the SS28(1-12) sequence at its C-terminus. Similarly, microsequencing showed that the N-terminal sequence of the 8K peptide is APSDPRLRQF. This observation together with amino acid analysis data and antibody specificity indicate that the 8K is a 76 amino acid peptide containing the 5 K at its C-terminus. The 8K most probably represents the whole prosomatostatin sequence without Arg-Lys-SS14.

We propose that (1) rat prosomatostatin represents a 92 amino acid protein of 10,386 mol wt which generates several polypeptides, five of which are now characterized; (2) at least three of these peptides do not contain the SS14 sequence; (3) generation of the 5 K peptide from prosomatostatin involves cleavage of a Leu-Leu bond. Prosomatostatin could be envisioned as a multivalent molecule highly preserved through evolution and possibly endowed with more than one role in neurotransmission.

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220.1 EFFECTS OF PRETECTAL LESIONS ON COMPONENTS OF THE HORIZONTAL OPTOKINETIC RESPONSE. V. Matsuo, J. Buettner-Ennever*, B. Cohen, J. Fradin* and H. Blumenfeld*. Dept. of Neurology, Mt. Sinai Sch. of Medicine, New York, N.Y. (SPON: G. Cohen).

Electrolytic and chemical lesions were made in the pretectum (PT), superior colliculus (SC) and mesencephalic reticular formation (MRF) of monkeys to determine their effect on the various components of optokinetic nystagmus (OKN) and optokinetic afternystagmus (OKAN). OKN can be divided into two components that reflect processes which produce a rapid and slow rise in slow phase velocity. The rapid rise is thought to be mediated through the cerebral cortex and the flocculus, but the interconnecting pathways are unknown. The slow rise reflects activation of a velocity storage mechanism and is associated with production of OKAN. The slow component is thought to be mediated through the pretectum via NRTP to the vestibular nuclei in rabbit, cat and rat (Precht, 1981); PT participation in OKN and OKAN has not been studied in the monkey. Destruction of SC with kainic acid caused ipsilateral spontaneous nystagmus, but did not affect either OKN or OKAN. It seems unlikely that SC participates in production of OKN or OKAN. Findings were similar after discrete electrolytic lesions of the MRF, leading to a similar conclusion. Small electrolytic and vascular lesions of the medial pretectum centered near the region of the nucleus of the posterior commissure transiently abolished the rapid rise in OKN, leaving the slow rise in OKN and OKAN intact. This suggests that activity related to production of the rapid rise in OKN slow phase velocity may be processed through this area. A large kainic acid lesion involving PT, SC and MRF caused ipsilateral spontaneous nystagmus, a loss of the slow rise in contralateral OKN, and abolished contralateral OKAN. However, the rapid rise in OKN was unaffected and time constants of vestibular nystagmus were unaffected. Findings were similar after a large electrolytic lesion that involved the lateral portions of PT and the MRF. We interpret these data to indicate that the pretectum is an important processing center for OKN and OKAN in the monkey as in other species. It is inferred that cells are located in lateral PT whose axons project activity toward the vestibular system for production of the slow rise in OKN slow phase velocity and for OKAN. The loss of the rapid rise in OKN after vascular or electrolytic lesion of PT, but its preservation after a kainic acid lesion that destroyed PT cells indicates that activity related to the rapid rise in OKN is probably carried through medial PT in a fiber tract. The data also indicate the region of the MRF previously identified as being related to both slow and rapid eye movements is not primarily related to generation of horizontal OKN and OKAN.

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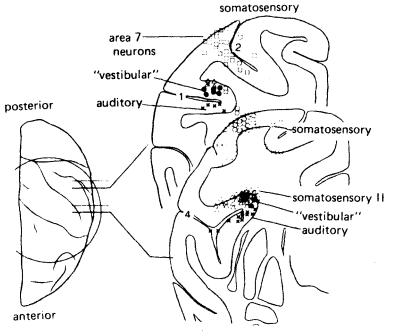
220.2 LOSS OF VERTICAL RAPID EYE MOVEMENTS AFTER KAINIC ACID LESIONS IN THE ROSTRAL MESENCEPHALON IN THE RHESUS MONKEY, V. Henn*, H. Schnyder*, K. Hepp* and H. Reisine (SPON: H. Krieger). Dept. of Neurology and Brain Research Institute, University, Zürich, and Physics Dept., ETH, Zürich, Switzerland.

The rostral interstitial nucleus of the MLF contains medium-lead burst neurons with vertical on-directions. Numbers of spikes per burst and burst frequency determine vertical components of rapid eye movements. Anatomical evidence indicates that neurons in the rostral nucleus of the MLF are directly connected with the oculomotor complex and eye movement triggering zones in the paramedian pontine reticular formation. To decide what role these neurons play in the causal chain of events to generate eye movements, they were destroyed chemically. Their precise location was determined by single neuron recordings. Local injection of kainic acid through a microsyringe destroyed all neurons but left fibers en passage unaffected. With a bilateral lesion of the rostral interstitial nucleus of the MLF all vertical rapid eye movements were lost: the animal made rapid eye movements only in the horizontal plane. During rotation in the vertical plane, the slow compensatory movements induced by the vestibular stimulus were intact.

Clinical data show that rapid eye movements might be lost in an upward or downward direction only. This proves that generation sites or pathways for vertical movements are separate. Work is in progress to place smaller bilateral lesions in the rostral mesencephalon to delineate these areas.

220.3 A NEW VESTIBULAR AREA IN THE PRIMATE CORTEX. O.-J. Grüsser*, M. Pause*, U. Schreiter*. Dept.Physiol.,Freie Univ.Berlin,Germany.

Microelectrode recordings were performed in 6 awake Java monkeys (M. fascicularis) sitting in a monkey chair mounted on an electronically controlled turntable and surrounded by a vertical striped cylinder (1.15 deg. period). Recordings were obtained from single units located in an insular and retroinsular area. All neurons responded directionally selective to horizontal and/or vertical head-in-space movement in the dark. Most of the units were also directionally selectively activated by trunk movements (neck receptor input) and by visual movement signals when the striped cylinder was rotated either to the left or to the right. The units exhibited predominantly very large, directionally selective, visual receptive fields. Visual-vestibular interaction was studied: "agonistic" and "antagonistic" interaction of the input signals from these two modalities was found. The same was true for the interaction of the responses to horizontal semicircular canal receptor stimulation with the responses to neck receptor stimulation. A considerable percentage of the vestibular



neurons also responded to vaguely defined somatosensory stimuli, such as tactile stimulation of the skin of neck and arms, of joint receptors of the extremities, slapping of the soles of the feet or general body vibration. On completion of the neurophysiological experiments, the recording sites were determined histologically. A region described in cytoarchitectonic studies by Pandya and Sanides (Z.Anat.Entwicklungsgesch., 139:127,1973) as a candidate for vestibular cortex was found to be the area from which our recordings were obtained (fig. 1).

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220.4 PREFRONTAL, FRONTAL EYE FIELD, AND AREA 6 PROJECTIONS TO THE PARAMEDIAN PONTINE RETICULAR FORMATION (PPRF) IN THE MONKEY. G.R. Leichnetz and D.J. Smith*. Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond,VA 23298.

The paramedian pontine reticular formation (PPRF) has been recognized as having a central role in eye movement, since stimulation of the region results in ipsilateral horizontal eye movements whereas unilateral lesions of PPRF produce ipsilateral gaze paralysis. In this study we made small transcannular implants of solid horseradish peroxidase (HRP) polyacrylamide gel in the paramedian pons in four capuchin monkeys (Cebus apella) to determine the principal source of cortical input to this region. In other monkeys, HRP gel implants were made in frontal and parietal cortex to anterogradely label corticopontine projections. The brains were processed according to the TMB protocol of Mesulam and the tissue was studied under dark field illumination.

Both paramedian tegmental and basilar pontine HRP gel implants resulted in retrogradely labelled small lamina V pyramidal neurons located predominantly in the ipsilateral rostral frontal lobe. Large numbers of labelled cells were observed in area 6, area 8 (frontal eye field, FEF), dorsal prefrontal convexity and dorsal medial prefrontal cortex. A lesser, but still considerable number of labelled cells were present in the sulcus principalis cortex, ventral prefrontal convexity, and medially in the cingulate gyrus. In the largest pontine tegmental implant where orbitofrontal cells were also labelled, there was an almost uninterrupted line of lamina V corticopontine neurons surrounding the perimeter of rostral frontal sections. Implants in the paramedian tegmental pons (involving nuc. ret. pontis oralis, nuc. ret. tegmenti pontis and superior central nuc.) resulted in heavier prefrontal labelling, whereas paramedian basilar pontine implants resulted in heavier labelling of area 6, particularly in postarcuate cortex. There were considerable numbers of FEF cells labelled bilaterally even where there was clearly no possibility of spread across the midline of the pons.

Anterograde studies following frontal (area 6), prefrontal, and area 8 (FEF) implants, demonstrated the paramedian orientation of corticopontine terminal fields with a partial decussation of FEF-pontine projections in the rostral PPRF, whereas parietal implants led to labelling of predominantly lateral basilar pons with a small terminal field in NRTP. The anterograde findings confirmed the retrograde results and raised the possibility of convergence of prefrontal and parietal projections in the paramedian pons, but clearly showed the rostral frontal lobe to be the main source of cortical influence on the region.

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220.5 COMPARISON OF AFFERENTS TRACED TO THE SUPERIOR COLLICULUS FROM THE FRONTAL EYE FIELDS AND FROM TWO SUB-REGIONS OF AREA 7 OF THE RHESUS MONKEY. J.C. Lynch and A.M. Graybiel. Dept. of Anatomy, U. Miss. Med. Center, Jackson, MS 39216 and Dept. of Psychology and Brain Sci., Mass. Inst. of Tech., Cambridge, MA 02139.

The inferior parietal lobule (IPL), frontal eye fields (FEF) and superior colliculus (SC) are major elements of the central mechanism controlling eye movements and behavioral attention in visuomotor tasks. The FEF are known to project massively to the intermediate and deep layers of the SC and fiber-degeneration findings suggested that these layers also receive a significant input from the IPL. In recent axon transport studies, however, only sparse projections from the IPL to the SC have been reported. We therefore studied parietal efferents in detail, comparing them with frontal projections to the brain stem. Autoradiographic tracing was used in 9 rhesus monkeys, and horseradish peroxidase (HRP) transport in one. Altogether, 12 hemispheres were injected.

The major finding of these experiments is that the cortex of the inferior bank of the intraparietal sulcus projects heavily to the intermediate and deep layers of the SC, whereas the cortex on the convexity of the IPL sends only sparse projections to the SC. Both sub-regions were included in area 7 by Brodmann and in area PG by von Bonin and Bailey. However, the bank cortex has been proposed as a distinct cytoarchitectural zone (POa) by Seltzer and Pandya, who also reported that input from visual area 19 to the IPL is restricted to this same sub-region. We observed both anterograde transport to the SC and retrograde filling of area 19 cells after placing HRP in area POa. The fibers from POa reach the entire rostrocaudal extent of the SC. Anteriorly, they are concentrated in the intermediate gray layer (IV); in the posterior SC they extend from layer IV into the deep gray (VI). Only rarely do they extend into the stratum opticum (III). FEF fibers terminate similarly, although there is more consistent invasion of layer III. In addition, the FEF distribution is characterized by "holes" where labelling is weak, whereas the POa distribution is more homogeneous. POa and convexity PG (PGc) both project to medial and lateral pulvinar, to n. lateralis posterior, pretectum, and lateral pontine nuclei. POa contributes also to a discrete horizontal band of label in the periaqueductal gray matter.

POa receives the vision-related input sent from prestriate cortex to the IPL, contains a larger proportion of eye-movement related neurons than PGc, and contains all of the active reach and hand-eye coordination cells that have been observed in the IPL. The present demonstration of a selective projection from POa to a major preoculomotor center supports the proposal that POa represents a functionally independent sub-region of the IPL.
(Supported by NIH grants EY02640, EY04159, EY02866, EY02621, and NSF grant BNS 81-12125)

220.6 **DEFICITS IN EYE MOVEMENTS AFTER INJECTION OF GABA-RELATED DRUGS IN MONKEY SUPERIOR COLLICULUS.** **R.H. Wurtz and O. Hikosaka. Lab. of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20205.**

Our previous studies (Hikosaka and Wurtz, J. Neurophysiol., 49: 1230, 1983) suggested that cells in the monkey's substantia nigra pars reticulata (SNr) exert tonic inhibition on saccade-related cells in the superior colliculus (SC) and release the inhibition before a saccade to a visual stimulus or to the remembered position of the stimulus. The nigro-collicular inhibitory connection has been suggested to be GABAergic.

We now report that microinjections of muscimol (GABA agonist) and bicuculline (GABA antagonist) in SC produce severe deficits in eye movements in the alert, trained monkey. Through a guide tube directed to the SC we introduced a glass pipette filled with a drug solution which could be pressure injected. The pipette also contained a fine tungsten microelectrode which allowed stimulation and recording of single cell activity. The pipette was positioned in the intermediate layers of the SC where cells discharged before a saccade to a particular part of the visual field (movement field).

Immediately after injection of muscimol (0.2-2.0 ug) saccades to visual or remembered targets in the movement field had increased latency (frequently over 300 msec), and were hypometric. Saccades to areas outside the movement field were relatively unaffected suggesting that the drug effect was restricted within the SC. Spontaneous saccades, particularly to the movement field contralateral to the side of injection, became infrequent; the eye stayed in a small area in the orbit usually symmetrical to the movement field.

Bicuculline (0.2-1.0 ug) dramatically increased the frequency of saccades to the movement field of cells near the injection site. Its injection was immediately followed by repetitive, uniform saccades which brought the eye from the center of the orbit to the contralateral periphery. The repeated saccades then took the form of square wave jerks: a saccade to the movement field was followed by a saccade in the opposite direction. The eye tended to stay in a small area in the orbit which corresponded to the movement field. Under these conditions the monkey could not suppress contralateral saccades and therefore could not perform the task which required fixation of gaze. The effects of muscimol and bicuculline lasted no more than a day. Injection of saline in the SC produced no apparent change in eye movements.

The present results confirmed the important role of the SC in saccade generation and further emphasized the role of SNr-induced, GABA-mediated, inhibition in saccade initiation.

220.7 EFFECTS OF SUPERIOR COLLICULUS LESIONS ON SCANNING HEAD MOVEMENTS IN HAMSTERS. J.-C. Lecas*, C. Thinus-Blanc* (SPON : P. Ellen), Laboratoire de Physiologie Nerveuse, C.N.R.S., 91190 Gif-sur-Yvette, France.

The aim of this study was to assess in adult hamsters the effects of bilateral colliculus lesions on scanning head movements during a visual discrimination learning. We used a new detection system involving the presence of two light-emitting diodes, affixed on the animal's head, and a computer analysis of the video signal from a TV camera installed above the training apparatus (Lecas & Dutrieux, in press).

With this equipment, we were able to continuously monitor (resolution 60 milliseconds) the head position and the horizontal head movements of free-moving hamsters subjected to a simultaneous visual discrimination task.

Collicular hamsters do not differ from control ones in head movement patterns but with regard to the occurrence of stops during which these movements are made. Normal animals exhibit slower and more irregular progression, intermingled with scanning movements, whereas collicular hamsters make straighter runs. Although the latter did not show any drastic impairment in the rate of learning, our data strongly suggest that they use different orienting and learning strategies to master the task.

Additional testing provides evidence that collicular hamsters do not ignore changes in the surrounding environment but react to novelty in a different way from normal animals.

220.8 TECTAL ABLATION ARRESTS EYE MOVEMENTS ELECTRICALLY EVOKED FROM OCCIPITAL AND PARIETAL EYE FIELDS. E.G. Keating, S.G. Gooley*, S.E. Pratt* and J.E. Kelsey*. Department of Anatomy, S.U.N.Y. Upstate Medical Center, Syracuse, New York 13210

Electrical stimulation of several cortical areas in monkeys evokes saccadic eye movements. We have set about tracing the routes by which signals from these areas gain access to the midbrain and pontine premotor structures generating eye movements.

Eye movements of cynomolgous monkeys were measured with the magnetic search coil. Monopolar surface stimulation of the cortex (1 sec trains of 0.5 msec monophasic pulses at 200 Hz) confirmed the presence of a frontal eye field (FEF) along the anterior bank of the arcuate sulcus. There was also a group of posterior fields mapped across striate, prestriate and inferior parietal cortex where stimulation currents between 0.5 - 3 mamps reliably evoked a stereotyped staircase of saccadic eye movements.

These eye fields were remapped 2-6 weeks after surgery intended to remove the superior colliculus (SC) on one or both sides. Removing the SC silenced eye movements normally evoked from striate cortex (Schiller, 1977). In addition, eye movements could no longer be evoked from prestriate cortex (only V_4 was examined) or from area 7a of the inferior parietal cortex. Currents 2-3x threshold failed to trigger eye movements from these areas. However, saccadic eye movements could still be evoked from the frontal eye fields. If the tectal surgery was unilateral, eye movements could also still be evoked from posterior fields contralateral to the lesion. All of the tectal lesions invaded pretectum and medial thalamus to some extent, but ablations in one control monkey that mimicked the diencephalic pathology did not silence evoked eye movements. So, for their control of eye movements, posterior cortical visual areas seem to share a common dependence on a circuit that passes through the superior colliculus.

In tracing the network prior to the tectum we have preliminary evidence that neither FEF nor area 7a serves as a critical funnel of visuomotor control from occipital lobe to the superior colliculus.

These results continue the notion of there being 2 pathways of oculomotor control descending from the forebrain. One passes through FEF and does not require SC for its access to the oculomotor core. The second pathway arises in the posterior cortical eye fields, does not require FEF but does depend on superior colliculus for its access to brainstem premotor structures.
EY02941 and EY04005.

220.9 NEURONS IN ATTENTION-RELATED REGIONS OF THE CEREBRAL CORTEX WHICH PROJECT TO INTERMEDIATE AND DEEP LAYERS OF SUPERIOR COLLICULUS OF THE RHESUS MONKEY. L.J. Lobeck*, J.C. Lynch, and N.L. Hayes. Dept. of Anatomy, U. Miss. Med. Center, Jackson, MS 39216.

Both anatomical and physiological evidence suggest that the superior colliculus (SC) plays an important role in the control of eye and head movement and the modulation of selective attention. The superficial layers derive their primary input from the retina and visual cortex, the deeper layers from a multitude of cortical and subcortical regions. In the course of an autoradiographic study of the efferent connections of the inferior parietal lobule (IPL), a cortical area with functional characteristics similar to those of the SC, we observed (Lynch and Graybiel, this meeting) that only the cortex of the posterior bank of the intraparietal sulcus (IPS) (area POa of Seltzer and Pandya) projects to the SC, whereas the cortex on the convexity of the IPL (PGc) does not. In order to study these connections of the IPL in more detail, we injected horseradish peroxidase (HRP; 0.02 to 0.3 µl, 30% in distilled water) in the SC of three rhesus monkeys, using physiological criteria to center the injections in the intermediate and deep layers of SC. After 48 hours animals were sacrificed by perfusion with mixed aldehydes and the brains cut frozen at 50 µm. Alternate sections were reacted with either TMB or PPD:PC as chromagen; sections reacted with PPD:PC were counterstained with cresyl violet for cytoarchitectonic analysis.

The cortical regions with the highest density of labelled neurons include the inferior bank of the IPS, the superior bank of the superior temporal sulcus (STS), and the anterior bank of the arcuate sulcus of the ipsilateral hemisphere. All of these areas are considered to be involved in the modulation of selective attention. There was no reliable difference in the average density of labelled cells among these 3 regions. Few labelled neurons were observed on the convexity of either the IPL or the temporal lobe. Almost all labelled neurons were layer V pyramidal cells. Significant numbers of labelled cells were also seen in the inferior bank of STS, in the superior bank of IPS, both banks of the principal sulcus, the superior bank of the lateral fissure, and in dorsal prestriate cortical areas. A few cells were observed in area 17, including giant pyramids in layer 6, indicating that the effective injection sites encroached upon the superficial layers of SC.

The present demonstration of neural connections between POa, FEF, the bank of the STS, and intermediate and deep SC supports the proposal that these regions may function as physically separate components of a single distributed system concerned with the control of selective attention and related eye and head movements.
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220.10 ATTENTION-RELATED CHARACTERISTICS OF CELLS IN THE FRONTAL CORTEX IN THE RHESUS MONKEY. E. Bakay Pragay*, A.F. Mirsky, R.K. Nakamura and R.U. Esposito. Lab. of Psychology and Psychopathology, NIMH, Bethesda, MD 20205.

We have previously examined task-related units in the brainstem and in various forebrain areas. Some of these units, called Type II, showed peristimulus activity in relation to rewarded go and no go trials. Controls ruled out simple sensory or motor explanations for the responses and indicated their relation to the attentional demands of the task. Type II units were often found intermixed with Type I units; these responded to go trials only.

We report here characteristics of units related to a go-no go task in the region of frontal cortex extending from the rostral bank of the arcuate sulcus to the central sulcus and extending medially to the cingulate sulcus. To manipulate preparatory sets, we varied the fixed pre-stimulus waiting period (1,2,2.5, 3 sec). To test the stability of the no go related activity, we recorded during blocks of no go trials as well as during blocks with semi-randomized go-no go trials (standard task).

Type II and Type I units were found in all areas; their proportion and predominant subtypes varied across areas, largely following an anterior-posterior gradient.

The rostral areas, expecially the anterior cingulate region, contained "symmetrical" Type II units. These showed phasic post-stimulus bursts nearly equal in size in go and no go trials. The majority showed anticipatory activity to the stimuli with marked sensitivity to changes in the intertrial interval. Their anticipatory response could be increased by using long (2.5 or 3 sec) waiting periods or eliminated by using a 1 sec waiting period. Virtually any task-event encoded by Type II units could be made anticipatory by the appropriate task-manipulation.

The posterior regions, especially the rostral bank of the central sulcus, contained predominantly Type I units.

In intermediate areas (dorsal convexity at the level of the superior precentral sulcus) Type II and Type I cells were equally numerous. Type II units in this area were typically "asymmetrical" showing more intensive and/or longer lasting response in go trials. The no go response, although small, did not deteriorate during homogenous no go series.

The "asymmetrical" Type II activity may have stimulus-response association functions; the "symmetrical" activity is best explained as attention to or anticipation of behaviorally significant stimuli.

220.11 CORTICAL REGULATION OF SACCADIC EYE MOVEMENTS IN MAN DEMONSTRATED BY POSITRON EMISSION TOMOGRAPHY, R.M.Burde, P.T.Fox* and M.E.Raichle. Depts. of Neurology and Ophthalmology and Div. of Radiation Sciences, Washington Univ. School of Med., St. Louis, MO 63110.

Positron emission tomography (PET) is a non-invasive measure of regional cerebral blood flow (rCBF) able to quantify changes in regional cerebral activity due to motor tasks and sensory stimuli. We are employing PET to study the pattern of cortical activation during saccadic eye movements in man.

Each subject undergoes a series of 8 $H_2{}^{15}O$ PET scans during a single session. The initial and final scans of a series are unstimulated with eyes and ears occluded. The remaining 6 conditions are presented in random order and include: continuous bilateral peripheral target lights with eyes immobile at midline; continuous bilateral peripheral targets with 2Hz alternating saccades to the targets; peripheral targets alternating sides at 2Hz with eyes immobile at midline; peripheral targets alternating sides at 2Hz with saccades to the alternating targets; 2Hz alternating saccades with visual deprivation; bilateral 2Hz finger movements. Percent change in rCBF from unstimulated control is calculated for each pixel in the PET image. Cortical regions activated by the test condition are localized by projecting the region coordinates onto a template of the cerebral hemisphere utilizing a lateral skull radiograph to establish the horizontal reference plane.

Peripheral visual stimulation without saccades or foveation induces no cortical response. Foveation during 2Hz saccades consistently induces a 16-20% activation of striate cortex. Saccades without visual stimulation produce no detectable activation of striate cortex, nor of any occipital or parietal cortex. Saccades under any condition produce rCBF activation of 10-20% in the posterior third of the middle and superior frontal gyri and of 11-17% in pre-Rolandic cortex within the inter-hemispheric fissure. These areas correspond well to the presumed positions of the frontal eye fields and of supplementary motor cortex. Finger movements, used as an internal anatomic reference, activated both the supplementary motor cortex and the precentral gyrus immediately posterior to the frontal eye fields.

Several conclusions may be drawn. 1) $H_2{}^{15}O$ PET permits repeated non-invasive quantitative measurements of rCBF during sensory and motor activation in man. 2) Saccadic eye movements consistently produce regional cortical activation in the frontal eye fields and in supplementary motor cortex. 3) No parietal or occipital regions were measurably activated by saccades. Notably Brodman areas 7, 18 and 19 could not be demonstrated to take part in saccadic eye movements.

220.12 DISCHARGE PATTERNS OF TECTO-BULBO-SPINAL NEURONS DURING VISUO-MOTOR REACTIONS IN THE ALERT CAT. A Grantyn* and A. Berthoz* (SPON: R. Hudgin). Lab. de Physiologie Neurosensorielle, CNRS, Paris, France.

According to morphological criteria, neurons of the cat superior colliculus (SC) projecting to the tecto-bulbo-spinal tract (TBSN) represent a homogeneous population (Grantyn & Grantyn 1982). They also display similar active membrane properties. Experiments on anaesthetized cats have shown that all TBSNs generate grouped discharges (extra spikes) when stimulated by constant transmembrane currents of intermediate intensity (Grantyn & Grantyn 1983). The present study was undertaken to examine the activity of this class of neurons in alert cat. Intra and extracellular recordings were taken from axons of TBSNs, within the predorsal bundle at caudal pontine levels. TBSN axons were identified by their short latency (0.3-0.5 ms) direct orthodromic response to SC stimulation and antidromic response to stimulation of the spinal cord at C_2 level. Behavioral tests consisted in presenting a variety of visual stimuli to experimentally naive animals while monitoring eye movement and EMG of neck muscles. TBSN activity was also studied during spontaneous eye movements and vestibular nystagmus.

Out of 40 TBSNs, 29 were activated by discrete moving (15-100 deg/s) visual stimuli. Most neurons were directionally selective. Twelve of them discharged more vigorously to presentation of real objects than to spots of light and 15 showed an additional enhancement of discharge if the animal oriented towards the stimulus as judged from eye movements and contractions of neck muscles. A few cells only were identified as true presaccadic neurons. Eleven TBSNs were silent or generated sporadic spikes unrelated to sensory-motor performance. The absence of sustained spontaneous activity was a typical feature of TBSNs in alert cat. Phasic discharges usually lasted 50-100 ms and attained mean frequencies of 50-100 imp/s. For a majority of neurons, one could find a behavioral situation during which they entered the range of doublet generation. The transition to this mode of repetitive firing occurred at critical mean frequencies of 70-100 imp/s.

It is concluded that the mechanism of extra spike generation which represents a characteristic active membrane property of TBSNs is mobilized during naturally occurring visuo-motor reactions. With respect to the behavioral correlates of their activity TBSNs cannot be regarded as a uniform population. Some of them appear to be engaged in motor and/or attentional aspects of orienting while the others transmit visual information independently of whether or not it is used for the triggering or guidance of gaze shifts.

220.13 DYNAMIC PROPERTIES OF DIFFERENT CLASSES OF PURSUIT-RELATED PRE-MOTOR NEURONS IN THE PRIMATE BRAIN STEM

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In a new search for possible input neurons to the final common pathway for the neural control of foveal pursuit eye movements (Eckmiller,R.,Mackeben,M.,Brain Res., 184:210,1980; Sparks,D.L., Sides,J.P.,Brain Res., 77:320,1974), single unit activity was recorded in the vicinity of the abducens nuclei in alert monkeys (Macaca fascicularis). With their head attached to a primate chair, animals had been trained to pursue a horizontally moving visual target(8 min of arc in diameter) under three conditions: a)pursuit during sinusoidal target movement(10 deg amplitude at frequencies between 0.4 and 1.1 Hz), b)pursuit during head rotation with the same amplitude and frequencies as in a) (chair rotation about the vertical axis in front of the stationary target), c)fixation of the target which moved with the sinusoidally rotating chair. Only those neurons which exhibited an impulse rate(IR) modulation in correlation with pursuit at least under condition a) and were clearly distinguishable from eye position coded neurons were considered.

Three main classes of pursuit-related neurons were found:
1.Pursuit Neurons which had recently been described by Eckmiller and Mackeben(1980) were found to be modulated in phase with eye velocity ipsilateral to the recording site(ipsi-$\dot{\theta}_e$) only under a). Under b) max.IR dropped considerably and occurred 20 to 40 deg before contra-$\dot{\theta}_e$. Under c) max.IR exceeded the value under a) but had the same phase relationship as under b).
2.Pause Neurons were(with a few exceptions) pursuit-related at variance with several earlier reports. For individual neurons max.IR was in phase with either ipsi(or contra)-θ_e or $\dot{\theta}_e$ under a), but it was typically in phase with or even leading contra-$\dot{\theta}_e$ under b) and c). For some neurons IR modulation was considerably stronger during the two conditions with vestibular stimulation.
3.Tonic Neurons were quite often pursuit-related and will need further sub-classification with respect to their activity changes during saccades or vestibular stimulation. For individual neurons max.IR typically occurred 20 to 40 deg before contra-θ_e or contra-$\dot{\theta}_e$ under both conditions a) and b).

The results suggest that only pursuit neurons have optimal dynamic properties as input neurons to the common pathway to assure foveal pursuit by generating the total required velocity signal under a), the necessary difference signal to supplement the vestibular velocity signal under b), or a strong compensatory signal under c). The functional role of pursuit-related pause neurons and tonic neurons in the neural control of foveal pursuit remains to be studied.

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CIRCUITRY AND PATTERN GENERATION II

221.1 SELECTIVE RECORDING AND STIMULATION OF IDENTIFIED NEURONS IN FREELY-BEHAVING APLYSIA. David W. Parsons* and Harold M. Pinsker. Marine Biomed. Inst. and Depts. of Physiol. & Biophys. and Psychiat. & Behav. Sci., Univ. TX Med. Br., Galveston, TX 77550.

Three criteria currently used to establish the behavioral importance of an identified nerve cell are: 1) it is "necessary" if the normal behavior is abolished or severely disrupted when it is selectively destroyed or prevented from firing; 2) it is "sufficient" if the normal behavior is elicited when it is selectively stimulated; and 3) it is "appropriate" if the putative pattern of activity actually occurs during normal behavior. The last two criteria require a technique for selective stimulation and chronic monitoring of an identified neuron in an intact animal. We recently developed a simple technique for gluing a fine-wire electrode into the connective tissue above an identified cell body in Aplysia. By recording during implantation, signal-to-noise properties of the waveforms and selectivity of stimulation are maximized and the identity of the cell confirmed by its characteristic firing pattern. A cuff electrode implanted on an appropriate peripheral nerve or central connective provides an additional criterion (1:1 correlation between soma and axonal spikes) for identifying the target neuron during surgery. Rigorous behavioral criteria (including attachment to the substrate, normal withdrawal reflexes, and feeding) are used to establish that postoperative behavior and neuronal activity are normal.

Representative selective cell-body and simultaneous whole-nerve recordings in intact animals are shown for several different identified neurons in Aplysia central ganglia. We illustrate activity recorded during implantation; the signal-to-noise properties of soma and axonal spikes; the selectivity of soma compared to whole-nerve stimulation; subsequent impalement of the same soma in the exposed nervous system; and more prolonged monitoring of normal neuronal activity. It may be possible to record from identified cell bodies whose axons are too small for their spikes to be recorded with whole-nerve electrodes. The assumptions and requirements for using this technique in other preparations will be discussed. It combines the selectivity of intracellular approaches with the noninvasiveness of extracellular approaches and provides a direct link between neuronal activity in intact animals and reduced preparations. Selective soma stimulation and recording provide two additional methods for assessing the behavioral function of an identified neuron in an intact animal, and complement recently developed techniques for selectively destroying individual identified nerve cells.

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221.2 CIRCUITRY UNDERLYING BURST GENERATION IN THE LOCUST FLIGHT SYSTEM. R.M. Robertson and K.G. Pearson, Department of Physiology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

The flight motor pattern of the locust is generated at the interneuronal level. Many of the interneurons responsible for driving flight motoneurons and for generating the rhythm have now been described (Robertson and Pearson, J.Comp.Neurol. 215: 33-50 (1983). Currently we are investigating connections between these interneurons. In a deafferented preparation of Locusta migratoria able to express the flight rhythm the neuropile processes of interneurons were penetrated with Lucifer Yellow-filled microelectrodes. Neuronal interconnections were established with simultaneous double penetrations and the neurons were filled with dye for subsequent identification. Results obtained using these techniques enabled us to construct a partial circuit diagram of the locust flight system involving 18 interneurons.

Of primary importance in this circuit is a bilateral pair of mesothoracic interneurons (301s). Each 301 has inhibitory connections (latency 2ms; duration 15ms) with other flight interneurons. More important, however, is that every spike in 301 is followed by a powerful delayed excitation (latency 5ms; duration 47ms) in a number of other flight interneurons. The basis of this postsynaptic potential is unknown at present but the fact that it can be reversed by passage of hyperpolarizing current is consistent with the idea that it is produced by a decreased conductance across the postsynaptic membrane (i.e. a direct decreased-conductance synapse or disinhibition with graded release of transmitter). The importance of the delay in the connection from 301 to other interneurons is that 301, which fires in the elevator phase, can drive neurons to fire in the depressor phase. One of the depressor-type interneurons excited in this way feeds back with a direct inhibitory connection to 301 thus establishing an elementary circuit by which burst activity may be generated. Support for this proposal is given by the observation that sustained suprathreshold depolarization of 301 with constant current can induce rhythmical activity resembling flight activity.

This burst-generating circuit is linked to depressor motoneurons by other interneurons driven by 301. One of the interneurons involved in the activation of 301 has been identified. This interneuron (504) also excites elevator motoneurons. Thus we have found additional circuits which may underlie the generation of the basic elevator-depressor sequence seen in deafferented preparations.

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221.3 IS LAMPREY RESPIRATION A ONE-PHASE RHYTHM?
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The respiratory system of lampreys resembles that of fossil pre-vertebrates, and may use basic mechanisms conserved in higher vertebrates. Water moves tidally in and out of a series of 7 gill sacs along each side of the head during respiration (1).

The coordination of the system was studied here by simultaneous electromyographic records from several segments, in lightly anesthetized young adult Petromyzon marinus. EMG electrodes were placed subcutaneously between pairs of gill pores, recording from branchial constrictor muscles and possibly others. During quiet breathing (Fig. 1), there was good burst synchrony between the two sides, and between rostral and caudal segments. The cycle period was 0.6 sec (at 10 °C); the duty cycle of bursts was 0.15 or less.

A less complete synchrony was seen during another type of breathing, in which an intense discharge could occur every 10-30 cycles, with significant but variable delays between the MN discharges of different sides or segments.

A pattern of brief MN discharges forming a one-phase rhythm was also seen in suction electrode records from the vagus nerve, made intracranially in eviscerated in vitro preparations of the head (2). These continued after 1-2 h exposure to 2-4 mM strychnine (Fig. 2).

The synchronous discharges of all the branchial constrictor MNs for all the segments, and their continuation under strychnine, tend to exclude models of the CNS pattern generator invoking inhibitory synaptic networks. Instead, as for the one-phase rhythm of the crustacean heart (3), I propose that lamprey respiration may be driven by the endogenous bursting cellular properties of certain pacemaker neurons in the medulla.

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(1) T.D.M. Roberts, Proc. R. S. Edinburgh B64: 235 (1950).
(2) C. M. Rovainen, J. comp. Physiol. 94: 57 (1974).
(3) K. Tazaki & I. M. Cooke, J. Neurophys. 42: 975 (1979).

Fig. 1. Intact animal - EMG

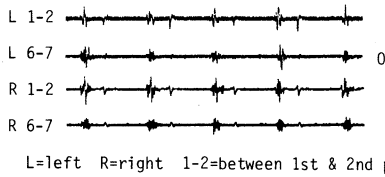

Fig. 2. 90 min in 4 mM strychnine

L=left R=right 1-2=between 1st & 2nd pores, etc.

221.4 STRYCHNINE INDUCES "FICTIVE GALLOPING" IN THE ISOLATED SPINAL CORD OF THE LAMPREY. Avis H. Cohen and Ronald M. Harris-Warrick. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The motor program for swimming in the lamprey is characterized by 1) bursts of motor activity in the left and right ventral roots (VRs) of a single segment alternating strictly out-of-phase, and 2) a constant phase delay between the VR bursts of segments along the cord. This pattern can be activated in the isolated spinal cord by bath application of excitatory amino acids such as D-glutamate (Cohen and Wallén, Exp. Br. Res., 41:11-18, 1980). Pretreatment of the cord with low concentrations of the glycine antagonist, strychnine (5-10 μM) results in alteration in the motor pattern evoked by D-glutamate. The altered pattern, called fictive galloping, is characterized by in-phase bursting between left and right sides of a single segment. Such synchronized bursting is always more rapid than controls ($\bar{X}$=0.99±.36 Hz to $\bar{X}$=3.8±1.3 Hz) and can be highly stable, lasting 5 to 45 minutes. Pretreatment with lower concentrations of strychnine (0.5-3 μM) increase the burst frequency but retains alternation of VR activity between right and left sides, as was found by Grillner and Wallén (Acta Physiol. Scand. 110:103-105, 1980). These effects are in contrast to seizures that can be induced by higher concentrations of strychnine (>10 μM). Seizures are generally long intense VR discharges followed by periods of complete silence of the motoneurons lasting several seconds.

Strychnine can produce a variety of changes in the inter-segmental constant phase coupling. In all cords, the phase lag after strychnine is more variable than in controls. The mean phase value can be longer, shorter, or unchanged, compared to controls. In some cords, the phase value can decrease to zero, with the bursts along the cord fully synchronized.

The most likely interpretation of these results is that the central pattern generator for locomotion generates the synchronized bursting which follows strychnine pretreatment. On the basis of mathematical modeling studies (with R. Rand and P. Holmes) we suggest that the normal pattern is generated by a pair of independent right and left neuronal oscillators in each segment (or small group of segments). In this view, the oscillators within a segment are reciprocally coupled via strong inhibitory and weak excitatory connections. Strychnine presumably acts by blocking the glycinergic inhibitory coupling. This exposes the weak excitatory coupling, which is apparently adequate to synchronize the pair of segmental oscillators. Strychnine-sensitive intersegmental coupling is demonstrated by the results, but at present its role remains unclear.

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221.5 SEROTONIN MODULATES FICTIVE SWIMMING IN THE LAMPREY SPINAL CORD. Ronald M. Harris-Warrick and Avis H. Cohen. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Upon bath application of excitatory amino acids such as D-glutamate, the isolated spinal cord of the lamprey, Petromyzon marinus, generates the complete motor pattern for swimming (Cohen and Wallen, Exp. Br. Res. 41:11, 1980). To aid in the analysis of the central pattern generator (CPG) for swimming, we sought endogenous compounds that modulate the CPG output. Serotonin (5-HT) is one such agent. Two changes are seen in the D-glutamate-induced swimming motor pattern following bath application of 5-HT: 1) the frequency of ventral root (VR) motor bursts is reduced; 2) the intensity of each ventral root discharge is increased. This follows from an increase in the discharge rate of previously active motoneurons and recruitment of new motoneurons. These effects are dose-dependent; the threshold is 1-3 μM, and with 10^{-4}M 5-HT, the burst frequency is slowed 20-fold. Intracellular recordings from myotomal motoneurons show synaptically driven membrane potential (MP) oscillations during fictive swimming (Russell and Wallen, Acta Physiol. Scand. Abst., 1979). Serotonin induces a large increase in epsp frequency during the depolarizing phase of the motoneuronal oscillation, thereby depolarizing the MP further than usual and increasing spiking. During the hyperpolarizing phase of the oscillation, an increase in ipsp frequency strongly hyperpolarizes the cell. There appear to be no direct effects of 5-HT on myotomal motoneurons themselves: in the absence of fictive swimming (without D-glutamate in the bath), 5-HT does not directly affect the resting potential, input resistance or threshold for action potential initiation. However, in some preparations, in the absence of fictive swimming 5-HT induces a slow oscillation in the motoneuron resting potential. This oscillation has a period of about 1 minute and is due to periodic high frequency inhibitory synaptic input. In summary, 5-HT affects the CPG for fictive swimming by slowing and enhancing the motor output. While this may be due to an effect of 5-HT directly on the CPG, we cannot exclude a possible effect of 5-HT on other inputs to the CPG, thus only indirectly affecting CPG output. The lamprey could modulate the activity of a single CPG in this way to generate a family of related behaviors used in different environmental circumstances, such as swimming, crawling and burrowing, which have been described in the lamprey (Ayers, 1982). Supported by NIH Grant No. NS-17323 and an MDA Research Grant.

221.6 SEROTONIN IS PRESENT IN NEURONS AND FIBER TRACTS OF THE LAMPREY SPINAL CORD. Joan A. Filler*, Kathryn Simmons* and Ronald Harris-Warrick. (SPON: M. Salpeter) Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In an accompanying abstract (Harris-Warrick and Cohen), we show that serotonin (5HT) modulates fictive swimming generated by the lamprey spinal cord. Here we show that 5HT is present in neurons and fiber tracts in the spinal cord of the lamprey, Petromyzon marinus. Formaldehyde induced fluorescence has labeled indolealkylamine-containing ventromedial cell bodies and endings along the ventral surface of the lamprey spinal cord (Baumgarten, Progr. Histochem. Cytochem., 4:1-90, 1972; Ochi et al., Arch Histol. Jap., 42:327-336, 1979). We report that additional putative 5HT-ergic projections are revealed in the cord with indirect immunofluorescent labeling techniques. Furthermore, we have biochemically demonstrated the presence of 5HT in the cord. To determine 5HT content, spinal cords were extracted in acid. The extract was fractionated by HPLC on a reverse-phase C-18 column, and analyzed by electrochemical oxidation of the eluate. Serotonin is detected in spinal cord extracts as a substance which coelutes with authentic 5HT in several running buffers, and which has an oxidation voltage dependence identical to that of authentic 5HT. The lamprey spinal cord contains 26 ± 7 pmol 5HT/mg wet weight (n=9). For anatomical localization of 5HT-like immunoreactivity (5HT-LIR), spinal cords were processed as whole mounts or in 20μm cryostat sections using the methods of Beltz and Kravitz (J. Neurosci. 3:585-602, 1983). 5HT-LIR was localized in ventromedial cell bodies with processes running laterally and ventrally. At the ventral surface of the cord, just lateral to the midline, are regions of intense punctate labeling. Labeled processes dotted with large varicosities, possible release sites for 5HT, are seen running longitudinally in the lateral and medial fiber tracts and occasionally traversing the medial gray region. Some of the labeled fibers in the medial fiber tracts may originate from the ventromedial cell bodies; however, most labeled fibers do not appear to arise locally. While we do not know the origin of these fibers, they may constitute a previously unidentified descending 5HT-ergic input to the lamprey spinal cord. 5HT-LIR was specific and was eliminated by preadsorption of the first antibody with 5HT or 5HT-BSA, but not by BSA or other amines. These results show that 5HT is present and appropriately localized in the lamprey spinal cord to have a normal role in the modulation of the central pattern generator for swimming.

Supported by an MDA Research Grant.

221.7 EXCITABILITY OF THE CRAYFISH LATERAL GIANT ESCAPE CIRCUIT IS DEPRESSED DURING FEEDING. F. B. Krasne. Dept. of Psychology and Brain Res. Inst., University of Calif., Los Angeles, 90024.

The probability of a given behavior occurring in response to a suitable stimulus is often modulated by factors such as motivational state or ongoing activities of an animal. In invertebrate nervous systems there is a real possibility of discovering the sites at which, and the cellular mechanisms whereby such control is effected. The lateral giant (LG) tailflip escape reaction of the crayfish provides particularly desirable material for this sort of investigation because the mediating circuitry is well analyzed and is organized in such a way that one can plausibly identify certain neurons (mechano-sensory neurons and first-order interneurons) as being part of stimulus processing circuitry, others (the LGs themselves) as being decision and command elements, and still others (fast flexor motor neurons and interneurons that are interposed between the LGs and the motor neurons) as motor pattern generating elements. Thus, discovery that the operations of particular neurons in this circuit are subject to modulation could readily be equated with control of particular processing tasks. However, the priority of LG escape, which is an emergency evasion tactic, might be so high that its likelihood of occurrence would be unaffected by motivation or other behavior. The experiments to be reported show, to the contrary, that excitability of LG escape is consistently depressed when a crayfish is feeding. This opens the possibility for experiments on locus and mechanisms of control. Stimuli for LG escape were single brief shocks to roots 2-4 of the last abdominal ganglion via chronically implanted electrodes in freely behaving animals. Tailflip behavior was monitored visually, and firing of the LGs and first-order sensory interneurons were recorded with chronically implanted electrodes on the abdominal nerve cord. During feeding the stimulus threshold for LG-mediated escape responses and for firing of the LGs themselves almost invariably rose, though not always to a great extent. Thus, there is a site of modulation at or antecedent to the LGs. Experiments to determine more precisely the site(s) of control, which are in progress, will be reported at the meeting.

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221.8 ELECTRICALLY COUPLED PACEMAKER NEURONS RESPOND DIFFERENTLY TO THE SAME PHYSIOLOGICAL INPUTS AND NEUROTRANSMITTERS. J.S. Eisen and E. Marder. Biology, Brandeis Univ., Waltham, MA 02254.

The 2 pyloric dilator (PD) motor neurons and the single anterior burster (AB) interneuron form the pacemaker for the pyloric central pattern generator of the stomatogastric ganglion of the lobster, Panulirus interruptus. The PD and AB neurons are electrically coupled and always depolarize and release transmitter synchronously. We now show that in spite of their electrical coupling and synchronous activity, the AB and PD neurons differ dramatically in their physiological responses to known neuronal inputs and to a number of exogenous substances which are candidates for neurotransmitters in pathways likely to be important in regulation of the pyloric output. The PD and AB neurons were studied in isolation from one another using the Lucifer yellow photoinactivation technique (Miller and Selverston, Science 206: 702-704, 1979). Bath application of 10^{-4}M dopamine activated slow wave depolarizations (bursts) in the isolated AB neuron (following inactivation of the PD neurons), but hyperpolarized and inhibited the isolated PD neurons (after inactivation of the AB neuron). Bath application of 10^{-4}M serotonin activated bursts in the isolated AB neuron, but was without detectable effect on the isolated PD neurons. Bath application of the muscarinic, cholinergic agonist, pilocarpine (10^{-4}M) activated bursts which had very different waveforms and frequencies in isolated AB and isolated PD neurons. Stimulation of the IVN input fibers produced an enhancement of the bursting properties of the isolated PD neurons but not of the isolated AB neuron. Previous work has demonstrated that the pyloric pacemaker is composed of neurons which differ in a number of ways and evoke distinct physiological actions on follower neurons (Eisen and Marder, J. Neurophysiol. 48: 1392-1415, 1982). The data presented here suggest that neurons of the pacemaker can be activated and modulated in a number of different ways, by a variety of neuronal inputs. Supported by NIH NS 17813 to E.M.

221.9 SEROTONIN AND LEECH SWIMMING: THE RETZIUS CELLS ARE NOT REQUIRED. W.B. Kristan, Jr. & J. C. Glover*. Dept. of Biology, B-022, UCSD, La Jolla, CA 92093.

The production of normal swimming movements in the leech has been shown to be critically dependent on serotonin in that destruction of all serotonin-containing neurons (5HT neurons) in the nerve cord renders the animal incapable of normal swimming (Glover & Kramer, Science 216:317-319, 1982). In investigating the essential role of serotonin in this behavior, it is important to know whether all or only some of these neurons are required. There are 5 types of segmentally iterated 5HT neurons in the nerve cord which can be distinguished from each other on the basis of position morphology. The selective ablation of the full complement of one of these cell types would determine whether that cell type is exclusively responsible for the serotonin needed in the expression of swimming behavior. Such an ablation has been achieved for the Retzius cells, the largest and best characterized 5HT neurons in the leech. Because the Retzius cells are the first of the 5HT neurons to develop a high-affinity serotonin uptake system (see Glover & Stuart, this volume), and 5,7-dihydroxytryptamine, the toxin used to ablate these neurons, exerts its effects via this uptake system, toxin-treatment of embryos at an early stage results in the selective ablation of the Retzius cells while most of the other 5HT neurons are spared. Subsequent behavioral examination of these embryos reveals that swimming is normal. Thus, the Retzius cells are not a requisite source of serotonin for swimming behavior, even though they are capable of releasing enough serotonin to initiate swimming in isolated nerve cords (Willard, J. Neuroscience 1:936-944, 1981). Later toxin-treatment of embryos results in the inability to produce normal swimming movements, and histological examination of these embryos reveals that in addition to the Retzius cells, at least 80% of the lateral 5HT neurons (cells 61 & 21) are ablated. There are two possible explanations for these results. One is that none of the 5HT neuron types is a requisite source of serotonin, and that ablating enough 5HT neurons of all types drops serotonin below some critical level necessary for normal swimming. The second is that one or both of the lateral 5HT neurons, which are known to have inputs to the swimming central pattern generator (Nusbaum, this volume), are required for swimming. Selective ablation of these neurons is being attempted to distinguish between these possibilities.

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221.10 EFFECTS OF A SEROTONIN-CONTAINING NEURON (CELL 61) ON THE LEECH SWIM CENTRAL PATTERN GENERATOR. M.P. Nusbaum Dept. of Biology, UCSD, La Jolla, CA 92093.

Stimulation of the serotonin-containing neuron designated as cell 61 causes the initiation and maintenance of the leech swim motor program in the completely isolated nervous system (Nusbaum & Kristan, Soc. Neurosci. Abstr. 8: 161, 1982). Sometimes stimulation of cell 61 causes only a localized generation of the swim motor program, initiating the swim pattern in only several adjacent ganglia of a longer chain of segmental ganglia. In contrast, in the same preparations stimulating the swim-initiating interneuron cell 204 (Weeks & Kristan, J. exp. Biol. 77: 71-88,1978) always initiates swimming in the entire chain of ganglia. These findings suggest that cell 61 can turn on the swim central pattern generator (CPG) independent of activity in cell 204. This difference in the swim-initiating abilities of these two cells may be due to the more limited extent of the axons of 61 as compared to cell 204. Each cell 61 has processes that extend over only three ganglia whereas the processes of a single cell 204 extend over at least fourteen, and perhaps all twenty-one, segmental ganglia. It may be that what determines whether cell 61 initiates a localized or cord-wide expression of the swim motor program is the strength with which it excites the local CPG interneurons to which it has access. Only if they are stimulated strongly would they provide enough excitation to cell 204 to activate the swim motor program along the entire nerve cord. Additionally, cells 61 and 204 have additive effects on the swim CPG so that in isolated nerve cords where stimulation of neither cell initiates swimming, simultaneous stimulation of both cells does initiate swimming.

That cell 61 does have direct access to the swim CPG is indicated by pairwise intracellular recordings showing that cell 61 causes a strong, apparently monosynaptic excitation of a swim pattern generating interneuron, cell 208. Preliminary results suggest that this EPSP is caused by the closing of voltage-sensitive channels selective to an ion with an equilibrium potential that is more hyperpolarized than the resting potential. Cell 61 activity does not excite cell 208 when this cell is hyperpolarized, either by inhibitory synaptic potentials or by current injection. In contrast to this, monosynaptic excitation of cell 208 from cell 204 is mediated by an increase in conductance in cell 208. It appears that the entire excitatory synaptic input to cell 208 during swimming comes from the serotonin-containing cells and cells 204. The relative contribution to swim initiation of the respective decrease and increase in conductance underlying the synaptic excitation of this CPG interneuron by cells 61 and 204 is being investigated.

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221.11 Leydig Cell Activity Modulates Heartbeat Pattern Generator Cycling in the Leech. Edmund A. Arbas & Ronald L. Calabrese Biological Laboratories, Harvard Univ., Cambridge, MA 02138.

Bursts of action potentials in heart excitor (HE) motor neurons comprise the fundamental motor output of the central pattern generator that drives heartbeating in the leech. HE cells themselves tend to fire tonically, but their activity is shaped into rhythmic bursts through coordinated inhibition by a network of heart interneurons, the HN cells. All 7 pairs of HN cells contribute to the rhythm and coordination of the broader network, but the most rostral 4 pairs alone control the timing of network cycling through their ability to reset and entrain activity of all cells in the heartbeat system.

The pattern generator cycles continuously, and in the absence of apparent perturbations, HN and HE bursts can be very regular, varying from the mean cycle period by 5% or less over long time intervals.

In prevous studies on rate control in this system, we have identified behavioral conditions and neural pathways by which pattern generator cycling is accellerated, i.e. HN and HE cell burst periods are reduced.

We report here that spiking activity in the electrically coupled network of Leydig neurons leads to an increase in HE cell burst period, that is, a transient decelleration of motor bursts.

When any of the bilaterally paired Leydig cells of ganglia 3 or 4 is made to fire by intracellularly injected current, HN cells of the stimulated ganglion are made to fire spikes in concert and their normal side to side reciprocity is disrupted. They alternate in firing clusters of action potentials rather than bursts. This concomittant firing prolongs the duration of inhibition to HE cells, lengthens their burst period, and resets the rhythm of the network.

Leydig cells in the anterior 4 ganglia apparently exert their influence by acting on HN cells of the timing oscillator while Leydig cells of other ganglia influence the timing of the network to the degree that their activity conducts to the anterior Leydig cells via their electrical connections.

Leydig cell influence on HN cells has the following properties. 1) Onset of bilateral HN excitation is delayed by as much as tens of seconds from onset of Leydig firing, and for short bursts, outlasts Leydig firing. 2) No discrete psp's related to Leydig firing are evident in HN recordings.

Leydig cells are suspected to exert their actions in a neurosecretory manner. Experiments are underway to test this possibility. Supported by N.I.H. 5 F32 NS 06453 (EA) and N.S.F. BNS-8121551 (RLC)

221.12 INTERGANGLIONIC COORDINATION OF THE LEECH SWIMMING RHYTHM. R.A. Pearce* and W.O. Friesen. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The central pattern generator (CPG) underlying swimming in the leech Hirudo medicinalis produces its characteristic output even when the leech nerve cord is removed from the animal. The output pattern of the isolated nerve cord exhibits many of the features characteristic of swimming in the intact leech, including a rearward-directed metachronal progression of motor nerve activity as recorded from peripheral nerves. The motor neurons are driven by a small number of oscillatory interneurons whose interactions generate the rhythm and provide for intersegmental phase lags. It is known that some of the oscillatory interneurons project at least five segments and possibly ten (Friesen et al., J. exp. Biol., 75:25, 1978; Weeks, J. comp. Physiol., 148:265, 1982). The present study was undertaken to determine the span over which interneurons coordinate swimming pattern generation in the isolated nerve cord.

Motor neuron spikes were recorded simultaneously from up to seven dorsal posterior nerves. Nerve cords included segmental ganglion two through ganglion twenty. One to several ganglia in the middle of the cord were bathed in modified leech saline containing 20 mM Mg and 1 mM Ca, a solution which blocks chemically-mediated synaptic transmission in the leech (Baylor and Nicholls, J. Physiol., 203:591, 1969). In this way, the CPGs in those ganglia which were "blocked" could not contribute to the oscillations in other ganglia, although action potentials in through-fibers were not blocked. It was found that the cord still exhibited normal coordination when up to four mid-body ganglia were blocked, with the following exception. The motor output of one or two ganglia immediately anterior to the block was delayed and of those immediately posterior was advanced. In one case this led to a reversal of the phase relationships across the block, even though normal phasing was maintained between the two ends of the cord. When five ganglia were blocked, the cord usually exhibited normal phase relationships, although occasionally the ends became uncoordinated. When more than five ganglia were blocked, swimming could not be elicited.

We conclude from these results that information sufficient to coordinate the CPGs in different ganglia travels at least six segments, and possibly many more. It is curious that the ganglia adjacent to the synaptic blockade could express altered phase relationships, while normal phasing was maintained between the two ends. It is possible that the change in phase occurs at the motor level only and not at the level of the CPG. Supported by grants from NSF and NIH.

221.13 THE ROLE OF SWIM-INITIATING INTERNEURONS IN LEECH SWIMMING ELICITED BY MECHANOSENSORY STIMULATION. E.A. Debski and W.O. Friesen. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Intracellular stimulation of an individual N cell in an isolated leech nerve cord reliably initiates the patterned neural activity characteristic of swimming (Debski and Friesen, Neurosci. Abstr. 8:529, 1982). However, the effectiveness of N cell stimulation fatigues rapidly. We are currently investigating swim-related interneurons to define the pathway leading from the N cell to the swim oscillator and to identify the labile elements in that pathway.

Cells 204 and 205 are the only well-described interneurons whose depolarization reliably elicits swimming activity (Weeks, J. exp. Biol. 77:71, 1978 and J. Neurosci. 2:972, 1982). Both cell 204 and cell 205 monosynaptically excite cell 208, a candidate component of the central pattern generator for swimming (Weeks, J. Comp. Physiol. 148:265, 1982). Kristan has proposed that sensory cells activate cell 204 in several ganglia as well as cell 205 . Furthermore, it is the summed activity of these swim initiating interneurons, acting through cells 208, which produces swimming (TINS 6:84, 1983). In order to determine if swim initiation by N cell stimulation occurs via this pathway, we have investigated the input to cells 208 and 204 when stimulation of an N cell leads to swimming and then subsequently, when this response has fatigued. This has been done with the goal of first ascertaining whether the input to these cells differs in the two cases and second, determining if this input is predictive of whether or not the preparation will initiate swimming.

Intracellular stimulation of an N cell eliciting swimming activity evokes weak inhibition in cell 208 of the same and immediately adjacent ganglia. Neither the amplitude nor the duration of this inhibition appears altered when the ability of the N cell to initiate swimming has fatigued. We found that, as reported by Weeks, any single 204 receives only weak polysynaptic input from intracellular stimulation of individual N cells. Input from N to cell 205 is also polysynaptic but somewhat greater in magnitude. As with cell 208, the level of input into cells 204 in the same and the immediatedly adjacent ganglia does not predict subsequent swim initiation. In contrast to the data so far obtained for cells 204 and 208, preliminary results for cell 205 show that this cell does undergo a large change in input that is correlated with the ability of the N cell to initiate swimming. This suggests that cell 205 may be more directly involved than cell 204 in this swim initiation pathway. Supported by NSF grant BNS 81-0243.

221.14 SELECTIVE CELL KILLING BY PHOTOINACTIVATION DISTINGUISHES INDIRECT FROM DIRECT CONNECTIONS BETWEEN MOTOR NEURONS IN THE LEECH. B. Granzow and W.B. Kristan, Jr. Dept. of Biology, UCSD, La Jolla, CA 92093.

In the leech Hirudo medicinalis longitudinal muscles of the body wall are innervated by both excitatory and inhibitory motor neurons. During swimming, contractions in dorsal longitudinal muscles alternate with contractions in ventral longitudinal muscles. This occurs because the central pattern generator for swimming drives alternating bursts of action potentials in dorsal and ventral longitudinal muscle excitatory motor neurons. In addition, alternating bursts occur in the dorsal and ventral inhibitory motor neurons whose activity contributes to the relaxation of their respective target muscles between contractions. Each inhibitory motor neuron also centrally inhibits the excitatory motor neurons whose target muscles they antagonistically innervate; i.e., dorsal inhibitors inhibit dorsal excitors and ventral inhibitors inhibit ventral excitors. Several lines of evidence indicate that this inhibition is monosynaptic. A complicating feature of this system, however, is that depolarizing current injected into dorsal inhibitors also causes an inhibitory post-synaptic effect in the ventral excitors; likewise between ventral inhibitors and dorsal excitors. This effect is usually not as strong and is much more variable than that which occurs between dorsal inhibitors and dorsal excitors or ventral inhibitors and ventral excitors. We hypothesized, therefore, that the connections from dorsal inhibitors to ventral excitors, and from ventral inhibitors to dorsal excitors were indirect and mediated by an electrical connection known to exist between the dorsal and ventral inhibitors. In order to test this hypothesis we photoinactivated the dorsal inhibitors, by injecting them with Lucifer Yellow and then illuminating them with blue light (Miller & Selverston, Science 206:702-704, 1979). Prior to inactivation of the dorsal inhibitors, depolarizing current injected into ventral inhibitors caused a substantial hyperpolarization in dorsal excitors. Following photoinactivation, this effect was no longer present although the ventral inhibitors still strongly inhibited the ventral excitors. We are now using the same photoinactivation method to test the contribution of the inhibitor motor neurons to the phasic inhibition of excitor motor neurons during swimming. Judging by the strength of the connections between inhibitors and excitors, dorsal inhibitor elimination should significantly weaken or even eliminate the oscillations which underlie phasic bursting in the dorsal excitor motor neurons.

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222.1 LATEX BEADS COATED WITH FRAGMENTS OF THE FIBRONECTIN MOLECULE AS PROBES OF NEURAL CREST MIGRATORY PATHWAYS.

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Latex beads with defined surface coats can be used as probes of interactions involved in neural crest cell localization. In previous studies, latex beads were microinjected into embryos at the time of neural crest migration. The beads distributed along the ventral neural crest pathway in an analogous manner to endogenous crest cells. However, when the beads were coated with fibronectin (FN), the FN-beads remained at the implantation site and did not distribute along the ventral route (Bronner-Fraser, Devel. Biol., 91: 50, 1982). Fibronectin is a glycoprotein with multiple binding domains. In the present study, latex beads were coated with various fragments of the fibronectin molecule. Because each fragment represents a different functional domain, these studies examine specific binding interactions which are responsible for immobilization of the beads and their subsequent restriction from the ventral neural crest pathway.

Three proteolytic fragments which represent various parts of the fibronectin molecule (kindly provided by Dr. Erkki Ruoslahti) were used for these studies. The "120K" fragment contains the cell binding site. The major components of the "66K" fragment include the collagen-binding site and the amino terminal end of the polypeptide. The fragment referred to as the "45 + 32K" fragment represents the heparin-binding domain and the carboxyl terminal.

Latex beads were coated with either the 120K, 66K, or 45 + 32K fragment. The coated beads were subsequently injected into the trunk somites of avian embryos at the time of neural crest migration. In the majority of embryos injected with 120K-coated beads, the beads did not translocate along the ventral pathway, but remained at the implantation site. In contrast, beads coated with the 66K or 45 +32K fragments did translocate along the ventral pathway in the majority of cases.

Thus, the 120K fragment, which contains the cell-binding moiety of fibronectin, appears to be the major fragment involved in bead immobilization. The results suggest that the cell-binding domain of the fibronectin molecule may be responsible for the restriction of fibronectin-coated beads from the ventral neural crest pathway.

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222.2 PROPOSED GREEK KEY FOLDING PATHWAY FOR β-STRUCTURE IN MYELIN BASIC PROTEIN (MBP).

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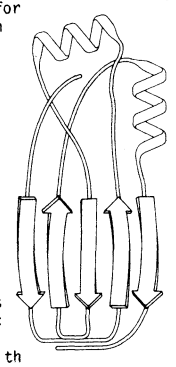
Phosphorylation of nascent MBP in the cytoplasm of the oligodendrocyte may permit the cationic polypeptide to fold easily into a compact structure by reducing the positive charge at critical sites (Stoner, Trans. Am. Soc. Neurochem. 14:164, 1983). Secondary structure prediction by algorithms of Robson, Chou-Fasman, and Lim shows 5 hydrophobic β-strands in MBP (Martenson, J. Neurochem. 36: 1543, 1981). The strands are ideally located for folding as a Greek key β-structure (right) from the type of hairpin intermediate proposed by Ptitsyn and Finkelstein (Quart. Rev. Biophys. 13:339, 1980). The hairpin loop contains the Pro-Pro-Pro (100-102) sequence, phosphorylated Thr-99, and methylated Arg-108. P-Thr-99 could form salt links with Lys-106 and with Arg-108 across the loop. The N-methyl of Arg-108 may interact with the side chain of Pro-97. With the 3 Pro residues in the trans conformation, models show that a reverse turn may occur at residues 102-105 (Pro-Ser-Gln-Gly). Each large loop could accomodate an α-helix. The proposed β-sheet (below) contains only 26% of the 170 residues in human MBP, but it has 69% of the hydrophobic residues (underlined). The strands are aligned for maximum interstrand hydrophobic interaction (dashes). Following dephosphorylation, MBP interacts as an extrinsic protein with the cytoplasmic surface of the myelin membrane. If the highly polar large loops are extended as shown above, the hydrophobic residues in the β-sheet remain available for interaction with non-polar constituents of the membrane surface. Six cationic residues on the faces of the β-sheet (brackets) may be part of binding sites for phospholipid head groups. The prediction of a β-sheet in MBP is at variance with CD studies of MBP in solution which show little, if any, β-structure. However, MBP has usually been extracted from the myelin membrane under denaturing conditions. If phosphorylation of nascent MBP were essential for the folding of the protein into its native structure, the purified protein, which is only minimally phosphorylated, would not be expected to refold correctly in aqueous solution.



(13)	(118)	(87)	(45)	(150)
[Lys]	Gly	Val----Phe		Thr
Tyr----Trp----Val----Phe----Leu				
Leu	Ser	His	[Arg]	Ser
Ala	Phe----Phe		Gly	[Lys]
Thr	[Arg]	Phe----Ile----Ile		
Ala	Ser	[Lys]	Ser	Phe
Ser	Leu	Asn	Asp	[Lys]
Thr	Ser	Ile----Leu----Leu		
Met----Leu----Val----Ile				Gly
(21)	(110)	(95)	(37)	(158)

222.3 SCHWANN CELL-AXON INTERACTIONS STUDIED IN VITRO.

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Schwann cells (SC) are known to interact specifically with axons. The basis of this interaction is poorly understood. We report here preliminary studies on the development of an assay for examining this interaction in vitro.

Schwann cells from newborn rat sciatic nerves were isolated by a modification of the procedure of Raff et al. (Nature 273:672, 1978). Approximately 4 x 10^6 cells were obtained from 10-12 Sprague Dawley rat pups of 1 to 3 days of age. By indirect immunofluorescence using anti-Thy 1.1, 10% of the cells were fibroblasts. One day after seeding, the rapidly dividing fibroblasts were killed with cytosine arabinoside (10^{-5} M). The remaining fibroblasts were removed using a complement mediated killing step with a monoclonal anti-Thy 1.1 (IgM antibody) and rabbit complement. The SC thus obtained were of greater than 99% purity. They were grown on bovine endothelial cell extracellular matrix with Bottenstein-Sato's N2 medium with 1 ug/ml of cholera toxin which elevates intracellular cAMP. The Schwann cells grew as monolayers with a doubling time of 48 hours, but did not elaborate a basal lamina.

Schwann Cell recognition and adhesion to the axon is required for synthesis of basal lamina components and eventual synthesis of myelin. We investigated this process by utilizing two different neuronal cell cultures; rat spinal cord explant cultures and dissociated chick spinal cord anterior horn cell cultures (Schnaar et al. J. Neurosci. 1:204, 1981). Schwann cells, when co-cultured with these two types of tissues, appeared to bind to the neurites, elongate to adopt a spindle shape and form into bands. About one week after co-culturing, the SC processes became refractile but there was no convincing evidence of myelination. In order to quantitate the adhesion phenomenon, the Schwann cells were labeled by culture with [^{32}P]orthophosphate and the method of McClay et al. (Proc. Nat. Acad. Sci. 78:4975, 1981) was used. In summary, we have obtained cultured neurites and pure populations of SC and have demonstrated their interaction in vitro. Furthermore, we have developed a method for radiolabeling the SC so that we can now begin to quantify this interaction and examine its characteristics. By use of these techniques, the molecular requirements for this interaction may be probed.

(This research was supported by a grant from the KROC Foundation.)

222.4 ADHESION BETWEEN NERVE MEMBRANES AND GLIAL CELLS IS INHIBITED BY MONOCLONAL ANTIBODIES.

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Studies on nervous tissues have suggested the hypothesis that the development of the nervous system and its mechanical and biochemical support are greatly dependent on interactions between nerve cells and glial cells. To test this hypothesis further, we studied the interaction between nerve and glial cells in vitro. Heterotypic cell-cell binding experiments indicated that neurons in suspension bound to monolayers of glial cells but not to fibroblasts or to cells from the meninges during a 25 min incubation period. Fab' fragments of rabbit antibodies against neuronal membranes inhibited adhesion of neurons to monolayers of glial cells, but Fab' fragments from preimmune rabbit sera or sera from rabbits immunized with the neural cell adhesion molecule (N-CAM) did not inhibit adhesion. In order to search for cell surface antigens that may function as adhesion molecules between nerve and glia, an assay was developed that allowed quantitative measurement of adhesion between neuronal membrane vesicles and glial cells. As in the case of heterotypic cell-cell adhesion, neuronal membrane vesicles bound to glial cells but not to fibroblasts, and adhesion was inhibited by Fab' fragments prepared from anti-nerve membrane antisera, but not by Fab' fragments from preimmune sera or anti-(N-CAM) sera. Fractions extracted from neuronal membranes neutralized the inhibitory activity of the anti-nerve membrane Fab'. In this manner, the neutralization assay was used to select fractions rich in neutralization activity during purification processes. Antigenic determinants were released from 14d chick embryo brain membranes with trypsin, partially purified by chromatography on DEAE-cellulose, lentil lectin Sepharose, and Sephacryl S300 columns, and used to generate monoclonal antibodies in mice. Monoclonal antibodies that inhibited neuronal membrane vesicle binding to glial cells were obtained. Monoclonal antibodies that were coupled to Sepharose beads by the CNBr method, depleted neutralization activity from solution. Indirect immunofluorescence staining of cultures of live brain cells showed that neurons but not glial cells were recognized by the monoclonal antibodies. These results suggest that a nerve cell surface antigen may mediate adhesion between nerve and glial cells in vitro. The monoclonal antibodies described here are being used to isolate the antigen on neural cells and to determine the role of such a molecule in the adhesion between nerve and glial cells.

(This work was supported by NIH grants HD-16550, AI-11378, AM-04256, and a postdoctoral fellowship to M.G. AI-06414).

222.5 NONERYTHROID SPECTRIN-LIKE PROTEINS: STRUCTURAL CHARACTERIZATION OF A SPECTRIN ANALOGUE FROM MOUSE BRAIN. S.R. Goodman*, I.S. Zagon, C.F. Whitfield*, L.A. Casoria*, P.J. McLaughlin and T.L. Laskiewicz*. (SPON: T.A. Lloyd) Depts. of Physiology* and Anatomy, The M.S. Hershey Medical Center, Hershey, PA 17033.

Spectrin is a long fibrous $(\alpha\beta)_2$ tetrameric (α = 240 K Dal, β = 220 K Dal) protein, which along with actin and several other proteins forms the erythrocyte membrane skeletal network. Our laboratory was the first to demonstrate that immunological and structural analogues of spectrin exist is diverse nonerythroid cells (Goodman et al, PNAS 78 7570-7574, 1981), and it is now known that these spectrin analogues exist almost ubiquitously in the cortical cytoplasm of eukaryotic cells, where they probably mediate the association of actin to the plasma membrane. Mouse brain demyelinated membranes contain two large spectrin-like peptides of 240 K and 235 K Dal which are present in a 1:1 mole/mole ratio, constitute 2.3% of the total membrane protein, and cross-react with antibodies against mouse rbc spectrin. The membrane-associated brain spectrin has been purified by low ionic strength extraction of demyelinated membranes (37°C, 1h) followed by rate zonal sedimentation through a 5-18% sucrose gradient (286,000 x g, 15h, 2°C), and removal of minor actin contaminants on an anti-actin-Sepharose 4B affinity column. The purified mouse brain spectrin contains the 240 K (α) and 235 K (β) subunits in a 1:1 mole/mole ratio and has a M.W. $=_o$972,000 calculated from its hydrodynamic properties (R_s = 220 A, $S_{20,w}$ = 10.5, partial specific volume = 0.73). The mouse brain spectrin is therefore structurally similar to erythrocyte spectrin as a large asymmetric protein which is an $(\alpha\beta)_2$ tetramer. While brain and erythrocyte spectrin share common functional regions such as an actin, calmodulin, and syndein binding sites, two dimensional peptide mapping analysis of ^{125}I-labelled tryptic peptides indicates only limited overlap between their α and β subunits. This suggests that erythrocyte and brain spectrin are distinct gene products, and is supported by our finding that sph/sph mice with a severe inherited spherocytic anemia due to a 95% deficiency in erythrocyte spectrin have normal levels of membrane-associated brain spectrin. Only the 220 K β subunit of rbc spectrin and the 235 K β subunit of brain spectrin are phosphorylated by [^{32}P]orthophosphate in the intact animal, and in vitro analysis suggests that both are phosphorylated by cAMP independent protein kinases. Brain and rbc spectrin are therefore distinct gene products, which share structural and functional homology. (Supported by NIH grants HL-26059 and NS-19357 to S.R.G., and DA-01618 to I.S.Z.; S.R.G. is an Established Investigator of the American Heart Association).

222.6 NONERYTHROID SPECTRIN-LIKE PROTEINS: IMMUNOCYTOCHEMICAL LOCALIZATION IN MOUSE BRAIN TISSUE. I.S. Zagon, S.R. Goodman*, P.J. McLaughlin and L.A. Casoria*. Depts. of Anatomy and Physiology, The M.S. Hershey Medical Center, Hershey, PA 17033

Nonerythroid spectrin-like cytoskeletal components are actin-binding proteins found in the cortical cytoplasm of diverse eukaryotic cells and tissues (review by Goodman and Shiffer, Am. J. Physiol. 244: C121-141, 1983). We have raised antibodies in rabbits against mouse red blood cell (rbc) and mouse brain spectrin, and have demonstrated by immunoautoradiography that both antibodies crossreact with the α and β subunits of rbc and brain spectrin, with preferential staining of the α subunit. Both antibodies have been used to localize an immunoreactive analogue of spectrin in the adult mouse brain. Indirect immunofluorescence studies of the cerebellum with either rbc or brain spectrin antibodies revealed an intense fluorescence of the perikarya of internal granule neurons, with the pattern of staining being most intense at the cell membrane. The observed staining appeared to be localized within the cell rather than on the cell surface since specimens which were not permeabilized did not stain. Granule cell nuclei were unstained. Other neuronal cell types such as Purkinje, basket, and stellate cells also demonstrated fluorescence of the cytoplasm, and unstained cell nuclei. The molecular layer was less intensely stained than the internal granule layer. Except for long strands of fluorescent material that resembled nerve fibers, the medullary layer exhibited no staining. Oligodendrocytes and astrocytes in the cerebellar cortex were often difficult to detect, having little cytoplasmic fluorescence. The localization of spectrin analogues in other parts of the brain was further examined by study of the hippocampus. The cytoplasm of the granule cells of the dentate gyrus had intense staining, but cell nuclei were unstained. Although the pattern of staining within anti-brain and anti-rbc spectrin IgG were often similar, a more intense staining was observed with brain spectrin antibody as would be expected from immunoautoradiographic studies. The observed staining patterns were specific for brain spectrin, as anti-brain spectrin preabsorbed with a 10-fold excess of brain spectrin, anti-rbc spectrin preabsorbed with a 10-fold excess of rbc spectrin, and preimmune IgG exhibited little or no staining in the cerebellum or hippocampus. Our results show that spectrin is an integral part of the cortical cytoplasm of neural cells. Moreover, while brain is a tissue rich in nonerythroid spectrin, the concentration of these immunoreactive analogues is quite variable within distinct cellular entities of the brain. (Supported by grant DA-01618 to I.S.Z. and grants HL-26059 and NS-19357 to S.R.G.; S.R.G. is an Established Investigator of the American Heart Association).

222.7 A MOLECULE RESEMBLING ACTIN IS INVOLVED IN CLUSTERING OF ACETYLCHOLINE RECEPTORS IN CULTURED RAT MYOTUBES. R.J. Bloch and W.G. Resneck*, Department of Physiology, University of Maryland School of Medicine, Baltimore, Md. 21201.

Acetylcholine receptor (AChR) clusters can be purified >20-fold from cultured rat myotubes, with little change in their organization: AChR-rich regions (AChR domains) and AChR-poor regions in close contact with the tissue culture substrate (contact domains) remain clearly distinguishable. Cytoskeletal proteins associated with contact domains in intact cells are lost during cluster purification, however. This raises two questions. What structural proteins remain associated with the isolated AChR clusters? And, are they involved in maintaining cluster organization? Here we report that an actin-like molecule is present in isolated cluster preparations, and that its removal disorganizes AChRs.

The major band in SDS-PAGE analyses of isolated AChR clusters has an apparent molecular weight of 43,000. It is metabolically labeled with ^{35}S-methionine, but not with ^{3}H-sugars, and it is degraded by proteolytic enzymes. It is therefore a protein, which we call p43. At low ionic strength (a condition which solubilizes actin from skeletal muscle) most of the p43 is extracted from isolated AChR clusters, together with two other, slightly larger proteins. AChRs remain associated with the membrane phase, but lose their characteristic domain organization. This suggests that p43 and other polypeptides extracted at low ionic strength are involved in maintaining isolated AChR clusters.

In immunofluorescence experiments, a monoclonal antibody prepared against actin (kindly provided by Dr. R. Anthony, Univ. of Md. Sch. of Med.) stains isolated AChR clusters. Staining is only over AChR domains. Upon extraction at low ionic strength, immunofluorescent staining is eliminated. Chymotryptic digestion, which also disrupts the AChR organization of isolated AChR clusters, simultaneously destroys the ability of isolated clusters to stain with antibody. After dispersal of AChR clusters in intact cells using azide, AChR levels and staining by anti-actin of isolated clusters preparations are greatly reduced. These results suggest that an actin-like molecule is involved in maintaining clusters in isolated preparations and in intact myotubes. Preliminary evidence indicates that the antibody reacts well with p43 and with erythrocyte actin, but only poorly with rabbit skeletal muscle actin. Thus, p43 may be a membrane-bound form of actin which is involved in AChR clustering.

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222.8 SIALIC ACID REGULATES CALCIUM EXCHANGE IN SYNAPTOSOMES. S. Lin-Liu, W.R. Adey and E.M. Helm*. VA Medical Center and Loma Linda University, Loma Linda, CA 92357

Sialic acids occupy the outermost position of the carbohydrate chains of cell surface glycoproteins. Their strategic position and charge properties may have led to their ability to regulate calcium exchange across the plasma membrane of cultured heart cells. We are now testing the possibility of a similar role for sialic acid in synaptosomes. Synaptosomes were prepared from Sprague-Dawley rats and calcium exchange was first studied by investigating the uptake of $^{45}Ca^{++}$ at 32°C. Synaptosomes (0.6 mg/ml) were incubated in a physiological medium (0.2 mM $CaCl_2$) containing either low K^+ (5 mM) or high K^+ (40 mM) for various time intervals (10s - 10 min). Incubation was terminated by dispersing aliquots of synaptosomes on Millipore filters under vacuum filtration and rinsing immediately with 10 ml of an ice cold physiological medium containing either high Na^+ (135 mM) or low Na^+ (5 mM). In general, the time course curve exhibited two phases with a latency of 2.5 min for the second phase if high Na^+ is present in the washing medium. No delay was observed with the low Na^+ washing medium. High K^+ enhanced both the rate and level of $^{45}Ca^{++}$ uptake of the first phase, but was rather ineffective in changing the rate of uptake of the second phase. Neuraminidase treated synaptosomes can be differentiated from the controls only with the high K^+ incubation and high Na^+ washing. The latency for the second phase was decreased (shifted to the left) in the neuraminidase treated synaptosomes, resulting in an apparent increase in $^{45}Ca^{++}$ uptake during the second phase. Since no difference was observed with a low Na^+ washing medium, the total level of calcium exchanged was probably not altered by neuraminidase treatment. Since high K^+ increases intracellular calcium level and Na^+ enhances calcium efflux in synaptosomes, the difference between neuraminidase treated and control synaptosomes may be explained by the ability of sialic acid to regulate calcium fluxes across the plasma membrane.
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222.9 A TORPEDO MONOCLONAL ANTIBODY DEFINES A SUBSET OF MAMMALIAN NEURONS. P.D. Kushner and D.T. Stephenson* ALS Research Center, Pacific Medical Center, San Francisco, CA 94115.

Neuronal cell surface molecules coordinate the development and insure the maintenance of the nervous system in its organization and in the execution of its remarkable tasks. Some of these molecules may be found on all neurons, playing roles fundamental to neuronal function, such as axonal elongation or adhesion. Other molecules are found on some neurons and not on others. Such molecules, such as ion channel receptors, may play roles specific to those neuronal types on which they occur.

We have generated a library of monoclonal antibodies to the cholinergic synaptosome from the marine ray (Kushner and Reichardt, Neurosci. Abs. VII 40.4). From this collection of 141 synaptosome-specific antibodies we have identified an antibody which cross-reacts strongly with the mammalian nervous system, but is found associated with only a very limited population of neurons. Histochemical localization of this antibody's binding within the rat nervous system is distinguished by the fact that, although the antigen appears to be rare, where it is found, it is highly concentrated along the membrane of individual neurons. These neurons include both cholinergic ventral horn motor neurons of the spinal cord and noradrenergic principal neurons of the sympathetic ganglion.

We report that the mammalian antigen defined by this antibody is a protein and has an apparent molecular weight of 180,000 daltons. Our studies indicate this antigen is evolutionarily conserved from elasmobranchs to mammals. Sedimentation equilibrium studies reveal that the protein is peripherally associated with the membrane. The antibody binds the membrane fraction of freshly homogenized rat tissue centrifuged at moderate speeds (12,000 g). Both freezing and high speed centrifugation (100,000 g) dissociate the protein from the membrane. This loose association of the protein with the membrane indicates it is not an integral membrane protein. It is therefore not an ion channel receptor. Studies of this protein help address the question of whether there are neuronal subsets defined by cell surface components which correspond to transmitter type. Supported by an MDA grant to L.F. Reichardt and grants from MDA and ALSSOA toPDK.

222.10 PURIFICATION AND CHARACTERIZATION OF CALCIUM-ACTIVATED NEUTRAL PROTEASES FROM MOUSE AND POSTMORTEM HUMAN BRAIN. A. Vitto and R.A. Nixon. Harvard Medical School, McLean Hospital, Belmont, MA. 02178.

We have purified Ca^{+2}-activated neutral proteases (CANPs) from both mouse brain and postmortem human brain cortex using a rapid three-step column procedure that affords good recovery of stable protease, and avoids exposure of CANP to calcium or destabilizing ligand-affinity columns. Supernatants of brain homogenates are applied to DEAE-cellulose, and CANP activity against ^{14}C-azocasein eluted with a linear salt gradient. The main peak of activity eluting at 0.2-0.25 M NaCl is applied to Phenyl-sepharose and eluted with a low ionic strength buffer containing 15% ethylene glycol. Activity is pooled, concentrated, and applied to an Ultrogel AcA 44 gel filtration column. CANP eluted from Ultrogel is pooled, concentrated, and stored at -70°C in 10% glycerol. Molecular weights of 95-96K were obtained for both mouse and human enzymes by Sephacryl S-300 analytical gel filtration. SDS gel electrophresis indicated two subunits with molecular weights of 72-73 and 23-30K. The proteases were active at neutral pH but not at acid or alkaline pHs. No activity was detected in the presence of EGTA. Both enzymes were inhibited by leupeptin, antipain, TLCK, and by thiol-active reagents such as iodoacetamide, mersalyl, N-ethylmaleimide, and mercuribenzoate derivatives. PMSF, pepstatin, TPCK, benzamidine, diphenylcarbamyl chloride, and soybean typsin inhibitor did not inhibit protease activity. Mouse and human brain CANPs were sensitive to 0.5 mM Ca^{+2} and optimally active at 1-5 mM. Barium and strontium stimulated activity but were less effective than Ca^{+2}. Magnesium was without effect. Manganese slightly activated only the human enzyme. In addition to the major CANP from mouse brain, a second CANP of slightly lower molecular weight eluted from DEAE at 0.05 M NaCl. This lower molecular weight form partially co-eluted with an endogenous heat-stable inhibitor of CANP. The endogenous factor appeared more inhibitory toward this lower molecular weight form than the major CANP. The endogenous inhibitor from mouse brain is also active against the major CANP from human brain attesting further to the similarity between the mouse and human enzymes. Both proteases degraded endogenous substrates and were especially active toward high molecular weight proteins including neurofilament proteins. The foregoing results indicate that CANP can be assayed and purified from human brain and that the major CANPs from mouse and human brain are quite similar. These results will facilitate the analysis of Ca^{+2}-mediated proteolytic events in normal human brain and in a number of human neurological disorders. (Supported by NIH grants NS17535 & RR05484, the Alfred Sloan Foundation, and the Anna and Seymour Gitenstein Foundation).

222.11 PHOSPHORYLATION OF EXTRACELLULAR AND MEMBRANE PROTEINS OF GOLDFISH OPTIC TECTA IN VITRO: EFFECTS OF CALCIUM AND ELECTRICAL STIMULATION. V. E. Shashoua and G. W. Hesse*. Dept. of Biological Chemistry, Harvard Medical School, Mailman Research Center, McLean Hospital, Belmont, MA 02178

Goldfish optic tecta can be maintained in a defined medium in an atmosphere of O_2/CO_2 (99/1) for at least 72 hr in vitro. Stable extracellular field potential responses, identical to those obtained in vivo, are produced when the tectal optic nerve fibers or marginal fibers are stimulated. We have used this preparation as a model system for investigating the direct effects of electrical stimulation and alterations of Ca^{2+} levels in the incubation medium on the pattern of phosphorylation of proteins in an intact neural tissue. We focused our studies on the influence of depletion of Ca^{2+} in the extracellular environment, since, under physiological conditions, intact neural tissues normally become exposed to transient reductions in the levels of Ca^{2+} as a result of neural activity. Moreover, we used ATP [5'-(γ-thio)^{35}S phosphate] as the phosphorylating agent to restrict the reaction to external surface membrane proteins, since ATP does not usually enter cells unless it is first hydrolyzed to release phosphate, and the ^{35}S phosphorylated proteins, once formed, do not tend to be as readily subjected to enzymatic breakdown as ^{32}P-labeled products.

Under such conditions, ATP (5'-[γ-thio]^{35}S-phosphate labeled), added to the incubation medium, was found to selectively label two tectal protein bands electrophoretically migrating at 45 kD and 45 kD on SDS-polyacrylamide gels. Subcellular fractionation of the labeled tecta, followed by one- and two-dimensional gel electrophoresis, indicated that both proteins were largely associated with the membrane and crude synaptosomal fractions. In addition, certain proteins released into the extracellular medium migrating at 32 kD and 26 kD were heavily labeled. If the normal extracellular calcium level of 2.5 mM was lowered by the addition of 1.5 mM EGTA, then the incorporation of phosphate into the 54 kD and 45 kD bands increased by over 400%. The labeling of these same bands was also enhanced by 30-40% if the tecta were electrically stimulated at 20 Hz for up to 30 min via the marginal fiber input to the tecta.

These findings suggest that the tectal extracellular environment may have an important role in the post-translational modification of specific membrane proteins. Thus long-term depletion of Ca^{2+} by chelation with EGTA or the transient local removal of Ca^{2+} that occurs during stimulation can result in an enhanced phosphorylation of the same membrane components. (This research was supported by NINCDS grant No. 09407.)

222.12 ^{3}H-INOSITOL INCORPORATION INTO PHOSPHATIDYLINOSITOL IN A SYMPATHETIC GANGLION TREATED WITH 4-AMINOPYRIDINE. B.A. Patterson*, and R.L. Volle. Dept. of Pharmacology, Univ. of Connecticut Health Center, Farmington, CT 06032.

Enhanced phosphatidylinositol (PI) turnover has been associated with activation of certain receptors, however the nature of the interaction and its role are not well defined.

Rat superior cervical ganglia are incubated at 37° C in various drug containing and modified Locke's solutions, all of which contain 20 μM myo-inositol (MI). After an initial 30' equilibration in MI containing Locke's solution, ganglia are incubated for an additional 30' with blocking agents, when used, then transferred to test solutions containing ^{3}H-MI for 30' of uptake. Ganglia are then homogenized and the phospholipids extracted by a modification of the method of Lapetina and Michell (Biochem. J. 126: 1141-1147, 1972) and chromatographed against standards on silica gel TLC plates according to the method of Weiss et al. (Biochem. J. 204:587-592, 1982).

The ^{3}H label migrates almost exclusively with the PI spot on TLC, typically accounting for 97% of the label applied to the plate, the remainder of the label being found at or near the origin. Aliquots of extract counted directly, without resort to TLC, consequently give results that are like those derived from TLC. Therefore, results are routinely obtained by that method.

Ganglia exhibit a dose dependent increase in the incorporation of ^{3}H-MI into PI in the presence of 4-aminopyridine (4AP), an inhibitor of K^+ conductance. Treatment of ganglia with 10^{-3} M 4AP results in a 100% increase in the labeling of PI. The response is eliminated by incubation with 10^{-5} M atropine and reduced by 73% in 10^{-6} M atropine. Hexamethonium (5×10^{-4} M) reduces the response by 40%. Tetrodotoxin, however, is without effect. Experiments in which all Ca^{2+} in the bathing media is replaced by Mg^{2+} are complicated by the fact that Ca^{2+} free media alone produces a 50% increase in label incorporation into PI and inclusion of 1 mM EGTA in the Ca^{2+} free media results in a 120% increase. However, when 10^{-3} M 4AP is added to the Ca^{2+} free media, a further increase in uptake is clearly evident. Treatment of ganglia with 10^{-4} M bethanechol brings about a 230% increase in uptake of ^{3}H-MI into PI. Ganglia denervated for 7-10 days exhibit a 30% increase in uptake into PI when compared with their contralateral controls. Nevertheless, denervated ganglia show increased incorporation of ^{3}H-MI into PI in response to 10^{-3} M 4AP. Atropine (5×10^{-6} M), while ineffective in blocking the increase resulting from denervation alone, blocks 90% of the additional increase observed with 10^{-3} M 4AP.

These results suggest that acetylcholine is not involved in the atropine sensitive stimulation of ^{3}H-MI uptake into PI brought about by 4AP. (Supported by NS 07540-16).